ABSTRACT BOOK

SFTCG Annual Meeting 2025

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Invited Speakers

INV03

Challenges for CRISPR-Cas9 genotoxicity: detection and prevention

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CRISPR-Cas9 is a breakthrough that has propelled regenerative medicine into a new era. Important advances are illustrated by several ongoing clinical studies using CRISPR-Cas9 nuclease in fields such as immunotherapy, regenerative medicine, and monogenic diseases. Nevertheless, a major concern is the potential genotoxicity of DNA double-strand breaks (DSB), which arise from incorrect or ineffective DNA repair and DNA damage response. The risk of genomic instability seems to be the Achilles heel of CRISPR–Cas9 nuclease. It is mandatory to find solutions to prevent these side effects.

Our team was pioneer to describe the presence of chromosomal rearrangements induced by CRISPR-Cas9 nuclease. We are developing innovative methods for detecting these unwanted events, making it possible to decipher the mechanisms involved in their appearance and to test the effectiveness of new editing protocols (using new DSB-less CRISPR tools or modifications of culture protocols). For example, we recently highlighted the role of cell cycle. G0/G1 cell cycle phase blockade during DSB drastically reduces chromosomal rearrangement rate, without impairing DSB efficacy. Importantly, palbociclib exposure during editing prevents genotoxicity in cell line and in clinical-relevant hematopoietic stem cells. These results contribute to the understanding of genotoxicity mechanisms and the development of safe editing protocols with this powerful CRISPR-Cas9 tool for tomorrow's medicine.

Oral Presentations

OR01

POLYPRIME: A novel DNA-Polymerase based prime editing strategy

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Prime editing (PE) is a DSB-free method that edits DNA using a Cas9 nickase fused with reverse transcriptase (RT), and a modified guide RNA (pegRNA) with a 3' extension. However, PE efficiency depends on the target, cell type, and edit size, with inefficient long insertions or replacements (>50bp). In addition, the scaffold sequence from the pegRNA can be incorporated into the genome.

Our goal is to develop safer and more efficient PE tools. We created a DNA Polymerase-based method called POLYPRIME. We replaced RT with DNA polymerases (DNApols), which could help to install long edits and reduce scaffold incorporation. POLYPRIME uses a hybrid RNA-DNA molecule, pegDNA, which we produce by ligating a standard gRNA to a DNA extension.

We evaluated POLYPRIME *in vitro*, comparing three DNApols. Our results showed that pegDNAs can generate target nicks more efficiently than standard pegRNAs, and the 3' DNA extension is efficiently copied by Cas9-DNApol fusions. Next, we optimized POLYPRIME in cells using a reporter system based on BFP to GFP conversion (due to C to T conversion). Different lengths and chemistries of the 3' DNA extension were tested to optimize the pegDNA design, finding that the 3'extension should be much longer in pegDNAs compared to pegRNAs, typically 20nt compared to 10. We tested 3 DNA-pol fusions with optimized pegDNAs, all showing equivalent POLYPRIME activity. We validated the optimized POLYPRIME design on endogenous targets and found equivalent or superior activity to standard PE, especially with long insertions (30 to 54 bp).

OR02

Zip Editing: an easy-to-use tool to increase CRISPR-Cas9 HDR-editing efficiency

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Genome editing using CRISPR-Cas9 holds great promise in the field of gene therapy. However, the use of CRISPR-Cas9 for precise genome editing through HDR repair pathways remains a challenge. This is mostly due to the lack of availability of the exogenous template at the site of editing at the moment of the repair. To increase the presence of the exogenous template

delivered as single-stranded oligodeoxynucleotides (ssODN), we develop ZIP-Editing (ZE), a new tool that brings the ssODN with the RNP complex by annealing an extension of the ssODN template with an extension of the gRNA (patent in progress). We tested ZE in different cell lines and primary cells (including human fibroblasts and HSPCs) to edit several loci (*eGFP*, *CFTR*, *UROS* and *HBB*) with the nuclease and nickase Cas9s to challenge its universality. Thus, we demonstrated that this system, very easy to design and that uses only commercially available components, increases HDR-editing efficiency up to 12-fold as compared to a condition where ssODN template is not annealed to the gRNA. These results position ZE as a new tool to precisely edit the genome and as part of the gene editing toolbox for treating or modelling diseases. Moreover, ZE is a safer tool because it does not rely on the modification of the cell cycle, the opening of the chromatin nor the modulation of the DNA repair pathways, which can have deleterious genotoxic effects and are a major safety concern in the development of gene therapies.

OR03

Correcting Autoinflammation in STING-Associated Vasculopathy with Onset in Infancy (SAVI) by Human Stem Cell Genome-Editing

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Type I interferonopathies comprise a large group of Mendelian autoinflammatory diseases that generally lack effective treatments. STING-associated Vasculitis with onset in Infancy (SAVI) is a severe autosomal dominant disease due to gain-of-function (GOF) mutations in the gene *STING1*, encoding for the Stimulator of Interferon Response CGAMP Interactor 1 (*STING1*). SAVI leads to a severe multisystemic disease comprising systemic vasculitis and lung fibrosis. A possible therapeutic approach is to engineer the patient's hematopoietic system to restore interferon homeostasis, thereby preventing chronic inflammation and organ damage and halting disease development. Here, we describe a "universal" genome editing correction strategy using CRISPR/Cas9 that targets the *stimulator of interferon response cGAMP interactor 1* (*STING1*) gene at the endogenous locus in SAVI patient-derived induced pluripotent stem cells (iPSCs) and in human $CD34^+$ hematopoietic stem and progenitor cells (HSPCs). The engineered SAVI-iPSCs express normal levels of STING1 protein following differentiation into monocytes and macrophages and no longer produce interferon-alpha (IFN-a) at a basal state. Using SAVI-gene-engineered HSPCs, we determined the minimum fraction of mutant alleles required to induce spontaneous IFN-a production, thus establishing the threshold of genome correction necessary to rescue the disease. Finally, we demonstrated engineered $CD34^+$ HSPCs at the *STING1* locus retain regenerative potential by supporting long-term repopulating capacity and multi-lineage differentiation potential following transplantation into immunocompromised mice. Together, these studies established the rationale for the clinical translation of SAVI genome editing and a therapeutic framework for correcting other type I interferonopathies.

OR04

CRISPR activation of utrophin as a mutation-independent approach for Duchenne Muscular Dystrophy therapy

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Duchenne muscular dystrophy (DMD) is a severe genetic disorder affecting 1 in 5.000 newborn males. It is caused by mutations in the *DMD* gene, leading to dystrophin deficiency and progressive muscle degeneration. Current dystrophin-based therapies, such as exon-skipping, AAV-delivered mini/micro-dystrophin, and CRISPR editing, aim to restore truncated dystrophin forms. However, these approaches can elicit immune responses and some are mutation-specific, limiting their therapeutic scope. An alternative approach, potentially applicable to all DMD patients regardless of their genetic mutation, involves upregulating utrophin (UTRN), a structural and functional paralogue of dystrophin. UTRN reactivation has shown therapeutic potential in preclinical models, improving the dystrophic phenotype without triggering immune responses. Here, we employed CRISPR activation (CRISPRa) systems with catalytically inactive Cas9 (dCas9) fused to transcriptional activators or epigenetic modulators, enabling targeted *UTRN* activation without DNA cleavage. Using *in silico* chromatin analysis of ENCODE muscle tissue data, we identified 12 candidate sgRNAs targeting the *UTRN-A* promoter and distal enhancer (DUE). Testing dCas9-activators with each single sgRNAs in HEK cells, alongside a positive control sgRNA for *β-globin*, we achieved up to a 5-fold increase in *UTRN* mRNA. Combinations of sgRNAs and editors are currently being evaluated in immortalized myoblasts from DMD patients. Given the inefficiency of plasmid transfection in these cells, we are comparing the delivery of activators via FACS sorting of plasmid-transfected myoblasts and nucleofection of in vitro-transcribed mRNA to enhance delivery efficiency. Our findings highlight UTRN-targeted CRISPRa as a promising mutation-independent therapy for DMD, offering broader applicability and reduced immunogenicity compared to dystrophin-based approaches.

OR05

Efficient *in vitro* correction of a highly recurrent *COL7A1* pathogenic variant using Cytosine Base editing to treat recessive dystrophic epidermolysis bullosa

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a rare and severe genetic skin disease resulting in blistering of the skin and mucosa after minor trauma. RDEB is caused by a wide variety of variants in *COL7A1* encoding type VII collagen (C7), the major component of anchoring fibrils that form key attachment structures for dermal-epidermal adhesion.

Here, we achieved highly efficient *COL7A1* correction in primary RDEB cells using Cytosine Base editors (CBEs). We designed four guide RNAs (gRNAs) targeting c.425A>G (p.K142R), a highly recurrent variant changing the last nucleotide of exon 3.

Four *in vitro* transcribed CBEs together with four chemically modified gRNAs were delivered as mRNA by nucleofection to RDEB keratinocytes and fibroblasts (K and F) in culture. Two gRNAs showed up to 73 and 91% editing in RDEB cells at the gDNA level, as evaluated by Sanger and high-throughput sequencing. RT-PCR and sequence analysis showed the presence of a correct transcript in gene-edited RDEB-F. Assessment of C7 protein expression and secretion after editing revealed levels of restored C7 similar to C7 in normal human cells. Rescued C7 protein expression was also confirmed by immunofluorescence staining.

We concluded that *COL7A1* editing and desired phenotypic correction (up to 91%) could be achieved in primary RDEB-K and F by base editing using combined gRNA and CBE mRNA delivery.

Grafting of genetically corrected 3D skin equivalents onto nude mice is ongoing to demonstrate functional correction for future ex vivo clinical applications.

OR06

CRISPR-Cas9 mediated endogenous utrophin upregulation improves Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an incurable progressive neuromuscular disorder due to mutations in the *DMD* gene, loss of dystrophin expression and muscle wasting. Dystrophin-recovery approaches only restore truncated proteins, might be immunogenic and some are mutation-dependent. Another potentially universal approach consists in upregulating utrophin (UTRN), a paralogue of dystrophin able to compensate for the deficit without inducing immunogenicity. We developed a CRISPR-Cas9 strategy to increase endogenous utrophin. We disrupted the binding site (BS) of miR-Let-7c, a known UTRN repressor, in human DMD and murine myoblasts. This induced a ~3.5-fold increase of expression. Results were confirmed in three-dimensional human DMD cultures, where editing resulted in UTRN upregulation and functional improvements. We showed the gRNA has no major off-targets. Finally, we evaluated this strategy in the mdx mouse model of Duchenne. We confirmed that miR Let-7c expression is not affected in the skeletal and cardiac muscles of this model compared to wild-type mice. We then performed intravenous injection of two recombinant adeno-associated viruses type 9 (rAAV9s) encoding for Cas9 and gRNA. DNA editing in the tibialis anterior (13%), heart (21%) and diaphragm (5%) were sufficient to upregulate UTRN expression by 1.5-2-fold. This allowed an amelioration of muscle histopathological phenotype. To increase vector delivery and editing, we are currently treating mdx mice with a more myotropic AAV. The strategy has been applied to other loci, validated in human DMD myoblasts and is currently tested in 3D organoids. These findings provide the foundations for a universal gene editing therapeutic strategy for DMD.

OR07

Bioconjugation of the capsid of adeno-associated viruses for osteoarthritis treatment by gene therapy

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This project focuses on chemically engineering adeno-associated viruses (AAV) through bioconjugation to enhance specificity toward cartilage tissue to treat osteoarthritis.
Cartilage is a non-vascularized tissue in which chondrocytes, the sole extracellular matrix (ECM) producing cells, generate a highly negatively charged matrix due to its major component, glycosaminoglycans. In osteoarthritis, chondrocyte dysfunction leads to cartilage degradation, affecting over 500 million people worldwide. Despite its prevalence, osteoarthritis remains incurable, due to the challenges of delivering therapeutic agents to chondrocytes embedded in the dense ECM.

AAV are promising gene therapy vectors, as demonstrated by four ongoing clinical trials targeting cartilage. However, their broad tropism and limited ability to reach targeted cells reduce their efficiency. To address these limitations, our team developed a chemical modification strategy to enhance AAV specificity and efficacy for targeted cells or tissues.

The AAV capsid is chemically modified in two steps: biotin ligands are bioconjugated to capsid lysines, followed by non-covalent binding of avidin, a positively charged protein. This modification exploits the strong biotin-avidin interaction and imparts an overall positive charge to the vector. This charges facilitates electrostatic interactions with the negatively charged ECM, enhancing vector retention, adhesion, and penetration into deeper cartilage layers.

Preliminary results demonstrate that these modifications preserve AAV infectivity in human chondrocytes. Ongoing work assesses vector retention and penetration in cartilage explants, along with *in vivo* studies in a rat model to evaluate biodistribution and cartilage targeting. This approach presents a promising strategy to overcome challenges in cartilage-targeted gene therapy and develop osteoarthritis treatments.

OR08

AAV-mediated gene therapy of acid ceramidase deficiency by intravenous and CSF-directed vector administration

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Farber disease and spinal muscular atrophy with progressive myoclonic epilepsy are two ultra-rare lysosomal storage disorders resulting from loss-of-function mutations in the *ASAH1* gene encoding for acid ceramidase (ACDase). This enzyme catalyzes the conversion of ceramide, a

bioactive lipid, into sphingosine and fatty acid. ACDase deficiency leads to the intracellular accumulation of ceramides with an inflammatory response in tissues. These two diseases manifest differently but are part of a clinical continuum with variable severity affecting the nervous system and/or peripheral tissues. Currently, there is no curative treatment, highlighting a significant unmet medical need.

In the present study, we assessed the efficacy of a gene therapy approach with an AAV9 vector expressing human ACDase in a severe mouse model of acid ceramidase deficiency (*Asah1*$^{P361R/P361R}$) by two routes of administration, intravenous and intracerebroventricular. We performed a dose escalation study by intravenous injection with three vector doses and found that the low dose was suboptimal, whereas most pathological parameters were corrected at mid dose. Detailed histological analysis of treated mutant mice 6 months post-injection revealed that the high dose was able to correct the presence of inflammatory infiltrates in tissues, including the central nervous system (CNS). We also report that intracerebroventricular vector administration prolonged lifespan, improved body growth and enhanced motor activity. While treatment effectively mitigated CNS symptoms, it did not fully address peripheral organ manifestations.

Our findings provide proof-of-concept that systemic AAV-mediated *ASAH1* gene replacement can correct the whole-body phenotype of *Asah1*$^{P361R/P361R}$ mice and pave the way for clinical translation in patients.

OR09

Gene therapy for familial hemophagocytic lymphohistiocytosis related to Munc 13-4 deficiency

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Familial Hemophagocytic Lymphohistiocytosis type 3 (FHL3) is a rare genetic disorder, accounting for 30% of all forms of FHL. This condition is characterized by a defect in the cytotoxicity of T and NK cells, leading to hyperinflammation and the infiltration of activated cells in organs. FHL3 is caused by mutations in the UNC13D gene, which encodes the Munc 13-4 protein. Currently, hematopoietic stem and progenitor cell (HSPC) transplantation is the only curative treatment available. However, complications such as graft-versus-host disease or graft rejection are common when using partially HLA-compatible donors. Therefore, gene therapy may present a promising therapeutic option. We have applied this approach to human cells by utilizing a third-generation self-inactivating lentiviral vector. This vector expresses a codon-optimized UNC13D gene under the control of the EF1 alpha promoter. We successfully transduced HSPCs and CD3+ T-cells, and restored the cytotoxic functions. The average vector copy number achieved was 1.3 ± 0.7, making it suitable for a clinical application. Both in vitro and in vivo biodistribution studies showed no signs of toxic effects. Overall, our results demonstrate, for the first time, the effectiveness of UNC13D gene transfer into HSPCs and T-cells derived from patients with Munc 13-4 deficiency. A phase I/II clinical trial targeting Munc 13-4 deficiency has recently received

approval from regulatory authorities, and we expect to begin recruiting the first patients in the coming months.

OR10

In vivo CAR T cell therapy against angioimmunoblastic T cell lymphoma

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Angioimmunoblastic T cell lymphoma (AITL) represents a rare complex malignancy with no specific treatment available and a poor survival outcome. We have previously generated a unique preclinical mouse model for AITL by overexpressing glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exclusively in T cells, resulting in a T cell lymphoma closely mimicking the clinical and pathological features of human AITL disease, with $CD4^+$ follicular helper T cells being the drivers of the malignancy.

By lentiviral (LV) transduction, we generated $CD8^+$ T cells expressing a chimeric antigen receptor (CAR) against the CD4 epitope present on the malignant T cells. To allow the vector's exclusive entry into $CD8^+$ T cells, the anti-CD4CAR LV has been pseudotyped with a CD8 receptor-targeted measles virus envelope (anti-CD4CAR CD8-LV).

Anti-CD4CAR CD8-LV transduced murine AITL biopsies resulted in CAR expression on $CD8^+$ T cells and in their expansion, accompanied by an almost complete elimination of the neoplastic $CD4^+$ T cells, as compared to the control transduced with GPF-encoding CD8-LV. We then evaluated the anti-CD4CAR CD8-LV functionality and specificity *in vivo* by direct injection into the bloodstream of our preclinical mAITL model. This resulted in the generation of functional anti-CD4CAR $CD8^+$ effector T cells *in vivo* and in a significant reduction of the $CD4^+$ neoplastic T cells from the tumor, which correlated with an increased survival of the AITL mice.

This study might offer a new therapeutic perspective for patients suffering from a CD4-driven T cell lymphoma, which could surmount the main problems of current *ex vivo* CAR T cell therapy.

OR11

Combination of TCR-deficient CAR-Tregs and non-mitogenic antiCD3 to promote transplant tolerance

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CAR-regulatory T cells therapy holds promises for inducing long-term transplant tolerance while getting rid of immunosuppressive drugs. Our strategy aims to enhance the proportion of donor-specific CAR-Tregs, while debulking alloreactive T cells. We hypothesize that TCR-deficient CAR-Tregs would act synergistically with anti-CD3 therapy.

TCR-deficient HLA-A2-targeted CAR-Tregs were manufactured through lentiviral transduction and CRISPR-Cas9 gene editing. In a first model, NSG mice received HLA-A2+PBMC with CAR-Tregs, TCR+ or TCR-. In a second model, a mix of TCR+CAR-T and TCR-deficient CAR-Tregs, expressing different luciferases was adoptively transferred to mice, transplanted with HLA-A2+ skin grafts. Anti-CD3-induced depletion of TCR+ cells was assessed by *in vivo* bioluminescence and cytometry.

TCR-deficient CAR-Tregs, despite the absence of the TCR/CD3 complex, could still be activated in a HLA A2-specific manner. These cells retained key phenotypic and epigenetic Treg markers. Transcriptomic analysis revealed that gene editing predominantly affected *TRAC*-related genes, with minimal impact on other pathways. TCR+ cells, unlike their TCR- counterparts, were selectively eliminated from blood and lymphoid organs upon anti-CD3. Similarly, anti-CD3 induced a selective depletion of TCR+ CAR-T infiltrating HLA-A2+ skin allografts, while TCR-deficient CAR-Tregs persisted within the allografts. Ongoing transcriptomic profiling and imaging mass cytometry analysis of skin allografts are being conducted to further evaluate the effectiveness of this combination strategy and to map the location of graft-resident CAR-Tregs.

Our data demonstrate that anti-CD3 therapy can selectively advantage TCR-deficient CAR-Treg cells over resident TCR+ T cells *in vivo*. This approach significantly enhances the therapeutic potential of CAR-Tregs, while reducing the number of cells required.

OR12

A brain organoid model to study gene and cell therapy treatment for neuronopathic Gaucher disease

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Gaucher disease (GD) is one of the most common lysosomal storage disorders caused by biallelic mutations in GBA1 gene, which alter the folding and function of the β-glucocerebrosidase

(GCase) enzyme, leading to the accumulation of its glycolipid substrate within lysosomes. Enzyme replacement and substrate reduction therapies fail to control the neuropathology due to their inability to cross blood-brain barrier. Previous studies have shown that lysosomal enzymes can be secreted and taken up by surrounding cells (cross-correction process). Thus, overexpressing GCase in hematopoietic stem cells (HSC), which then colonize the CNS as microglia, may be more effective than transplanting unmodified allogenic cells. This can be achieved by genetically modifying HSCs to overexpress the enzyme. In this study, we successfully established an *in vitro* cerebral organoid model derived from induced pluripotent stem cells (iPSCs) of a healthy individual, a patient with the neurological form of GD ($GBA1^{L444P/L444P}$), and mutated cells re-expressing wild-type GCase. These organoids contain neurons, astrocytes, and microglia, with similar cell numbers. However, significant structural abnormalities in mitochondria and rough endoplasmic reticulum (RER) were observed in the mutated organoid. Additionally, colocalization studies showed that GCase enzyme accumulates within RER in mutated condition. Remarkably, these abnormalities were corrected in the mutated cells re-expressing the wild-type form of the enzyme, suggesting that the neuronopathic form of GD caused by the homozygous L444P mutation could be corrected by providing the wild-type protein. This model provides a promising tool for selecting appropriate GCase vectors via microglial cells transduced by lentiviral vectors.

OR13

Modelling Stargardt disease using three-dimensional retinal organoids

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Stargardt disease (STGD1) is an autosomal recessive genetic disorder caused by mutations in the ABCA4 gene, leading to a juvenile form of severe macular dystrophy. Accounting for nearly 7% of retinopathies, STGD1 currently has no treatment. To overcome the limitations of existing STGD1 models, the use of human induced pluripotent stem cells (hiPSCs) carrying disease-associated mutations represents a promising approach. Due to their differentiation potential, hiPSCs can generate multi-layered 3D structures resembling optic vesicles, known as retinal organoids (ROs), which recapitulate human retinal structure and physiology *in vitro*.

The objective of our project is to model the visual cycle—a critical process for vision that is dysregulated in STGD1—using hiPSCs and ROs derived from both patients and healthy donors. In our initial experiments, we successfully cultivated mature ROs that exhibited proper organization, discernible photoreceptors (PRs), and ABCA4 expression. Additionally, we obtained mature retinal pigment epithelium (RPE) cells with correct polarization, as evidenced by RPE65 expression and ZO-1 localization in confocal imaging. Differentiated RPE cells also participated in the visual cycle, as demonstrated by metabolite measurements using mass spectrometry. Finally, electron microscopy images of RPE cells revealed characteristic microvilli and melanosomes.

Comparative analysis between patient-derived and healthy control models will provide deeper insights into the cellular and molecular mechanisms underlying retinal degeneration in STGD1. Furthermore, our STGD1 models may serve as a platform for evaluating and optimizing gene therapy strategies in our lab.

OR14

Design of experiments assisted optimization of induced pluripotent stem cell (iPSC) directed differentiation toward airway progenitors for a cell and gene therapy against primary ciliary dyskinesia

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Primary ciliary dyskinesia (PCD) is a genetic disease causing ciliary function impairment and bronchial mucus accumulation. The mucociliary function of PCD patients may be restored using an autologous cell and gene therapy using induced pluripotent stem cell (iPSC) derived NKX2.1+ airway progenitors (AP). Our standard differentiation protocol (STD) avoid cell sorting which can damage the cells and be a severe bottleneck in a GMP-compliant bioproduction. STD consists in modulating pathways by introducing molecules in the culture medium only until the cells reach the definitive endoderm (DE) stage, followed by four days without cytokines. Some authors have documented that double inhibition of TFG-B and BMP pathways after the DE stage followed by activation of the RA, BMP and canonical Wnt pathways (DI protocol) can improve the differentiation process. Both protocols lead to the emergence of unwanted cell types such as hepatic progenitors. We improved our STD protocol by modulating the same pathways as DI protocol and optimizing molecules concentration and duration of exposure using a design of experiments (DOE) approach. This statistical method allows optimal value identification for each critical variable of a protocol, with a reduced number of experiments to perform. Compared to STD, the resulting protocol shows 50 times more NKX2.1 (AP marker) expression while neural and hepatic markers expression are respectively 20 and 100 times lower. To eliminate remaining contaminants, transcription factor modulation will be performed during the last differentiation step of the optimized protocol. The differentiated progenitors' homogeneity will be assessed by single cell RNA sequencing.

OR15

3-year Follow-up of Crigler-Najjar Syndrome Patients Treated with AAV-based Gene Therapy in the GNT-012-CRIG Study

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Patients with Crigler–Najjar syndrome (CNS) lack the Uridine diphosphoglucuronate glucuronosyltransferase 1A1 (UGT1A1), leading to severe hyperbilirubinemia that causes irreversible neurologic injury and death. Severe CNS requires prolonged daily phototherapy (PT). The only cure is liver transplantation.

We report the 3-year follow-up data of the dose-escalation part of a phase I/II/III study evaluating the safety and efficacy of a single intravenous infusion of an adeno-associated virus serotype 8 vector encoding UGT1A1 (GNT0003) in CNS patients treated with ≥6 hours/day phototherapy. Five patients received a single infusion of GNT0003: two received 2×10^{12} vg/kg, and three received 5×10^{12} vg/kg. The primary endpoints were measures of safety and efficacy; efficacy was defined as a serum bilirubin level ≤ 300 µmol/L without PT from week 16.

No serious adverse events related to GNT0003 were reported. Two patients receiving low dose of GNT0003 still require PT. Within week 48 after treatment, three patients on high dose could stop PT and had bilirubin levels below 300 µmol/L (mean [±SD] bilirubin at baseline, 301±43 µmol/L with PT; at week 48, 100±32 µmol/L without PT). Within month 36, two of the three high-dose patients still do not require PT, having bilirubin levels below 300µmol/L (199±30 µmol/L at month 36); the third stopped using phenobarbital at week 87 (pregnancy wish) and had to resume PT at week 107.

GNT0003 treatment was safe and well tolerated. These data showed preliminary evidence of sustained clinical benefit up to 36 months. The high dose 5×10^{12} vg/kg is currently evaluated in a pivotal trial.

OR16

Gene therapy for Artemis-SCID patients: preliminary results of the French ARTEGENE phase I/II clinical trial

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Genetic deficiency of the endonuclease DCLRE1C/Artemis, a key factor of the DNA repair machinery, causes Severe Combined Immunodeficiency (SCID) characterized by a complete lack of T and B cells. Allogeneic bone-marrow transplantation is the gold standard to cure the disease

but the outcome is not satisfactory with partial haplo-identical donors. In this context, autologous gene therapy (GT) for Artemis-SCID represents a good alternative. Recently, a GT phase I/II clinical trial (ARTEGENE -NCT05071222) was started at the Necker Hospital, Paris. The first patient (P1) included had a true SCID phenotype but maternal T cells requiring a corticosteroid treatment. During apheresis, we collected mobilized peripheral blood (mPB) and bone marrow (BM) cells for immunophenotyping. Our data demonstrated that the proportions of Hematopoietic Stem and Progenitor Cells (HSPCs) and CD34+CD19+ pro-B cells differs according to the cell source. We also collected P1-transduced HSPCs and after *in vitro* culture, these cells differentiated into pro-T cells and initiated T-cell rearrangements. In a xenotransplantation model, they colonized the thymus and differentiated until the CD3+TCRaB+ stage. At 6 months post-GT, P1 patients had a stable vector copy number of 1, similar to the one found in the drug product. The T and B cell reconstitution increased with time and reached normal values at 12 months post-transplant. Our data highlighted that ARTEGENE gene transfer strategy restore a functional T- and B-cell compartment in Artemis SCID patient. To date, three patients have been included in this trial and the follow-up is ongoing.

OR17

Enhancing anti-tumoral properties of specific extracellular vesicles via miRNA loading: A novel approach for gene therapy in breast and pancreatic cancer models

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Extracellular vesicles (EVs) are tiny, membrane-enclosed structures released by cells, playing a key role in intercellular communication by transporting biomolecules such as nucleic acids, proteins, metabolites, and lipids. They can exert both paracrine and endocrine signaling, traveling through biological fluids with low immunogenic properties, making them promising vehicles for targeted delivery of therapeutic agents. Although EVs are often associated with pro-tumoral effects, our previous work demonstrated that EVs produced by cells expressing the anti-tumoral transcription factor NFAT3 can suppress tumor growth and metastatic dissemination in a breast cancer model. We identified 15 microRNAs (miRNAs) within these EVs responsible for their anti-tumoral and anti-metastatic functions, validated as either anti-proliferative, anti-invasive, or both. To enhance the anti-tumoral properties of EVs from NFAT3-expressing HEK293T cells, they were used to perform an exogeneous loading of the 15 miRNAs. Following validation of the loading efficiency, we evaluated the impact on cancer cell proliferation using an in vitro 3D heterospheroid assay on two triple-negative breast cancer cell lines and a pancreatic cancer cell line. The results showed that exogenous loading of HEK293T EVs with the miRNAs enhances their anti-tumoral effect and that these EVs induce the maximum inhibition of growth across all cell lines, when compared to loaded ADSC EVs and liposomes. In vivo, only loaded HEK293T EVs demonstrated a significant tumor growth inhibition in athymic nude mice. These results provide proof of concept for miRNA-based therapies using EVs as delivery vehicles, laying the foundation for developing a potential gene therapy drug based on this approach.

OR18

Lentivector onco-targeting for solid tumor gene therapy

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Among the innovative therapeutic options in cancers refractory to current therapies, gene-based therapies show considerable promise. But first, cancer gene therapy needs efficient gene transfer into the tumors. Second, cancer gene therapy needs oncospecific transfer of the therapeutic genes, especially when bringing signals potentially harmful to healthy cells.

To obtain tumor-restricted oncotropism, we produced oncotropic lentiviral vector by engineering the E2 recognition glycoprotein of Sindbis virus (SINV-G) with a single-chain variable fragment (scFv), leaving intact the fusion E1 monomer. Unlike the scFv-engineered VSV-G which completely lost fusion activity, the scFv-SINV-E2 showed as efficient as the broad tropism VSV-G, but with exclusive specificity to cells expressing the targeted antigen. Moreover, with these envelope glycoproteins, the transduction efficiency was proportional to antigen expression by cancer cells, a crucial point when healthy cells display low expression of the targeted antigen. Intra-tumor injections of lentiviruses displaying scFv-SINV-E2 in mice bearing subcutaneous tumors produced transduction rates as efficient as lentiviruses displaying VSV-G. Importantly, while VSV-G lentivectors injected intravenously delivered the reporter gene at the injection site, in the liver, and in the bone marrow, scFv-SINV-E2 lentivectors reached only the tumors and its metastasis and no other detectable site, confirming the strong therapeutic anti-cancer value of lentivector onco-pseudotyping.

In conclusion, we confirmed that engineered oncotropic vectors target only the cancer cells, with high reproducibility and applicability for *in vivo* transfer of therapeutic genes. Considering the difficulty of reaching every tumor cell with toxic genes, this onco-specific targeting will be key for implementing intratumoral vulnerabilities.

OR19

Universal allogeneic regulatory T cell therapy from genetically engineered iPSCs

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Organ transplantation and autoimmune disease treatments require innovative immunotherapies to replace non-specific immunosuppressants, which often compromise patients' health. Cell therapy using autologous regulatory T cells (Tregs) has emerged a promising alternative. However, this approach is hindered by limited cell availability, extensive and costly procedures. To address these limitations, we aim to develop a universal allogeneic Treg therapy derived from induced

pluripotent stem cells (iPSCs). Using CRISPR/Cas9, we generated non-immunogenic iPSCs by targeting B2M and CIITA to silence MHC-I and MHC-II expression. To protect these cells from Natural Killer (NK) cell-mediated lysis and promote differentiation toward Tregs, we transduced them with HLA-E and FOXP3, respectively. These engineered iPSCs retained the ability to differentiate into CD34+ hematopoietic progenitors, we established several iPSC master cell banks to evaluate the benefits of each modification. Subsequently, we optimized T cell maturation from CD34+ cells from cord blood by comparing two methodologies: the Artificial Thymic Organoid (ATO) model and a Notch-dependent feeder-free system. Interestingly, the ATO model yielded robust numbers of mature CD8+ T cells. In contrast, the feeder-free culture produced lower yields, but demonstrated greater potential for clinical applications. Our results demonstrate the feasibility of generating universal allogeneic Tregs from iPSCs, offering a scalable and clinically relevant approach for transplantation and autoimmune diseases applications.

OR20

Treg empowering through gene addition

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Solid organ transplantation remains the most effective therapeutic option for organ failure, yet it is associated with significant complications inherent to life-long immunosuppressive therapy. CAR-Treg therapy holds promise for promoting transplant tolerance. However, CAR-Tregs are hindered by progressive attrition and dysfunction, limiting their long-term efficacy. As such, there is growing interest in stabilizing Treg identity and enhancing their suppressive function. Notably, the transcription factors BATF, JunB, and IRF4 play a critical role in driving Treg differentiation into highly suppressive cells. We hypothesized that the transgenic expression of a gain-of-function $IRF4^{F359L}$ variant, which shows enhanced cooperation with JunB, could improve CAR-Treg fitness and function. To test this, we generated $IRF4^{F359L}$-, $IRF4^{wild\text{-}type}$- and empty vector-transduced Tregs from naïve Tregs via lentiviral transduction. Phenotypic and functional analyses were performed at day 16 of culture. $IRF4^{F359L}$-expressing Tregs exhibited distinct phenotypic changes, as assessed by bulk RNA-sequencing and flow cytometry. Compared to controls, $IRF4^{F359L}$ Tregs showed increased expression of CD25, CTLA-4 and HLA-DR, indicating an activated effector phenotype. Importantly, $IRF4^{F359L}$-expressing Tregs retained the expression of key transcription factors, including FOXP3, IKZF2, IKZF4, as well as Treg-specific epigenetic marks. In addition to this core Treg signature, $IRF4^{F359L}$ Tregs exhibited elevated production of CXCL8 under steady-state conditions, which was further enhanced in pro-inflammatory conditions. Given that myeloid-derived suppressor cells (mDSC) express CXCR1 and CXCR2, the receptors for CXCL8, we are currently investigating whether the transgenic expression of $IRF4^{F359L}$ variant in Tregs could augment their tolerogenic potential by enhancing the recruitment of mDSCs.

OR21

Skeletal muscle organoids for preclinical gene therapy with recombinant AAV vectors

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Duchenne Muscular Dystrophy (DMD) is an X-linked genetic disease caused by mutations in the *DMD* gene, coding for dystrophin. Its deficiency causes muscle degeneration in young boys and premature death. Among possible therapeutic approaches, gene therapy using vectors derived from the adeno-associated virus (AAV) is promising. Efficiency and safety of this approach has been shown in animal models. However, during clinical translation, the efficiency was lower and several patients have developed serious adverse events that were not anticipated in preclinical studies. Therefore, new experimental models are needed to better predict the efficacy and toxicity of AAV vectors.

In this context, we developed a preclinical testing platform for DMD gene therapy using skeletal muscle organoids derived from human induced pluripotent stem cells (hiPSCs). We used a reporter AAV vector to determine the most efficient vector delivery strategy and the minimal dose for successful organoid transduction. We observed that transgene expression was maintained over 4 weeks, demonstrating that our engineered muscle model allows for medium-term monitoring of AAV transduction. We also compared the efficiency of 13 AAV serotypes in organoids at different stages of maturation. Interestingly, our results indicate that organoids subjected to mechanical tension to improve their maturation, are better suited to predict the efficiency of AAV variants recently engineered to target muscle cells. Altogether, our study demonstrates the relevance of a preclinical AAV vector screening platform based on human muscle organoids derived from hiPSCs.

OR22

Viral restriction factors impede the transduction efficiency of gene therapy lentiviral vectors and may be blocked to achieve therapeutic levels of gene transfer

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Gene therapy is used to treat a growing number of pathologies. HIV-1-derived lentiviral vectors (LV) are particularly versatile tools for genetic modifications to treat different diseases with engineered hematopoietic stem/progenitor cells (HSPC) or with T cells (e.g. CAR T cells). Major improvements in strategies and vector design have facilitated LV usage. However, LV remain sensitive to restriction factors, innate immune proteins that inhibit the transduction of specific cell lineages. In particular, the protein IFITM3 is a major blockage for LV-mediated transduction. Using overexpressing cells, we showed that IFITM 3 restriction effects are dependent on the pseudotype of the vector and affect the commonly used VSVG pseudotype. We also studied IFITM3 effects in HSPC that are used for gene therapy treatments. IFITM3 is expressed in HSPCs all along the transduction process. We successfully used cyclosporin H to counteract IFITM3 in

HSPC and other cells transduced with VSVG LV. Cyclosporin H reduces IFITM3 levels and enables the use of low concentrations of vectors to reach therapeutically-relevant levels of transduction. Thus, understanding the role of restriction factors in target cells enables the design of more effective vectors and transduction protocols that reduce vector doses and optimize the efficacy and safety of gene therapy.

OR23

Muscle-specific expression reduces early antigen presentation and promotes CD8 T cell tolerance after rAAV gene transfer

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Immune response against the transgene product can significantly reduce the efficacy of AAV-based gene transfer. Although numerous parameters controlling this immunogenicity have been identified, the in vivo mechanisms controlling $CD8^+$ T cell responses against the transgene product, particularly the initial events driving T-cell activation, remains poorly understood. In this study, we investigated in mice the kinetic of antigen presentation after intramuscular administration of AAV vectors. We found viral genomes in the lymph nodes draining the injection site as early as one hour post AAV administration and activation of capsid-specific $CD8^+$ T cells at 24h, suggesting rapid lymphatic drainage and degradation of AAV particles. Unexpectedly, the transgene product was also presented to $CD8^+$ T cells early, with an increasing percentage of activated T cells found between day 1 and day 4, and initial T-cell divisions detected by day 4. Moreover, experiments involving the removal of the injection site demonstrated that AAV particles reaching the draining lymph node within the first hour were sufficient to expand transgene-specific $CD8^+$ T cells with in vivo cytotoxic function. Finally, we tested different muscle-specific promoters and found that those promoting early transgene presentation efficiently primed $CD8^+$ T cells, whereas reduced transgene presentation correlated with an absence of $CD8^+$ T cells. Overall, our findings reveal an unexpected early transgene presentation to T cells which critically influences the efficacy of T cell priming. This paves the way for a new approach to assess immunogenicity potential of AAV constructs.

Poster Presentations

P01

Validation of a luminometric assay for the detection of human anti-AAV neutralization factors

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In AAV-mediated gene therapies, pre-existing anti-AAV humoral immunity can hinder gene therapy efficacy and induce safety concerns. This is a major challenge, particularly for clinical trials, since over 50% of patients present anti-AAV neutralizing factors (NFs) depending on AAV serotypes and can be excluded from treatment eligibility.

Anti-AAV NFs are typically detected using a cell-based transduction inhibition assay. However, protocols remain not standardized in the AAV gene therapy community. Over the past 15 years, our team worked on the detection of both pre-existing and induced AAV neutralizing factors, and we developed a luminometric qualitative anti-AAV NF assay using standardized controls. Using a luciferase reporter gene system, our assay is adaptable to several AAV serotypes and species and enable the detection of anti-AAV neutralizing factors at titers as low as 1:5, aligned with the selection criteria commonly used in gene therapy studies.

In this context, in the present study we validated our method according to regulatory guidelines such as EMA-ICH M10 and FDA guidelines for bioanalytical method validation guidance for industry. Specifically, the method was evaluated for the detection of AAVrh10 neutralizing factors in human sera, with the assessment of applicable parameters within-in run and between-run accuracy and precision, dilution linearity, serum and vector stability and batch to batch variability.

In conclusion, this study provides a validated and reliable assay for research grade applications as well as regulatory studies such as toxicology studies and clinical trials.

P02

A simple, rapid and robust bioluminescent assay for detecting anti-AAV neutralizing antibodies in serum samples

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Adeno-associated viruses (AAVs) are widely used vectors to deliver transgenes for gene therapy. Individuals with pre-existing immunity to AAVs are less likely to benefit from gene therapy owing to the presence of neutralizing antibodies (NAbs) that reduce cellular uptake. Widespread, pre-existing immunity in human and animal subjects increases the need for sensitive assays to detect NAb activity in serum samples. Here we describe a cell-based transduction inhibition (TI) assay

utilizing NanoLuc luciferase (Nluc), an enzyme engineered to be extremely bright and structurally stable. The superior brightness of Nluc (>700-fold brighter than firefly luciferase) enables the sensitive detection of NAb activity using low MOI values (100 – 10,000) and short incubation times (≤24 hours). The assay is tolerant to high levels of human serum, allowing dilutions as low as 1:3, and luminescence is measured following addition of an add-mix-read (homogeneous) detection reagent (Bio-Glo-AAV). We screened 1:3, 1:9, 1:27 & 1:81 dilutions of human serum samples (MOI = 10,000) to demonstrate the influence of sample dilution on hit rate. We also compared head-to-head with the analogous approach using firefly luciferase (Fluc) as the reporter, and we validated use of thaw-and-use HEK293 cells. Taken together, this rapid, highly sensitive and reliable assay enabled by Nluc AAV reporter technology precisely measures NAbs against AAVs in both human and animal serum.

P03

A highly sensitive nanoluciferase reporter enables efficient assessment of functional and genomic biodistribution of AAV in mice

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Engineering of AAV capsids has been a favorable approach to enhance specificity toward target tissues while minimizing off-target delivery. AAV variant selection often comprise of large sample sets and a complex workflow that requires automation. Each animal is injected with a variant AAV capsid library, followed by the isolation, purification and sequencing of DNA from each AAV infected tissue. Herein we report a simple, fast and automated workflow to enable extraction, purification and quantification of viral DNA from tissue samples in a high throughput fashion. We generated AAV9 viruses expressing a NanoLuc® reporter to examine tissue tropism in animals via bioluminescent live imaging. Two weeks after injection, bright and dose dependent bioluminescent images were captured via live whole-body imaging. Next, tissues were collected, and total DNA was extracted and purified using the Maxwell® nucleic acid extraction system. Total DNA quantity and purity were assessed, followed by determination of AAV viral titers by normalization to house-keeping gene Tert using digital droplet PCR. We found AAV titers of several organs correlated with their luminescent signals. The viral titers accumulated in targeted tissues were also strongly dependent on the injection dose. In summary, we report a versatile NanoLuc® reporter that is bright and well suited to examine tissue tropism in live animals. In addition, we provide a simple, single day workflow which enables high throughput compatible viral titer determination of AAV infected tissues. The combination of our reporter and our proposed workflow would advance efficiency and effectiveness when screening AAV capsid libraries.

P04

Characterization of pre-existing anti-AAV T Cells using spectral flow cytometry in a cohort of healthy donors

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Recombinant adeno-associated viruses (rAAV) are efficient tools for *in vivo* gene transfer as demonstrated by 8 FDA or EMA-approved drugs in the US and Europe. However, their immunogenicity in humans remains a major hurdles to overcome for a successful clinical translation. Indeed, adverse events related to immune system activation following systemic injection of high doses of rAAV into patients have been reported, resulting in some cases in clinical trial hold. This immunotoxicity is partly due to the reactivation of pre-existing anti-AAV T cells after gene transfer. In a previous cellular immune response prevalence study in a cohort of healthy donors (n=145), we showed that 24% and 46% of donors have a pre-existing cellular immune response to AAV8 and AAV9 respectively. Here, we aim at studying the phenotype of these anti-AAV8 (n=6) and anti-AAV9 (n=9) T cells by spectral cytometry. Preliminary data on this cohort showed that the cells involved in the anti-AAV8 and anti-AAV9 cellular response are mostly effector memory CD8 T cells (T_{EM}) followed by terminally-differentiated effector memory cell (T_{EMRA}). T_{EM} cells appear more involved in the anti-AAV9 response, while T_{EMRA} cells are more implicated in the anti-AAV8 response. Moreover, the co-expression of CD57 and CD27 markers by anti-AAV T_{EM} cells suggests a cytotoxic activity. An in-depth functional characterization of these cells is ongoing. This study will allow to better characterize the preexisting anti-AAV T cells that might be involved in clinical trial adverse events and to develop targeted immunomodulation to prevent deleterious immune responses after rAAV gene transfer.

P05

Let's standardize our AAV biodistribution procedures for CNS gene therapy

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Here, we propose a standardized method for assessing the AAV biodistribution of after intravenous or intracerebral delivery, to easily compare several preclinical studies.

It would allow to compare same AAV serotype carrying several payloads or several AAVs carrying the same payload to assess efficiently biodistribution even with different routes of administration.

It is a crucial step to fill regulatory agencies requirements before these gene therapy products can be used in humans based on the consideration that any new construct must undergo a full evaluation, including a biodistribution study in several animal models.

Our new method will lead to robust preclinical data evaluation that would build a library of AAV biodistribution to which we can compare independently of the Study Sponsor.

P06

Maximizing Upstream rAAV Yield: A Design of Experiments Approach to Plasmid Ratio Optimization

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Plasmids, essential for rAAV production via transient transfection, represent a significant expense and optimization target in early-stage development. This study aimed to identify optimal plasmid ratios for maximizing functional rAAV yield in preclinical settings using Design of Experiments (DoE), with potential applications in both discovery and early preclinical development stages.

We employed an R-based open-source DoE pipeline to reduce the number of 3-plasmid ratio combinations from 14,553 to 28, maintaining statistical power while minimizing experimental load. Using a HEK293F-derived clonal suspension cell line, we conducted transfections with the 3-plasmid system in 50 mL tubes containing 10 mL of cell culture. After 70 hours, cells were harvested, lysed, and clarified. Vector genome titers were quantified by ITR2-qPCR. Our analysis revealed optimal conditions for total plasmid DNA and ratios of pHelper, pRepCap, and pTransgene, yielding up to 4-fold higher productivity compared to other combinations. A Response Surface Model (RSM) was generated and validated through ridge analysis and comparison with previously established ratios. The optimized plasmid ratios resulted in up to 3-fold increase in vector genome titers across four common serotypes and two transgenes of varying lengths, with some serotypes achieving productivity exceeding 7E+14 VG/L of clarified cell culture. This method demonstrates potential for application in discovery stages to identify high-yield capsids and transgenes, as well as in preclinical stages to fine-tune plasmid ratios for improved empty:full capsid ratios. By optimizing plasmid usage, this approach contributes to enhancing rAAV manufacturing efficiency and potentially reducing development costs.

P07

Generation and characterization of Xcell™ Eng-HEK293: a suspension cell line for rAAV production

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The HEK293 cell line is widely used for the production of viral vectors, which serve as essential delivery vehicles in gene therapies. Several HEK293 derivatives are commercially available for R&D and GMP-manufacturing. However, we still have limited knowledge on the genetic composition of these cells and on how to improve their properties. Xcell™ Eng-HEK293 is a novel suspension HEK293 cell clone developed and characterized by NewBiologix SA. Deriving from a polyclonal parental HEK293 cell line, this clone was selected based on its high transfection efficiency, growth profile in suspension culture, suitability for efficient single-cell sorting, and ultimately for improved rAAV production efficiency following triple-plasmid transfection. Xcell™ Eng-HEK293 was fully characterized at the genomic level using PacBio long-read sequencing and Bionano's optical genome mapping, and at the transcriptomic level using high-throughput Illumina sequencing. Xcell™ Eng-HEK293 produced similar or higher rAAV titers as compared to either the parental polyclonal cell line or currently available leading HEK293 cell lines. This effect was demonstrated using various transgenes or culture volumes, and it was consistently observed over

55 generations of cultivation. In addition, Xcell™ Eng-HEK293 produced 2-fold higher ratios of DNA-containing vs empty capsids when compared to the materials produced by a leading HEK293 cell line, as well as comparable or higher degree of AAV genome integrity as assessed by high-throughput sequencing. In conclusion, we have generated and characterized Xcell™ Eng-HEK293, a novel cell line specifically optimized for the production of high quality rAAV gene therapy candidate vectors and which is available for licencing.

P08

Surface-modified AAV6 vectors combined with nanoblades allow high level gene knock-in in hematopoietic stem and progenitor cells without compromising cell survival

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Nanoblades are virus-like particles (VLPs) derived from murine leukaemia virus (MLV) or human immunodeficiency virus (HIV), loaded with Cas9-gRNA ribonucleoproteins and devoid of any viral genome. They represent a new promising technology to efficiently induce DNA double-stranded breaks, proven to be remarkably efficient for entry into human T, B and hematopoietic stem and progenitor (HSPCs) cells, thanks to their surface co-pseudotyping with baboon retroviral and VSVG envelope glycoproteins.

Incubation of HSPCs with nanoblades together with rAAV6 vector containing two homologous arms to the Wiskott-Aldrich syndrome (WAS) locus flanking a GFP expression cassette, resulted in 50% stable expression cassette knock-in into the WAS locus. However, high doses of rAAV6 caused HSPC cell death, while rAAV2, carrying the same donor template, was less toxic and achieved similar transduction levels.

To improve donor template delivery, rAAV2 and rAAV6 were chemically bio-conjugated with a mannose ligand, *via* the lysine or tyrosine amino-acid residues exposed at the capsid surface. Our results showed higher transduction levels of HSPCs with mannose-coupled rAAV6 vectors compared to mannose-coupled rAAV2, accompanied by a remarkable lower toxicity compared to the unmodified rAAV6, which led to an increased survival of HSPCs from 10% to 80%. Additionally, we revealed that the mannose-coupled rAAV6 reduced p53-mediated DNA damage response in $CD34^+$ cells, which might explain the lower cell toxicity.

Therefore, this provides an excellent tool for precise therapeutic correction in $CD34^+$ HSPCs, an important feature for clinical translation of HSPC-gene editing strategies.

P09

Targeting a shared neoepitope derived from non-canonical translation of c-Myc oncogene in cancer cells

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Translation is dysregulated in cancer cells with an increase of translational defects. We proposed that these translational alterations in cancer produce shared and immunogenic tumor-specific epitopes derived from oncogenes such as c-Myc, which represent a new family of targets for cancer immunotherapy. An *in silico*-based method identified potential neoepitopes derived from alternative open-reading frames of c-Myc and selected 2 candidates with evidence of specific translation in tumors in proteomic databases. Targeted immunopeptidomics confirmed the presence of the PR3 epitope on HLA molecules on the surface of tumor cell lines but not on normal primary cells. RNAseq analysis and a bicistronic reporter assay confirmed the translational origin of PR3. PR3-specific T cells were found among tumor infiltrating lymphocytes from 6/22 (27%) colon cancer samples, suggesting that PR3 is presented and immunogenic in cancer patients. PR3-specific CD8+ T cell clones of high functional avidity were generated by *in vitro* priming of T cells by dendritic cells from healthy donors. To assess the therapeutic potential of targeting the PR3 neoepitope, we genetically engineered T cells to express a PR3-specific TCR (TCR-T cells). After validating their functionality and TCR specificity, we confirmed that these TCR-T cells are cytotoxic against tumor cells endogenously presenting PR3, while sparing normal primary cells. We also demonstrated the *in vivo* antitumor activity of these PR3-specific TCR-T cells using the immuno-AVI-cellDX^{TM} model and NSG mice transplanted with tumor cells expressing PR3. Our results provide preclinical rationale for developing T-cell based immunotherapies targeting this *c-MYC*-derived neoepitope.

P10

Development of $\gamma\delta$ T cells immunotherapy against glioblastoma

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γδ T cells are unconventional T cells harboring features of innate and adaptive response. Indeed, they express both TCR receptors (although interacting with their target in a mostly MHC

independent manner) and NK receptors, conferring them cytotoxic characteristics. They are besides, specifically enriched in tissues and their number in tumors associates with better survival in different solid cancers. As such, clinical scale *in-vitro* expanded γδ T cells are currently in clinical trials. Their characteristics make them of great interest to develop as therapeutic agent particularly in tumors with low mutational burden or low HLA-I expression, such as Glioblastomas (GBM). So far, immunotherapies evaluated in clinical trials have failed to provide significant clinical benefit for GBM patients, notably due to the high genetic heterogeneity and immunosuppressive environment of the tumor.

Here, we aimed at developing a new cell therapy strategy in GBM, using *in-vitro* expanded γδ T cells (DOT cells).

Characterization of immune cells isolated from GBM patient samples highlighted γδ T cells recruitment within tumors. In parallel, using commercially available GBM cell lines and patient-derived glioblastoma stem cells, we showed that amplified γδ T cells, used in combination with interleukin-15, could efficiently recognize and kill GBM cells and spheroids *in-vitro*, relying on the granule exocytosis pathway. Finally, patient-derived xenograft mouse models of GBM and γδ T cells-based therapy are showing a delay in the tumor growth in preliminary results. Altogether, our results are providing encouraging results to develop allogeneic use of γδ T cell therapies in GBM patients.

P11

Systemic injection of recombinant AAV8 is able to rescue lysosomal acid lipase deficiency phenotype in a mouse model

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Lysosomal acid lipase (LAL) is a lysosomal enzyme implicated in the lipid's homeostasis, by hydrolysing triglycerides and cholesteryl esters into free fatty acids and free cholesterol. Recessive mutations in the LIPA gene lead to Lysosomal Acid Lipase Deficiency (LAL-D). Two forms of the disease are described, whose most severe one results in premature death. Liver is the most affected organ with hepatosplenomegaly, fibrosis and elevated triglycerides and cholesterol. Enzyme Replacement Therapy (ERT) consists of weekly injections of recombinant LAL and can improve patients' life but is not a curative therapy. Our aim is to develop a curative treatment using gene therapy. A recombinant AAV8 encoding the wt hLIPA gene under the control of a hepatocyte-specific promoter was evaluated following systemic injections in our *lipa-/-* mouse model. Stability and treatment efficiency were assessed in young adult mice. We observed the expression of a stable and functional enzyme in liver but also plasma and spleen, demonstrating an efficient secretion of LAL and the feasibility of cross correction. Moreover, transcriptomic and biochemical mitochondrial analysis supported the rescue of several critical parameters like triglycerides and cholesterol levels. Thus, we demonstrated that this AAV gene therapy approach is promising for long term correction and is an interesting alternative treatment to ERT for LAL-D patients.

P12

Modelling therapeutic strategies in Duchenne muscular dystrophy by CRISPR/Cas-based approaches using iPSC-derived cardiomyocytes

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Duchenne muscular dystrophy is X-linked genetic disease affecting about 1:5,000 boys. The mutations in *DMD* gene are usually out of frame and lead to absence of dystrophin, the structural protein in striated muscles. The patients loose ambulation in their teens, while heart failure is the major cause of death usually in the 3rd decade of life.

Human iPSC have been generated by reprogramming of blood leukocytes of DMD boys and healthy subjects. DMD mutations were corrected by CRISPR/Cas9 editing and HDR repair while relevant mutations have been introduced by *DMD* gene editing in healthy cells (*J Mol Cell Cardiol, 2021; doi: 10.1016/j.yjmcc.2021.07.007*). The studies revealed disturbance of structure and function of iPSC-derived DMD cardiomyocytes, Specifically, RNAseq and proteomic analysis showed disturbed gene expression of iron handling pathways. CISD1, encoding mitoNEET, the outer mitochondrial membrane exporting iron was identified as potential modulatory target. mitoNEET is decreased in DMD cardiomyocytes and CRISPR/Cas9 correction of *DMD* mutation restored mitoNEET level and corrected iron handling and ROS generation (*Cardiovasc Res 2024; doi: 10.1093/cvr/cvad182*).

In another study deactivated Cas9 (*Sa*dCas9) coupled with VP64 transcriptional activator delivered with AAV6 in a single vector harbouring also sgRNAs, upregulated utrophin expression in DMD cardiomyocytes, improving calcium handling and electrophysiological properties *(Mol Ther Nucleic Acids, doi: 10.1016/j.omtn.2024.102247*).

In sum, CRISPR/Cas-edited iPSC-derived cardiac cells allow to investigate DMD disease mechanisms revealing novel pathways for potential therapeutic exploitation.

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P13

Modeling fibrosis in duchenne muscular dystrophy organoids

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Duchenne muscular dystrophy (DMD) is an incurable severe muscle wasting disorder, caused by genetic mutations of dystrophin, a protein essential to preserve muscle fiber integrity after mechanical stress. The progression of the disease is dictated by detrimental consequences of lack of dystrophin such as muscle degeneration, chronic inflammation and fibrosis ultimately leading to loss of muscle function and impairment of gene therapy strategies. Fibrosis is primarily driven by

aberrant activation of fibro-adipogenic progenitors (FAPs) orchestrated by intercellular communications with muscle cells through secreted cytokines and myokines. Our data obtained in DMD-iPSC-derived human muscle organoids, including fibroblasts, indicate that the currently used short version of dystrophin, improves muscle function but only partially reduce profibrotic activity in fibroblasts. In particular, a persistent upregulation of mechanosignaling pathways was observed by transcriptomic and secretome analysis. We then optimized our system to include FAPs to analyze their cellular plasticity and fibrotic potential in DMD context. We aim to elucidate at single-cell resolution, perturbed signaling events leading to perturbation of muscle cells and FAPs secretome to identify new candidates enhancing gene therapy efficacy and more broadly anti-fibrotic targeted therapy. Comparative analyses between Control and dystrophic MYOrganoids including respective FAPs -will be performed using imaging, single-nuclei transcriptomics, and functional muscle force assays to support the physiological relevance of our system and its suitability as preclinical platform for therapeutic investigation.

Keywords: DMD, organoids, FAPs, Fibrosis

P14

ePsCas9 (eSpOT-ON): An Engineered High-Fidelity Genome Editor for Lipid Nanoparticle Delivery

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CRISPR-Cas technologies hold great promise for therapeutic genome editing, but many Cas enzymes lack sufficient in vivo activity for clinical use. Our previous work introduced PsCas9 (SpOT-ON), a high-fidelity Type II-B Cas9 from *Parasutterella secunda*, achieving comparable editing levels to SpCas9 with fewer off-target effects and reduced chromosomal translocations.

This study aimed to address reduced PsCas9 efficacy with non-adenoviral delivery methods. Cellular studies revealed PsCas9 activity was limited under low intracellular concentrations due to lower DNA-binding affinity compared to SpCas9. To enhance activity, we used structural insights to engineer the enzyme. While sgRNA optimization modestly improved activity, targeted mutagenesis increased editing efficiency up to 20-fold across multiple targets, enhancing DNA-binding affinity while maintaining high fidelity.

The optimized enzyme, ePsCas9 (eSpOT-ON), was tested for therapeutic genome editing in vivo. Delivered via lipid nanoparticles, ePsCas9 effectively edited the *Pcsk9* gene in mouse liver, reducing plasma Pcsk9 protein levels more efficiently than SpCas9.

ePsCas9's precision, efficiency, and fidelity make it well-suited for therapeutic applications. eSpOT-ON tools, including synthetic guide RNAs and purified nuclease, are now available through a Synthego-AstraZeneca partnership to advance CRISPR-based medicine.

P15

Proof-of-concept for a one-step CRISPR-based gene therapy approach for STAT1 gain-of-function

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Inborn errors of immunity (IEIs) are rare inherited disorders affecting the immune system presenting with increased susceptibility to pathogens and often associated with severe non-infectious comorbidities. The monogenic nature of IEIs converts them in a perfect target for precision medicine. Autosomal dominant (AD) signal transducer and activator of transcription 1 (STAT1) gain-of-function (GoF), which presents a wide range of mutations with a broad, mostly unexplained phenotype. STAT1 is a pivotal transcription factor in the immune response. Allogeneic hematopoietic stem cell transplantation, being the only curative option, comes with high morbidity and mortality. Gene editing offers a viable curative treatment for the patients given that STAT1 GoF is monogenic in nature. Here, we provide a proof-of-concept for a gene knock-in in STAT1 locus of genomic DNA by incorporating engineered virus-like particles and adeno-associated viral vectors to deliver CRISPR/Cas9 components and a donor template. Exploiting the homology-directed repair mechanism after Cas9-induced double-strand break, we report targeted integration in the STAT1 locus. Moreover, we are also able to achieve the endogenous regulation by STAT1 promoter. Our study highlights the potential of CRISPR/Cas9-mediated gene therapy for patients holding any mutation causing STAT1 GoF disease.

P16

TRPC3 inhibition reduces calcium entry in muscles of DMD^{mdx} rats

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Duchenne Muscular Dystrophy (DMD) is a severe, progressive muscle-wasting disease caused by mutations in the DMD gene, resulting in dystrophin deficiency and muscle degeneration. Patients typically lose ambulation during adolescence and face premature mortality in their 20s to 40s due to respiratory or cardiac failure. Excessive calcium (Ca^{2+}) entry into myocytes contributes to cellular damage and death. Previous research from our laboratory identified the TRPC3 (Transient Receptor Potential Canonical 3) channel as a key contributor to this abnormal Ca^{2+} influx. We also showed that treatment with a recombinant adeno-associated virus encoding micro-dystrophin (rAAV-MD) only partially corrects these abnormalities. The present study investigated whether inhibiting TRPC3 could reduce Ca^{2+} entry in DMD^{mdx} rat muscles, an animal model of DMD, and could have a synergistic effect when combined with a rAAV-MD. DMD^{mdx} rats injected systemically with a rAAV-MD or with vehicle, were treated during 6 weeks with pyrazole 10 (Pyr10), a TRPC3 inhibitor delivered via mini-osmotic pumps. rAAV-MD gene transfer was confirmed via digital PCR and micro-dystrophin expression via Western blot, while Pyr10 delivery was validated using mass-spectrometry. Finally, using Mn^{2+}-quenching of fura-2 fluorescence, we found that Pyr10 and rAAV-MD treatments individually reduced Ca^{2+} entry across

DMD^{mdx} muscle cell membrane, which is an encouraging outcome. However, no synergistic effect was observed, and neither treatment improved muscle strength or DMD histological features. As calcium dysregulation is an early and critical event in DMD progression, these results underscore the need for further studies to fully evaluate the therapeutic potential of TRPC3 inhibition for DMD.

P17

Therapeutic research in dysferlinopathies: Progress in gene therapy

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Dysferlinopathies are rare degenerative genetic muscle diseases that exist in two main forms : the Miyoshi Myopathy and the LGMDR2 (2B). Both are caused by recessive mutations in the DYSF gene and are characterized by muscular weakness, atrophy, and high levels of creatine kinase in the blood. Dysferlin is a large transmembrane protein whose key function is membrane repair in muscle cells. There is currently no etiological treatment for patients, only supportive care. Fifteen years ago, a large homozygous deletion in the gene was observed in a female patient with a late onset presentation and very moderate dysferlinopathy. This mini-gene enables the production of a truncated but partially functional protein. Given the limited encapsidation capacity of AAVs, this mini-gene would enable a gene therapy approach with only the part of the gene required for membrane repair. Following the discovery of this patient, we compared different constructs in vitro with a laser wound membrane repair assay in dysferlin-deficient cells. We are now testing our most effective mini-gene in a pre-clinical gene therapy trial in a mouse model that has already been characterized. Eight-week-old dysferlin-deficient mice were injected intramuscularly and we are analyzing their muscle strength every months post-injection. We are also studying protein expression and localization, the histology of their quadriceps and the possible hepatotoxicity of our treatment. If these initial results are promising, we will repeat the trial using systemic administration method and a new vector.

P18

Identification of regulatory factors of FKRP gene expression in skeletal muscle

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The Fukutin-related protein (FKRP) is a ribitol 5'phosphatase which contributes to the α-dystroglycan (αDG) glycosylation and allows the connexion of extracellular matrix to the cytoskeleton. In striated muscle, this linkage is crucial for stability of the fibers and protection against the mechanical stress induced by muscle contraction. Mutations in the FKRP gene cause αDG hypoglycosylation leading to disruption of the aforementioned functions. Depending on the nature of the mutation, FKRP deficiency results in a whole range of pathologies including Limb Girdle Muscular Dystrophy R9 (LGMDR9). To date, there is no cure for these diseases except

palliative care. We previously described the *in vivo* potential of rAAV mediated FKRP gene therapy in FKRP deficient mice, which led us to develop a gene therapy approach. A clinical trial is currently ongoing in LGMDR9 patients, showing promising preliminary results. Since the endogenous mutated FKRP can be expressed in patients and to define whether there is any possible feedback due to the transgene expression, we performed RNAseq and miRseq experiments following gene transfer. We combined the information of dysregulated genes with an *in silico* analysis of potential regulatory signals at the FKRP gene locus. This analysis allowed us to identify 20 transcription factors, 3 micro-RNAs and 19 RNA-binding proteins as potential FKRP regulators. We performed *in vitro* experiments by transfecting some of these factors and observed that 2 micro-RNAs modulate positively or negatively FKRP gene expression. Improving our understanding of FKRP regulation will help to understand the consequences of rAAV mediated FKRP gene therapy.

P19

NewBiologix Xcell™ Genomic Analytical Platform: a novel platform for the genomic characterization of cell lines

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Cell line characterization is critical to control biological product manufacturing. Confirming the identity, purity, and genomic stability of a cell line is of upmost importance for both viral vector manufacturing and cell therapy applications. The specific strategy for characterization of a cell bank varies depending on several factors including the source of the cell line itself, cultivation history, genetic manipulation, clonality, growth requirements, amongst others.

NewBiologix Xcell™ Genomic Analytical Platform is a proprietary platform specifically designed for the genomic and transcriptomic characterization of cell lines used in gene and cell therapies, based on three specific technologies: Optical genome mapping (OGM), long-read high-fidelity (HiFi) DNA sequencing and short-read, high-throughput RNA sequencing.

OGM using Bionano help determine clonality and genomic stability over many passages. OGM can reliably estimate cell ploidy and detect complex genomic rearrangements in a single, genome-wide workflow, providing with 10,000X higher resolution compared to standard karyotyping.

Whole genome sequencing using PacBio's HiFi long-reads technology eases the complex process of whole genome assembly and delivers highly accurate ultra-long reassembled DNA sequences, including difficult to resolve low complexity regions. HiFi reads also provide 5mC detection, allowing the analysis of the methylation status of the genome from a single sequencing experiment.

Whole-transcriptome analysis with total RNA sequencing using Illumina short-read sequencing can accurately measure gene and transcript abundance, giving information on the transcriptional capacity of the cell line.

NewBiologix Xcell™ Genomic Analytical Platform is available for characterization of cells and cell lines used in cell and gene therapy applications and in the production of biologics.

P20

Discovery of New Therapeutic Targets for Duchenne Muscular Dystrophy by Inference of Gene Regulatory Networks

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Duchenne Muscular Dystrophy (DMD) is an X-linked muscular disorder caused by mutations in the *DMD* gene. Patients are usually diagnosed at 3-4 years of age but early phenotypes have been described in fetuses and in animal models before the symptoms appear. Progressive degeneration of DMD muscles leads to severe disabilities, wheelchair dependency and a median life expectancy of ~30 years. In animal models, gene therapy with adeno-associated virus (AAV) vectors carrying a micro-dystrophin transgene leads to an almost complete disease correction. In DMD patients however, despite promising intermediary results, high doses of AAV have limited efficacy and lead to serious adverse events. This highlights the need for innovative, patient-centered gene therapy strategies together with relevant models to identify new therapeutic targets. In this context, our team uses induced pluripotent stem cells (iPSCs) to understand the initiation of DMD during muscle development. We first evaluated the impact of dystrophin deficiency on the transcriptome at single-cell resolution during the differentiation of iPSCs into skeletal muscle. Our data showed that DMD iPSCs diverge from control cells at the somite stage and acquire a different transcriptomic profile. Using a retro-engineering approach together with an augmented learning algorithm, we integrated the scRNA-Seq time series with RNA half-life and proteomic data and we inferred a gene regulatory network (GRN) composed of 6 genes interacting in a genotype-dependent topology. Modulation of the genes within the DMD GRN using RNA interference is currently under investigation to validate their potential as therapeutic targets in future gene therapy applications.

P21

Improving AAV Purification : A Novel Affinity Chromatography Approach

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Adeno-associated virus (AAV) vectors are highly promising for gene therapy applications, with numerous preclinical and clinical successes demonstrating their potential. However, clinical translation requires high doses of AAV, and scaling up production remains a significant bottleneck. Efficient purification is essential for isolating AAV particles from cell lysates, as impurities can compromise both therapeutic efficacy and safety. Chromatographic techniques, particularly affinity chromatography, are the gold standard for large-scale AAV purification. Most ligands used in this approach are nanobodies derived from single-domain antibody fragments of the Camelidae

family. However, these antibodies have several notable drawbacks, including high production costs, low yields and insufficient purity. Our project aims to develop innovative synthetic ligands designed to enhance AAV purification. These chemically synthesized ligands offer a cost-effective, scalable, and reproducible solution and are well-suited for GMP-compliant manufacturing.

P22

Genetic reprogramming systems for spatiotemporally regulated expression of immune effectors by transferred B cells to cure chronic diseases

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Cancer is a major public health problem, causing around 10 million deaths a year. Available treatments have numerous side-effects and often require multiple interventions; hence, the need over the long term for novel, more effective, more specific and even targeted therapeutic alternatives. In this study, we evaluated the potential of a new synthetic circuits to reprogram B cells for therapeutic purposes.

Such circuits encode a “sensor” corresponding to a membrane-anchored B cell receptor (BCR) targeting a model antigen ovalbumin (OVA) or mesothelin (MSLN) that are ectopically transduced in reprogrammed B cells to express BCRs specific to these antigens. The circuits also encode a “transducer”, which is a part of the NR4A1 promoter that can be induced by activated BCR, and “effector” molecules. When disease biomarkers are bound to the ectopic BCR, recognized here as inducing signals for the synthetic circuits, the NR4A1 promoter is specifically activated, leading to the expression of effector molecules.

We demonstrated the complete activation of the circuits in B cells following recognition of tumor cells expressing the target antigens *ex vivo*. To take further characterize these circuits, we established a humanized mouse model grafted with these tumor cells and, subsequently, with reprogrammed B lymphocytes to mimic adoptive transfer of autologous cells in a preclinical context. After validation of this cancer model, we confirmed the homing of reprogrammed B cells to the tumor sites. The potential of reprogrammed B cells is currently being assessed *in vivo* by the implementation of a therapeutic effector transgene.

P23

Optimising circular RNA production, expression efficiency, and delivery by viral pseudoparticles

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RNA delivery is a promising approach to achieve rapid and transient gene expression, in oncology, cell and gene-based therapies as well as vaccination. However, most mRNAs are rapidly degraded by cellular exonucleases, which might limit their use. To counteract this, using circular RNAs (circRNA) could be a promising strategy since they seem to be more stable by resisting to exonuclease degradation, in addition, circRNA can be translated.

In this context, we develop new tools to circularize RNA and drive their packaging into pseudoparticles. For efficient delivery we use MS2-chimeric retrovirus-like particles. This system enables RNA encapsidation via a specific interaction between a phage protein (MS2-coat) and a RNA stem-loop (MS2 aptamer).

We constructed vectors containing hammerhead ribozymes (HHR) sequences at the 5' and 3' end of a cassette containing an IRES, a reporter gene and six stem-loop of MS2 RNA. The 5' and 3' HHR sequences enable self-cleavage generating 5'OH and 3'P end substrates of cellular ligase.

RNA circularization is confirmed by RT-PCR using divergent primers and junction sequencing. Expression of reporter gene were detected in transfected HEK293 cells. We observed differences in fluorescence intensity according to the type of circRNA constructions.

Besides HHR, CircRNA production will be improved thanks to the use of Tornado (Twister-optimized RNA for durable overexpression). Their encapsidation will be evaluated after constructs modifications.

Our ongoing work focuses on refining circRNA production, improving the translation efficiency of circRNA and of the delivery systems to enhance their functionality and therapeutic potential.

P24

Biodistribution and immune response against CNS-targeting AAV vectors in non-human primates

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Adeno-associated viruses (AAV)-based gene transfer progressed in the last decade, up to the ability to treat single-gene disorders such as spinal muscular atrophy with great success. However, with the increase in clinical trials using AAV vectors, it appears that AAV are more immunogenic than previously estimated, when used at high doses. Several clinical trials were put on hold after severe adverse events occurred, related to hepatotoxicity and upregulated immune response. To better bridge the gap between preclinical studies and clinical trials, we evaluated the biodistribution and immune response against three of the most-used AAV capsids, i.e. 6, 9 and rh10, to target the central nervous system (CNS) in non-human primates (Macaca fascicularis). The animals received combinations of vectors, each containing a fluorescent reporter, detected by qPCR and immunofluorescence to evaluate their tropism and distribution. To target the CNS, we used intraparenchymal (IP) injection or intrathecal (IT) injection with 3E12vg and 3E13vg, respectively. IT delivery led to higher viral load than IP and broader distribution in the peripheral tissues, notably the lymphatic organs (spleen, lymph nodes, dorsal root ganglia), but also in non-lymphatic organs. For the IP group, the AAVs were detected in the injected areas, the spinal cord and dorsal root ganglia, but almost indetectable in the peripheral organs. The immune response

data is now under analysis. This work will help to better characterize the influence of serotype and injection route on vector biodistribution, and the related immune response, thus allowing the identification of targets for immunosuppression in clinical trials.

P25

Development of a gene therapy based on SMaRT technology for Huntington's disease.

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Huntington's disease is a genetic neurodegenerative disorder characterized by motor, cognitive and psychiatric symptoms that slowly but inevitably lead to the patient's death. It is caused by an abnormal expansion of a CAG repeat in the HTT gene, leading to toxic protein production and striatal neuron degeneration. There is currently no cure, but gene therapy has emerged as a promising option.

We are exploring SMaRT (Spliceosome-mediated RNA trans-splicing) technology, which replaces mutated exons in pre-mRNA using an artificial RNA called PTM (pre-RNA Trans-splicing Molecule). PTMs compete with endogenous splice sites to generate hybrid, mutation-free mRNA. A major challenge is ensuring efficient trans-splicing by optimizing the PTM's binding domain (BD), which targets the pre-mRNA intronic sequence.

To address this, we are developing a high-throughput strategy for BD selection. We generate reporter cell lines with fluorescent markers to quantify trans-splicing. BD libraries are screened, successful trans-splicing events are sorted by FACS and analyzed using next-generation sequencing (NGS) to identify the most promising BD candidates. The BD candidates will then be evaluated to select the optimal PTMs that may provide therapeutic benefit for Huntington's disease.

SMaRT technology has shown promise in other genetic disorders like retinitis pigmentosa and epidermolysis bullosa. With further optimization, it could offer a novel therapeutic approach for Huntington's disease.

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Sponsor's options for bioproduction site: advantages, risks and challenges

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1: INITS

The production of biological molecules (cell and gene therapy products, Extracellular vesicles, mAb…) is a complex and highly regulated process. Sponsors establish a roadmap crossing non-

clinical, CMC and clinical aspects and must decide fairly quickly whether they wish to invest in internal production capacities or subcontract to CDMOs, for the R&D and/or GMP part. . These approaches offer advantages but also risks and challenges. An alternative model is offered by Inits SMO (shared manufacturing organization) where the sponsor carries out its own production of DS GMP in the qualified and equipped premises of Inits SMO. Depending on the needs and expectations of the sponsor, all options can be evaluated and will condition the life cycle of the product.