Late-Breaking Abstracts

Oral Presentations

2000 Conservative Management of Acute Myocarditis and Thrombotic Microangiopathy after Elevidys (Delandistrogene Moxeparvovec)

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Introduction: Elevidys (delandistrogene moxeparvovec) is a recently approved rAAV-based gene therapy (GT) for Duchene muscular dystrophy (DMD). Reported toxicities of Elevidys include infusion reactions, hepatotoxicity, myositis, and myocarditis. Thrombotic microangiopathy (TMA) is an emerging rAAV toxicity that has been observed across several systemically administered rAAV drugs, but not previously after Elevidys. Here we report a 17-year-old male with DMD (dystrophin exon 45-52 deletion), who developed myocarditis, atrial fibrillation, and TMA within a week of Elevidys administration.

Case: He remained ambulatory with current DMD medications of deflazacort 36 mg daily and givinostat (held 2 weeks prior to GT). He had no history of cardiac dysfunction or arrhythmias, but 4 months prior to GT, cardiac MRI (CMR) demonstrated age-expected fibrosis with normal left ventricular (LV) ejection fraction (EF, 63%). Baseline cardiac medications were enalapril, eplerenone, and carvedilol.

He received 1.33 × 1014 vg/kg of Elevidys without complication. The day prior, he started 60 mg daily of prednisone for immunosuppression per the label. He remained asymptomatic, but on Day-3 after vector administration, his troponin level increased to 0.26 from <0.01 ng/mL (Figure 1A top panel) and had new T wave inversion on EKG, and a decline in his LV function (EF 52%), concerning for myocarditis. His blood pressure was also markedly elevated (maximum 170/100 mmHg). He was admitted for laboratory monitoring and blood pressure control. The next day (Day-4), he reported palpitations while telemetry showed atrial fibrillation, which resolved after 20 minutes of esmolol infusion. Blood pressure was controlled with the addition of amlodipine and increases of enalapril and carvedilol. CMR on Day-7 demonstrated new cardiac edema and regional increases of the extracellular volume (ECV), consistent with myocarditis; LV EF was 57%.

Upon admission, he was noted to have normal blood counts and complement studies, including normal sC5b9, C3, C4, and CH50. His cystatin-C on Day-4 was elevated, though it was unclear if this renal insufficiency was secondary to kidney hypoperfusion due to myocarditis or causative of his elevated blood pressure. While his myocarditis and arrhythmia improved, his platelets progressively decreased, and he developed mild thrombocytopenia (<150 k/μL) on Day-4. On Day-6, his sC5b9 increased to 266 ng/mL (ULN 247 ng/mL), which peaked at 555 ng/mL on Day-8; cystatin-C also continued to increase (Figure 1A). Also, on Day-8, his peripheral blood smear had ~1-2 schistocytes per high powered field and macrothrombocytopenia (Figure 2). The constellation of complement activation, schistocytes, thrombocytopenia, and renal insufficiency was indicative of TMA. Complement blockade with eculizumab was considered for rAAV-associated TMA, but our multispecialty gene therapy team consisting of neurology, cardiology, hematology, and nephrology recommended a conservative watchful waiting approaching. His sC5b9 began to decrease on Day-9, which coincided with his platelet nadir. Subsequently, these laboratory abnormalities mostly returned to baseline (Figure 1A).

Conclusions: TMA is a poorly understood rAAV toxicity. Complement activation is hypothesized to be a central component of its pathophysiology. However, the scope of rAAV drugs that incite TMA as well as its optimal management remains to be defined. This case demonstrates 1) Elevidys can trigger TMA and 2) mild cases of rAAV-associated TMA and myocarditis can be managed conservatively. Further, our experience highlights the importance of a multidisciplinary approach to managing rAAV toxicities. These considerations should be included in risk benefit discussions of Elevidys.

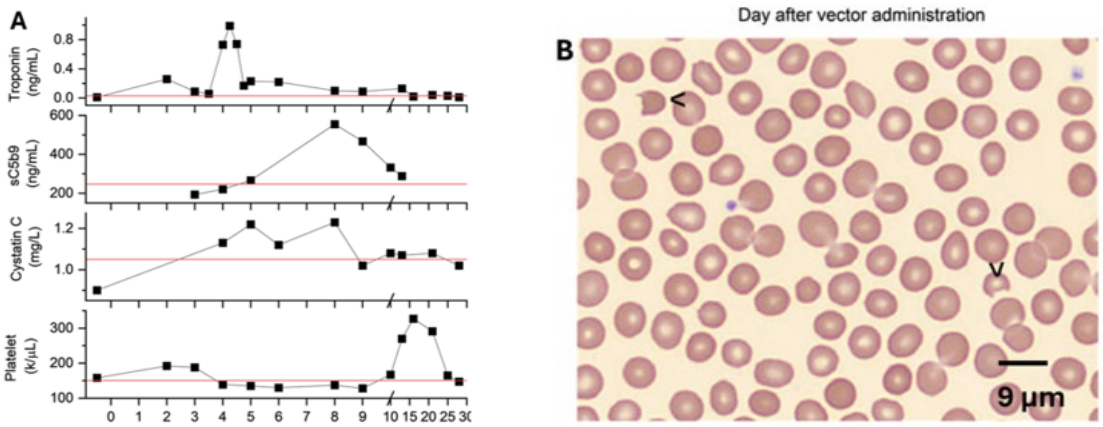

Figure 1: Acute Myocarditis and Thrombotic Microangiopathy after Elevidys (Delandistrogene Moxeparvovec) (**A**) Laboratory abnormalities concerning for myocarditis (*top panel*) and TMA (*bottom 3 panels*). Horizontal red lines demarcate upper or lower limit of normal values: 0.3 ng/mL, 247 ng/m:, 1.05 mg/L, and 150 k/μL, respectively. Baseline values imputed at Day -0.5 for clarity. (**B**) Wright-Giemsa-stained Day-8 peripheral blood smear demonstrating macrothrombocytopenia and 1-2 schistocytes per high powered field (*carets*).

2001 Evaluation of Clinical Safety and Efficacy of LY-M001: A Phase I/II Trial of AAV8-Mediated Gene Therapy for Gaucher Disease Type I

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Background: Gaucher disease (GD) is an inherited lysosomal storage disorder caused by mutations in the *GBA1* gene, leading to deficient glucocerebrosidase (GCase) activity and subsequent lysosomal accumulation of its substrate, glucosylceramide. LY-M001, a novel recombinant adeno-associated virus serotype 8 (rAAV8)-based gene therapy, is designed to address this deficiency by delivering a codon-optimized *GBA1* gene under the control of a hybrid liver-specific promoter, driving the expression of a modified glucocerebrosidase variant ($GCase^{LY}$). Several clinical trials are currently evaluating LY-M001 in China, including a Phase I/II dose-escalation trial (NCT06818838), a pilot study (NCT06162338), and the first trial for adolescent GD patients (NCT06528080).

In the ongoing Phase I/II dose-escalation trial (NCT06818838), both treatment-naïve and pre-treated GD type 1 (GD1) patients are eligible for enrollment. Pre-treated patients must discontinue standard-of-care (SoC) therapy—such as enzyme replacement therapy (ERT) or substrate reduction therapy (SRT)—at least two weeks before LY-M001 infusion. The primary objectives of the study are to assess the safety and efficacy of escalating doses of LY-M001. Participants are monitored for 52 weeks post-treatment before transitioning into a long-term follow-up study.

Results: Since August 2024, five participants have been enrolled in the Phase I/II clinical trial and administered LY-M001. Across two dosage groups, LY-M001 was well tolerated in all patients. Mild, transient elevations in liver enzymes were observed in some participants, which were effectively managed and resolved with immune modulators. No treatment-related serious adverse events (SAEs) were reported.

Rapid expression of GCase activity in peripheral blood was detected within the first week post-infusion, with levels continuing to rise and peaking between 8 and 16 weeks before reaching a plateau. This was accompanied by a reduction in Lyso-GL1 levels. Despite the substantial increase in circulating GCase activity, no anti-drug antibodies (ADA) targeting GCase were detected in any patient, indicating no immunogenicity of the modified $GCase^{LY}$ *in vivo*.

Clinical improvements were observed across all participants following LY-M001 administration. Specifically:

- Hemoglobin levels increased by 9 to 26 g/L.
- Platelet counts rose by 8 to 61 × 10^9/L.
- Spleen volumes decreased by 23.9% to 32.3%.
- Liver volumes reduced by 6.9% to 19.3% compared to baseline.

A participant in the high-dose cohort, who initially presented with hemoglobin levels near the lower limit of normal and platelet levels below normal, experienced significant hematologic improvements, with hemoglobin levels increasing substantially and platelets returning to the normal range. This was accompanied by a marked elevation in circulating GCase activity.

Additionally, most participants reported notable improvements in quality of life. Symptoms such as abdominal distension and fatigue were alleviated, and the majority resumed work or social activities following treatment.

Conclusion: LY-M001 represents a novel AAV8-based gene therapy approach for Gaucher disease. Preliminary clinical data across different dosage groups indicate a strong safety profile and compelling efficacy. Enhanced GCase expression facilitates substrate clearance, leading to meaningful clinical benefits, including hematologic normalization, organ volume reduction, and improved patient-reported outcomes. These findings support the continued clinical development of LY-M001 as a potentially transformative therapy for Gaucher disease.

2002 Preliminary Data from a Phase I Gene Therapy Trial of RP-A601 (AAVrh.74-PKP2a) for Adult Patients with PKP2-Arrhythmogenic Cardiomyopathy (PKP2-ACM)

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Introduction: Arrhythmogenic cardiomyopathy (ACM) is a progressive disorder characterized by increased risk of ventricular tachyarrhythmias, sudden cardiac death, and progressive heart failure. Up to 45% of ACM cases are caused by pathogenic autosomal dominant variants in the *PKP2* gene, which encodes for the desmosomal protein Plakophilin-2. AAVrh.74-PKP2a (RP-A601) is a recombinant adeno-associated viral (AAV) vector containing the coding sequence of human *PKP2* isoform A (PKP2a). Preclinical studies have shown that RP-A601 administration results in the delivery of the *PKP2* gene to cardiomyocytes. In a clinically relevant mouse model of PKP2-ACM (in which survival is ≤50 days post disease onset), a single RP-A601 administration after disease onset extended survival through 5 months, improved right ventricular function, decreased cardiac dilation and myocardial fibrosis, and reduced arrhythmia burden. Additional studies in rodents and nonhuman primates demonstrated RP-A601 safety. The preclinical data supported the initiation of a clinical trial in patients with PKP2-ACM. Here, we present preliminary safety and efficacy data from the phase I trial of RP-A601 in high-risk adult patients with PKP2-ACM.

Methods: This study (NCT05885412) is a multicenter, open-label, dose escalation trial evaluating the safety and preliminary efficacy of a single intravenous infusion of RP-A601 in adult patients (age ≥18 yrs) with clinical and genetic PKP2-ACM diagnosis and an implanted ICD. Patients with severe right ventricular dysfunction, left ventricular ejection fraction ≤50% (echocardiogram or cardiac MRI) and/or NYHA Class IV heart failure are excluded. The primary objective is to assess the safety and tolerability of RP-A601. Preliminary efficacy will be assessed during the months after RP-A601 infusion and includes evaluation of change in myocardial PKP2 genetic and molecular components and clinical markers of arrhythmia burden.

Results: As of February 28, 2025, three patients with PKP2-ACM have received a single infusion of RP-A601 at 8 x1013 GC/kg (cohort 1; low dose). The most common non-serious treatment emergent adverse events (TEAEs) were transaminase elevations and other laboratory abnormalities including decreased platelet count, the majority of which resolved to normal range within 2 weeks following RP-A601 infusion. Most TEAEs were not serious and mild/moderate in severity. One patient experienced treatment emergent SAEs of transaminase elevation, pancreatitis, sepsis, and bacteremia during the period when he was receiving immunomodulatory therapy. All SAEs have fully resolved without sequelae. No arrhythmia SAEs were identified during the initial 5-11 months of post-treatment follow-up. All patients discontinued immunomodulatory therapies at a median of 4.1 months (range: 3.3-4.8) following RP-A601 infusion. Patients showed evidence of demonstrable cardiac transduction and increased myocardial PKP2a protein expression at 3-6 months following RP-A601 infusion.

Conclusions: Initial results from this on-going phase I trial show treatment with RP-A601 was well-tolerated and conferred increased cardiac PKP2a expression in patients afflicted with this life-threatening and highly morbid disorder. Pending confirmation, these results represent the first demonstration of AAV-mediated genetic modification of an inherited ACM or any disorder predisposing to sudden cardiac death. Updated results will be presented.

2003 AAV-Mediated Gene Therapy Restores Retinal Structure and Function in X-linked Retinoschisis: From Bench to Bedside

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Background: X-linked retinoschisis (XLRS), a leading cause of macular degeneration in male adolescents, is characterized by retinal splitting and progressive vision loss. Despite advances in understanding its genetic basis, no approved treatments exist, with clinical management limited to addressing complications. Preclinical studies have shown the promise of adeno-associated virus (AAV)-mediated gene therapy in restoring retinal structure and function in XLRS mouse models. However, translating these findings into clinical success remains a significant challenge. This study evaluates the safety and efficacy of AAV2/8-mediated gene therapy in both preclinical XLRS mouse model and human patients.

Methods: A recombinant AAV2/8 (rAAV2/8) vector containing a CMV promoter, a human RS1 coding sequence (*hRS1*), and an eGFP reporter was constructed. P22±3 *RS1* homolog knockout (*RS1h*-KO) mice were intravitreally injected at a 2×109 vg/eye dose, with buffer solution as a control. Visual function was assessed using optomotor responses (OMR) and electroretinogram (ERG). The retinal structure was evaluated via histological analyses, fundus photography, and optical coherence tomography (OCT). Changes in proinflammatory and apoptotic markers were analyzed via qRT-PCR, TUNEL staining, and GFAP immunolabeling. In the clinical trial, XLRS patients received a single intravitreal injection of ZM-01 (rAAV2/8-CMV-*hRS1*) at a 2·07×1011 vg/eye dose. Safety, the primary endpoint, was assessed by monitoring the incidence of adverse events (AEs) and serious adverse events (SAEs). Secondary endpoints included best-corrected visual acuity (BCVA), fundus photography, macular OCT, and quality of life (QoL) over 12 months.

Results: AAV2/8-mediated gene therapy restored retinal structure and visual function in the XLRS mouse model 4 weeks post-injection. H&E staining revealed a near-normal retinal layer organization accompanied by the absence of retinal cavities in treated eyes. Restored morphology was observed in photoreceptor cells (PRCs), horizontal cells (HCs), rod-bipolar cells (RBCs), Müller cells (MCs), and their dendrites and axons, alongside RS1 expression throughout retinal layers. Treated mice demonstrated improved visual acuity and higher ERG parameters (a-wave and b-wave amplitudes) under both scotopic and photopic conditions. Retinal inflammation and apoptosis were reduced, with downregulation of proinflammatory and apoptotic markers, fewer TUNEL+ apoptotic cells, and attenuated fluorescence expression of GFAP in treated retinas. These benefits were sustained almost up to 12 months post-injection, with treated mice demonstrating superior retinal integrity and function recovery than the sham-injected.

In the clinical trial, both XLRS participants exhibited significant BCVA improvements. Participant 1 improved from logMAR 1.0/0.82 (20/200, 20/125) to logMAR 0.7 (20/100), and Participant 2 improved from logMAR 0.92 (20/166) to logMAR 0.4 (20/50) in both eyes. OCT imaging revealed reduced retinal layer separation and decreased cavity volume in the paracentral retina. Both participants reported enhancements in visual acuity and QoL during the 12-month follow-up, supported by family observation. No drug-related AEs or SAEs were reported.

Conclusions: The novel AAV2/8-mediated gene therapy demonstrated robust efficacy in restoring retinal structure and visual function in both XLRS mouse models and patients. The attenuation in retinal inflammation and apoptosis highlights its potential as a foundational treatment for XLRS. Clinically, ZM-01 contributed to improving retinal structure, visual acuity and QoL in pediatric patients with sustained durability over 12 months, offering a safe and effective therapeutic option. These findings mark an important step toward translating gene therapy for XLRS from bench to bedside and set the stage for further investigation and optimization.

2004 PRODYGY: A First-in-Human Trial of Rod-Derived Cone Viability Factor (RdCVF) Gene Therapy in Subjects with Rod-Cone Dystrophy

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Purpose: Rod-cone dystrophy (RCD) is a rare inherited retinal disorder leading to significant vision loss, and for which no treatment is currently available to most patients. SPVN06 is a gene-independent investigational gene therapy expressing the neurotrophic rod-derived cone viability factor (RdCVF) and the thioredoxin RdCVF-Long (RdCVFL), that aims at slowing down the progression of central vision loss in patients with RCD, regardless of the underlying pathogenic variant(s).

Methods: PRODYGY (NCT05748873) is a first-in-human Phase I/II trial enrolling subjects with advanced RCD due to a mutation in the RHO, PDE6A, or PDE6B gene. The study will assess the safety and tolerability of a unilateral subretinal injection of SPVN06, one year after treatment administration. The study design is comprised of an open-label dose-escalation phase (Step 1) followed by a controlled, double-masked, randomized, extension phase (Step 2). Step 1 has assessed 3 increasing doses of SPVN06 in subjects with severe advanced RCD, and an independent Data Safety Monitoring Board (DSMB) reviewed all available safety data to recommend two doses for assessment in Step 2. At time of DSMB review, the duration of follow-up was 1.5 years in the low-dose cohort (n=3), between 1 and 1.25 years in the medium-dose cohort (n=3), and between 3 and 9 months in the high-dose cohort (n=3).

Results: Three months after completion of the last dose-escalation cohort, no serious adverse events, dose-limiting toxicities, or study discontinuations had been reported in the 9 subjects of Step 1.

Ocular adverse events (AEs) (n=48) were predominantly reported in Study Eyes (n=41) and mild in intensity (n=35). Two severe AEs were reported, both in Study Eyes, and were deemed to possibly related to the procedure. A total of 9 ocular AEs were reported in Study Eyes of the low dose cohort, 17 in the medium-dose cohort, and 15 in the high-dose cohort.

Twelve (12) ocular AEs were considered possibly/probably or certainly related to SPVN06 (all were reported in Study Eyes): 7 of mild intensity, 4 of moderate intensity, and 1 of severe intensity; representing 3 in the low-dose

cohort, 4 in the medium-dose cohort, and 5 in the high-dose cohort. One non-ocular AE (38.5° body temperature) reported in a patient of the medium-dose cohort was considered possibly/probably related to SPVN06.

A mild to moderate and transient systemic humoral immune response against AAV8 capsid was observed in 7 out of 9 subjects tested so far. Total antibodies against the transgene products were detected in only 4 out of 9 subjects, at very low levels close to the background noise. No cellular immune response against SPVN06 peptides was observed.

Additionally, a positive net value in the number of ETDRS letters read —indicative of a more favorable outcome in the Study Eye— was reported at two consecutive time points (6 months apart) in 3 of 6 subjects with at least one year of follow-up post SPVN06 administration.

Conclusions: First administration of SPVN06 to nine subjects with severe advanced RCD showed a favorable safety profile at all 3 doses tested, with no significant immune response. After data review, the DSMB recommended to assess the medium and high doses of SPVN06 in Step 2 of PRODYGY, and to continue the trial without modification. The controlled, double-masked, randomized extension phase of PRODYGY was initiated, and SPVN06 was administered to the two sentinel subjects with intermediate advanced RCD.

Dr. Jose-Alain Sahel and UPMC have financial interests in the study sponsor, SparingVision.

2005 DNA Origami Vaccine (DoriVac) Nanoparticles Improve Both Humoral and Cellular Immune Responses to Infectious Diseases

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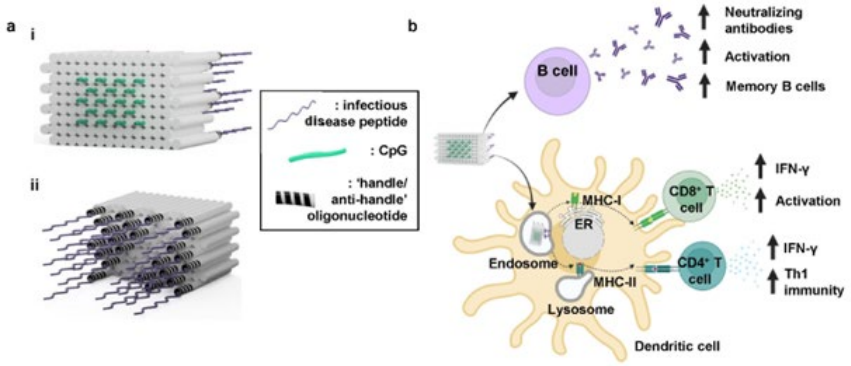
Current SARS-CoV-2 vaccines have demonstrated robust induction of neutralizing antibodies and CD4+ T cell activation, however CD8+ responses are variable, and the duration of immunity and protection against variants are limited. Here we repurposed our DNA origami vaccine nanotechnology, DoriVac, for targeting infectious viruses, namely SARS-CoV-2, HIV, and Ebola. The DNA origami nanoparticle, conjugated with infectious-disease-specific heptad repeat 2 (HR2) peptides, which act as highly conserved antigens, and CpG adjuvant at precise nanoscale spacing, induced neutralizing antibodies, Th1 CD4+ T cells, and CD8+ T cells in naïve mice, with significant improvement over a bolus control. Pre-clinical studies using lymph-node-on-a-chip systems validated that DoriVac, when conjugated with antigenic peptides or proteins, induced promising cellular immune responses in human cells. Moreover, DoriVac's flexible architecture accommodates full-length viral proteins, as demonstrated with SARS-CoV-2 spike-protein-conjugated DoriVac, achieving immune responses comparable to current mRNA vaccine platforms while potentially reducing immune exhaustion and storage constraints. These results suggest that DoriVac holds potential as a versatile, modular vaccine nanotechnology, capable of inducing both humoral and cellular

immunities. The programmability of this nanoparticle underscores its potential utility in addressing future pandemics.

Fig. 1 | DNA origami vaccines (DoriVac) fabricated with infectious-disease-specific peptides. ai–ii*, Schematic of DoriVac: a DNA origami square block (SQB) nanoparticle conjugated with CpG at a 3.5 nm spacing along with disease-specific peptides.* ***b****, Schematic of SQB delivering antigen and adjuvant at precise spacing to antigen-presenting cells, eliciting humoral and cellular responses.*



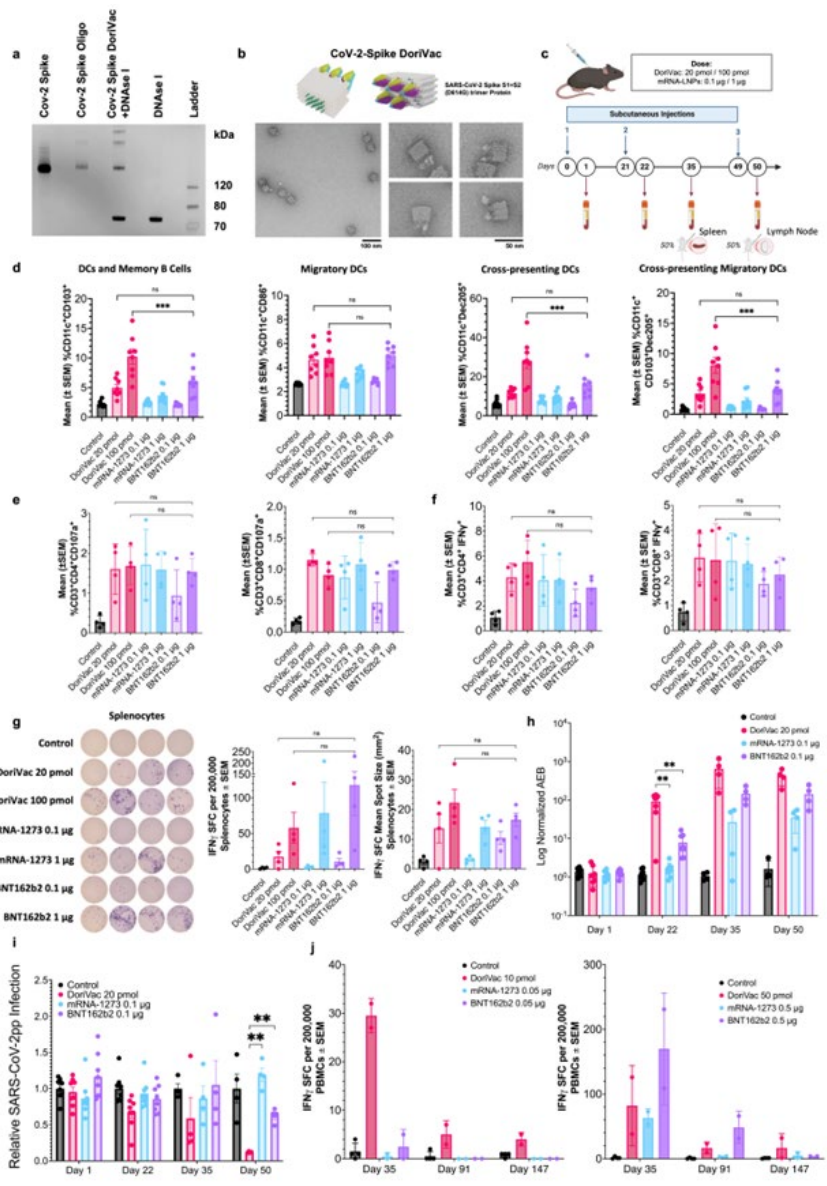


Fig. 2 | SARS-CoV-2 spike-protein-conjugated DoriVac induces potent cellular and humoral immune activation in mice. ***a,*** *SDS-PAGE confirming successful conjugation of SARS-CoV-2 spike proteins to oligonucleotides and their hybridization on the DoriVac SQB.* ***b,*** *Schematic of SARS-CoV-2 protein-conjugated DoriVac SQB with corresponding negative-stained TEM images.* ***c,*** *Schematic of the vaccine administration protocol for naïve C57BL/6 mice and data collection timeline. Blood was collected on days 1, 22, 35, and 50; plasma was used for anti-SARS-CoV-2 antibody quantification and pseudovirus neutralization assays, while PBMCs were isolated for flow cytometry and ELISpot; spleens and lymph nodes (LNs) were harvested on days 35 and 50, respectively.* ***d,*** *Percentages of CD103⁺, CD86⁺, Dec205⁺, and CD103⁺ Dec205⁺ double-positive CD11c⁺ PBMCs (n=8) on day 22 via flow cytometry. DoriVac treatment group showed enhanced immune activation compared to controls, in some cases surpassing that of mRNA-LNPs.* ***e–f,*** *Percentages of CD107a⁺ LN cells (n=4) and IFNγ-secreting cells (n=4) on day 50 via flow cytometry. DoriVac enhanced activation relative to control and was comparable to mRNA-LNP groups.* ***g,*** *IFNγ ELISpot showing antigen-specific splenocyte frequency (n=4, day 35) with quantification of SFUs and mean spot sizes. DoriVac increased SARS-CoV-2 antigen-specific T cell frequency versus control, with larger spots indicating robust cytokine release.* ***h,*** *Quantification of anti-spike IgG in plasma from control and DoriVac 100 pmol/mRNA-LNP 1 µg groups using SiMoA (n=8 on days 1, 22; n=4 on days 35, 50). DoriVac elicited higher antibody levels versus control at all time points; on day 22, levels were significantly higher than mRNA-LNP groups.* ***i,*** *SARS-CoV-2 pseudovirus (SARS-CoV-2pp) neutralization assay (n=8 on days 1, 22; n=4 on days 35, 50; 1:20 dilution) for DoriVac 20 pmol/mRNA-LNP 0.1 µg groups in ACE2-293T cells. DoriVac showed stronger neutralization from day 22 onward versus controls and, by day 50, compared to mRNA-LNP groups.* ***j,*** *Longitudinal IFNγ ELISpot of PBMCs following intramuscular administration shows lasting T cell responses in DoriVac-treated mice up to day 147, comparable to or exceeding mRNA-LNP groups (control n=4, others n=2). Data were analyzed by one-way ANOVA (with Tukey's test), with significance defined as a multiplicity-adjusted p value < 0.05. 'ns' indicates P > 0.05; '**'*

2006 ENTPD3-specific CAR Regulatory T cells for therapy of type 1 diabetes

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Despite advances in Type 1 Diabetes (T1D) management through hybrid closed-loop systems, patients continue to experience significant morbidities, reduced life expectancies, and abnormal glucose regulation compared to healthy individuals or those who have undergone pancreas transplantation. Inspired by the recent success of HLA-A*02-specific chimeric antigen receptor (CAR) regulatory T cells (Tregs) in inducing local immune tolerance, we developed beta cell-specific CARs targeting the antigen ENTPD3. Using a novel phage display methodology, we generated several CARs against properly folded ENTPD3 proteins expressed on cells. These CARs were transduced into natural Tregs to redirect their specificity independently of MHC restriction, aiming for local immune control.

ENTPD3 CAR Tregs exhibited preferential homing, local activation, expansion, and persistence within pancreatic islets in a spontaneous T1D model using NOD mice, effectively preventing T1D development. In contrast, control CARs showed no effect. Comprehensive characterisation of human ENTPD3 CAR Tregs revealed a stable regulatory phenotype, robust Treg activation, and suppression. Importantly, ENTPD3 CAR T cells recognised and were fully activated by human islets.

ENTPD3 CAR Tregs offer a promising, durable therapeutic strategy for achieving local immune control in patients with prediabetes, newly diagnosed T1D, or those undergoing biological beta cell replacement therapies.

2007 Glycan Shielding via Single SPPL3 Ablation Enables TCR-Sufficient Long-Lasting Allogeneic CAR-T Therapy in Patients

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Introduction: Allogeneic CAR T-cell therapy faces challenges such as limited persistence and host rejection. Our three unbiased CRISPR screenings in primary T cells and validation show that SPPL3 knockout T cells resist immune rejection by host T cells, NK cells, and activation-induced cell death. SPPL3, a sheddase, affects glycan formation and abundance by inhibiting glycosyltransferases and glycosidases. Knocking out SPPL3 in T cells

creates unique glycan profiles that cause steric hindrance, limiting accessibility of ligands like HLA, NK receptor ligands, TCR, and Fas, and blocking allogeneic immune effector activity. Importantly, this does not affect the function of synthetic anti-CD19 CAR molecules.

A key finding from our reverse clinical translational study (ET-901-Athena) is that maintaining TCR is important for the persistence of allogeneic T cells (NCT06014073). These findings redefine the current paradigm, as most universal CAR-T designs involve TCR deletion to prevent GvHD. We further demonstrated that SPPL3 single knockout T cells (ET-901-Herculas) exhibit low TCR detection, alongside extensive preclinical data showing low GvHD risk and a stable glycan-modified phenotype in vivo. The second translational study showed that allogeneic SPPL3ko TCR-sufficient CAR-T cells demonstrate robust persistence over 6 months and sustained tumor control for over six months without causing GvHD (NCT06323525).

Methods: ET-901-Athena (SPPL3ko/TCRko) and ET-901-Herculas (SPPL3ko) anti-CD19 CAR-T are being evaluated in r/r B-NHL patients at a single center. The 3+3 dose escalation design includes lymphodepletion with fludarabine (30-50 mg/m²/d) and cyclophosphamide (500-1000 mg/m²/d) for three days (day-5 to -3), followed by escalating doses of ET-901-Athena (1×10^6/kg, 3×10^6/kg, 10×10^6/kg) or ET-901-Herculas (1×10^6/kg, 3×10^6/kg, 6×10^6/kg). Primary objectives are safety assessment and RP2D determination.

Results: Of 20 screened patients, 9 received ET-901-Athena. All had advanced B-NHL (Ann Arbor stage III-IV) with median prior treatment lines of 4, two-thirds refractory disease, median SPD of 7,660 mm²(range: 1,710 to 43,400 mm²), and 4 with bulky disease. No DLTs or GvHD occurred. Objective response rate was 100% (66.7% complete responses). The CAR-T cells showed robust proliferation in allogeneic settings with manageable toxicities. Median peak CAR vector copy number was 123,529.4 copies/μg, and peak CAR T cell level was 49.2 cells/μl. Interestingly, clinical analysis revealed significant late-stage expansion of TCR-positive CAR-T cells, even though the initial infusion contained fewer than 0.3% TCR-positive cells. This suggests that residual TCR-positive cells had a persistence and expansion advantage.

Three compassionate-use patients received a high-dose ET-901-Herculas CAR-T infusion without HLA matching. SPPL3ko T cells demonstrated low TCR detection, causing manageable skin rash in 1 patient (Days 8-30), while the other 2 showed no signs of GvHD. No patients exhibited elevated bilirubin or transaminases, nor did they require continuous immunosuppressive drugs. These cells expanded and effectively suppressed B cell populations in the bone marrow, achieving sustained tumor control.

Conclusions: Glycan-shielded allogeneic CAR-T cells can persist for over six months, control tumors, resist immune rejection, and avoid GvHD. These findings position SPPL3 engineering as a transformative platform for universal CAR-T therapies.

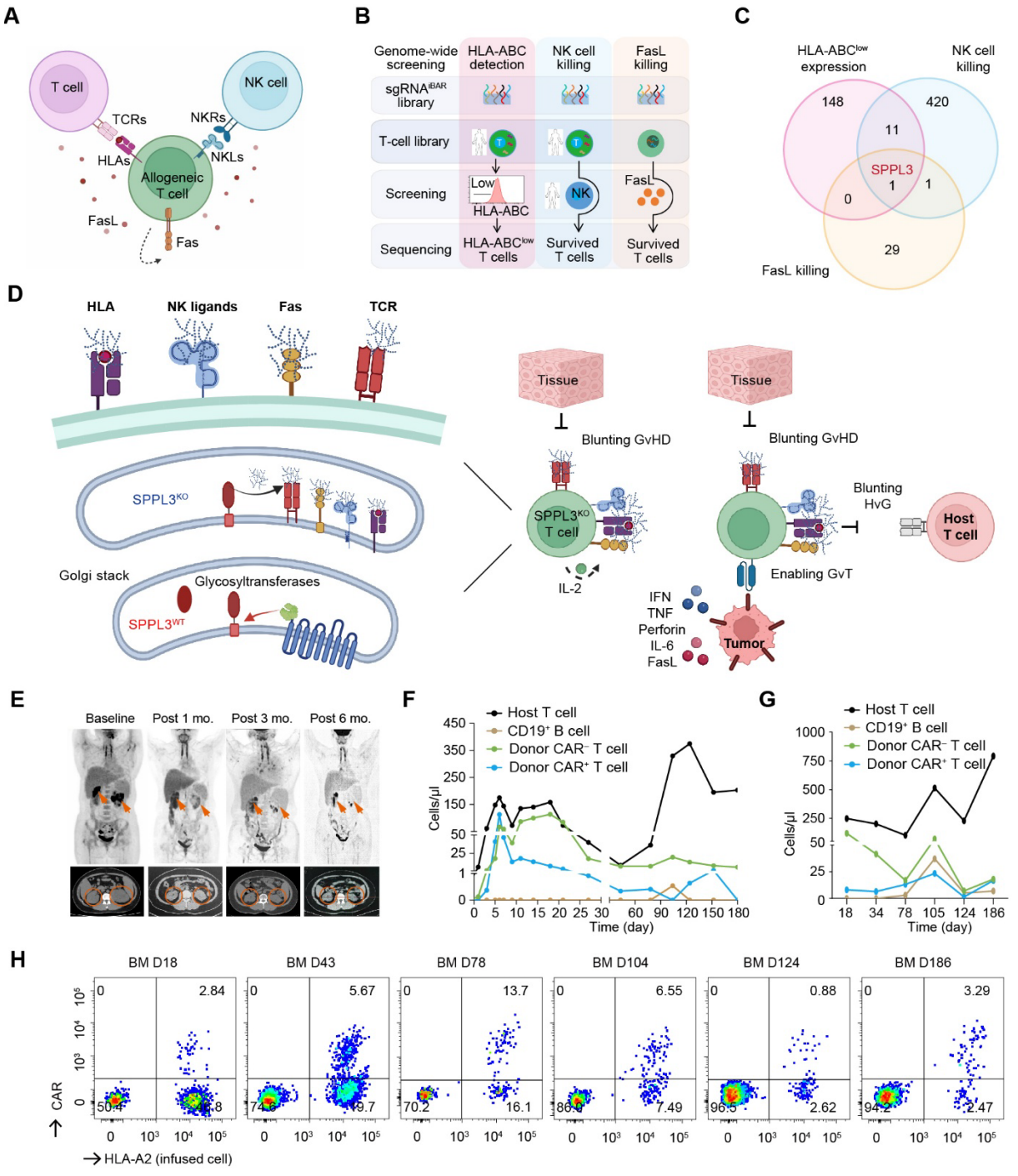

Figure. Glycan Shielding via Single SPPL3 Ablation Enables TCR-Sufficient Long-Lasting Allogeneic CAR-T Therapy.
A. Schematic illustrating key effectorsaffecting persistence of allogeneic T cells.
B. Bottom-up approach for whole-genome screenings.
C. Overlapping hits from the three screens.
D. SPPL3 is a key regulatorof alloimmunity, influencing the glycan modification of HLA, Fas, TCR, and NK cell ligands. The enhanced modification of these key immune proteins concurrently leads to engineered T cells that are resistant to alloimmune cell-mediated rejection and diminished GvHD. While the anti-CD19 CAR is not impacted by the modification, theanti-tumor capacity remains unaffected.
E. PET-CT scans of pre-infusion, and at 1, 3, and 6 months post-CAR-T infusion.
F. Monitoring the expansion of host T cells, donor T cells, and B cells in peripheral blood over time.
G. Tracking the host T cells, donor T cells, and B cells in bone marrow over time.
H. Infused T cell detection (HLA-A2 positive) in bone marrow at different time points.

2008 Novel AAV capsid selection approach: Development of Human-to-Human (H2H) platform

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Historically, practical limitations of wild type AAV capsids (wtAAV), such as limited transduction, lack of precise tissue/cell tropism, or susceptibility to neutralizing antibodies, served as drivers for AAV capsid engineering. Researchers have developed numerous strategies for engineering AAVs with altered properties: directed evolution, rational design, shuffling, and combinatorial approaches that integrate multiple methods and animal models. While we know a lot about tissue/cell tropism of wtAAV and engineered AAVs in various laboratory animals, despite >340 clinical trials which utilize AAV capsids, the exact biodistribution, and efficient transduction in human tissues/cells remains a critical uncertainty and often an unknown liability when treating rare disease patients. To fully unlock the potential of AAV as a broad therapeutic modality, akin to the small molecules and biologics, the field must break conventions and embrace a truly human-centric innovation selection strategy. To help achieve this, we established a unique and novel capsid screening paradigm that exposes capsid libraries to functional human immune systems, normal blood physiology, and clinically identical vascular biodistribution, utilizing the rare but unique setting of brain-dead patients, technically referred to as “decedents”. Below is a summary of this selection approach and early data readout.

Decedent (D) protocol: After the declaration of brain death, and while the decedent is still on full life support,

1. We first consent the next of kin for organ donation following established protocols
2. If organ donation is refused, we request consent for the participation in the AAV screening research study.
3. If yes, Decedents (2 males, 1 female to date) are administered within 1-24hr. with pooled high-diversity AAV peptide display libraries via intravenous injection.
4. At various times post administration (D1 66.9 hr, D2 134 hr, & D3 159.5 hr.), decedents undergo full autopsy for pathology analyses (> 100 unique tissues/decedent)
5. Pre-injection serum is collected and neutralizing (NAB) and neutralizing antibody (NAB) determined (D1 negative, D2 negative, D3 low at 1:10)

Results:

1. Three capsid libraries (AAV 5, AAV 9, & Rh10) were generated and administered to each decedent.
2. We also included a number of FDA approved and primate selected AAV capsids (AAV2, AAV2i8, AAV8, AAV9, Rh10, XL32, AAV-myo, Myo-AAV) now being used in research and in patients for detail biodistribution atlas.
3. We did not use the NAB/TAB status as exclusion criterion reasoning those identifying capsids capable of antibody escape would be advantageous for the field.
4. Liver was first tissue analyzed for validation of our decedent selection protocols (e.g. D1- 2086 and D2 - 2706 unique peptides identified with 109 overlap).
5. Currently we have derived candidate capsids from liver, heart, spinal cord and brain.
6. We identified unique capsid isolates that are present in the same tissues in all decedents,
7. We also identified isolates that distribute broadly to multiple tissues,
8. Additionally, isolates were identified that are decedent/tissue specific.
9. Based on recovered sequences, second generation libraries for additional rounds of screening have been prepared.

Further details of maximizing human decedents for human targeted AAV capsid isolation and future clinical therapeutics will be discussed.

2009 Development and First-in-Human Experience with LE051: An RNA Editing Drug for Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder arising from mutations in the dystrophin gene, leading to muscle weakness, disability, and premature mortality. Our previous research introduced the LEAPER 2.0 system, an innovative platform that enables efficient RNA editing in cells and living organisms through the use of circular ADAR-recruiting RNA (circ-arRNA). This system also supports exon skipping by combining ADAR-mediated RNA editing at critical adenosines for exon recognition, steric hindrance from ADAR binding, and additional steric effects from circ-arRNA functioning as an antisense molecule, collectively achieving highly efficient exon skipping.

LE051, an investigational therapy, incorporates a circ-arRNA expression cassette targeting human exon 51 and is delivered via adeno-associated virus. By inducing exon 51 skipping, LE051 holds the potential to treat approximately 13% of DMD patients. In a DMD mouse model with exon 50 deletion, a single systemic injection of LE051m, a surrogate for mouse study, triggered exon skipping, restored dystrophin protein expression, and enhanced functional outcomes. In wild-type non-human primates, LE051 induced dose-dependent exon 51 skipping. At a dose of 2.5E13 vg/kg, exon skipping ranged from 0.3% to 6.3% at 1 month and 0.2% to 5.2% at 3 months post-injection, while at 5E13 vg/kg, exon skipping increased to 0.9%–18.6% at 1 month and 0.6%–29.8% at 3 months post-injection in key muscles (gastrocnemius, quadriceps, triceps brachii, biceps brachii, latissimus dorsi, and diaphragm). At the highest tested dose (5E13 vg/kg), no adverse effects were noted, establishing the no-observed-adverse-effect level (NOAEL).

Building on these preclinical results, an investigator-initiated trial (IIT), approved by Institutional Review Board, was launched to evaluate the safety and tolerability of LE051 at two dose levels (2E13 vg/kg and 5E13 vg/kg). As of March 2025, two patients eligible for exon 51 skipping have received the 2E13 vg/kg dose. No dose-limiting toxicities were observed within the first month. All adverse events were classified as Grade 1, with transient abdominal pain, nausea, and vomiting determined by the investigator as possibly related to the study drug. Pharmacokinetic profiles in blood showed strong consistency with NHP data. In terms of efficacy, the first patient completed the 3-month follow-up and demonstrated notable improvements in motor function, as evidenced by a 3-point increase in the North Star Ambulatory Assessment score and a 61-meter increase in the 6-Minute Walk Test, alongside enhanced lung function compared to baseline. Additionally, exon skipping and dystrophin restoration were confirmed.

In summary, based on the LEAPER 2.0 RNA editing platform, LE051 demonstrated a favorable safety profile at the lower dose level, along with early signs of efficacy. Future efforts will focus on advancing to higher doses and further assessing the long-term benefits of LE051 for DMD patients.

2010 Revolutionizing Wilson's Disease Treatment: Clinical Safety and Efficacy of LY-M003, A Copper-Responsive AAV Gene Therapy Vector

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Introduction: Wilson disease (WD) is a rare autosomal recessive metabolic disorder caused by mutations in the *ATP7B* gene, leading to deficient ATP7B activity and progressive copper accumulation in key organs, including the liver, brain, and kidneys. LY-M003, an investigational adeno-associated virus serotype 8 (AAV8)-based gene therapy, is designed to restore copper homeostasis by delivering a modified miniATP7B^{LY} protein with functionality comparable to wild-type ATP7B, primarily targeting hepatic expression. LY-M003 utilizes the Copper Responsive Engineering of *ATP7B* Expression (CREATE™) platform, which dynamically regulates protein expression in response to hepatic copper levels. This novel approach aims to enhance both efficacy and safety in the treatment of Wilson disease.

Method: A clinical study (NCT06650319) is currently underway at the First Affiliated Hospital of Zhejiang University, China, to evaluate the safety and efficacy of a single intravenous infusion of LY-M003 in patients with WD. Stable treated WD patients are eligible for enrollment, with the option to continue standard-of-care (SoC) therapy post-administration until clinical improvements are observed. A prophylactic immune management regimen, including steroids and immunomodulating agents, is administered to all participants. The study's primary objectives are to assess the safety and efficacy of escalating doses of LY-M003. Participants will undergo a 52-week post-treatment evaluation, followed by a four-year long-term follow-up phase.

Results: Since September 2024, seven participants have received LY-M003, demonstrating a favorable tolerability profile across all dosage cohorts. No anti-drug antibodies (ADA) against ATP7B were detected, and no serious adverse events (SAEs) were reported. Mild elevations in liver enzymes were observed in some participants but were effectively managed with immune modulation therapy.

Key therapeutic outcomes include:

- **Hepatic Copper Transport Restoration:** Most participants exhibited significantly increased ceruloplasmin levels, with peak values exceeding 50% of the normal range in one patient, indicating reestablished hepatic ATP7B function.
- **Reduction in Chelation Therapy Dependence:** Four out of seven participants successfully discontinued chelation therapy following normalized urinary copper levels. Most patients initiated SoC reduction within one month post-LY-M003 administration, with one high-dose cohort participant achieving complete discontinuation of SoC therapy within six weeks.
- **Neurological and Psychiatric Symptom Improvement:** Participants experiencing neurological and psychiatric manifestations showed functional improvements within three months, as evidenced by reductions in UWDRS scores related to writing ability, language skills, and motor function.

Conclusion: LY-M003 is an innovative AAV8-based gene therapy for Wilson disease, currently under clinical investigation. Early clinical data across different dosage groups indicate a strong safety profile and promising therapeutic efficacy, with benefits observed in both peripheral and neurological symptoms. These findings highlight LY-M003's potential to revolutionize Wilson disease treatment, paving the way for a transformative therapeutic paradigm.

2011 Single Cell Analysis of AAV9 Transduction in a Patient that Succumbed to Acute Respiratory Distress Syndrome Following Systemic High Dose Gene Therapy

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Thrombotic microangiopathy, disseminated intravascular coagulation, and atypical hemolytic-uremic syndrome have been reported following high-dose systemic AAV9 clinical trials. In nonclinical studies, high-dose systemic AAV clade F administration can lead to severe hepatocellular and liver sinusoidal endothelial cell injury in nonhuman primates. We recently described the first reported case of acute respiratory distress syndrome (ARDS), another type of endothelial injury syndrome, following high-dose AAV administration in a Duchenne Muscular Dystrophy (DMD) patient who succumbed within 6 days of receiving 1 x 10^{14} vg/kg of AAV9-CK8e.dSaCas9.VP64.U6.sgRNA.

To gain insights into this unusual adverse reaction, we conducted further post-mortem analysis of vascular injury serum biomarkers (hyaluronic acid, von Willebrand factor) and of cell-specific biodistribution in lung, heart, and liver samples from this patient using *in situ* hybridization (ISH) and single-nucleus RNA sequencing (snRNAseq). Serum biomarker analysis highlighted acute elevations of serum hyaluronic acid and von Willebrand factor 3 and 5 days after vector administration, respectively, indicating early liver sinusoidal endothelial cell damage followed by non-specific vascular injury. Liver ISH indicated abundant DNA nuclear signals in the absence of cytoplasmic mRNA signals in hepatocytes. This finding suggests the presence of transcriptionally inactive vector genomes, which is expected based on the use of a CK8e promoter. Surprisingly, no ISH signal was observed in the heart, whereas the alveolar lining of the lungs was strongly positive for vector DNA (nuclear) and transcripts (cytoplasmic). This result was confirmed via snRNAseq, with the highest dSaCas9 expression measured in lungs (~11% of cells) compared to the liver and heart (1-2% of cells). Of the constituent lung cell types, ~20% of capillary endothelial cells expressed dSaCas9 transcripts.

In conclusion, our analysis highlighted an unexpectedly high level of pulmonary endothelial cell transduction in a DMD patient that developed ARDS post-AAV9 treatment. It is not clear why pulmonary endothelial cells in this patient were so permissive to vector transduction, or whether this contributed to the development of ARDS. It is possible, however, that primary effects of dystrophin deficiency or secondary effects of multi-organ failure could have played a role. Further work is required to understand the unusual biodistribution and toxicity profile observed in this patient, which has not been reported in any other high-dose AAV9 clinical trial. It is also important to note that ARDS was not diagnosed during the clinical course of this patient, but was only discovered at autopsy. Since post-mortem analysis has not been reported in other cases of fatal toxicity of AAV in DMD trials, it is not clear whether or not ARDS is an underrecognized complication of high dose AAV.

2012 Initial Experience From the INSPIRE DUCHENNE Phase I/II Study of SGT-003 Microdystrophin Gene Therapy for Duchenne Muscular Dystrophy

Kevin Flanigan[1], Perry Shieh[2], Hernan Gonorazky[3], Aravindhan Veerapandiyan[4], Patrick Gonzalez[5], Matthew Harmelink[6], Gabriel Brooks[6]
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INSPIRE DUCHENNE is a first-in-human phase 1/2, open-label, multicenter, clinical trial evaluating the safety, tolerability, and efficacy of a single intravenous infusion of SGT-003 microdystrophin gene therapy in pediatric Duchenne muscular dystrophy (Duchenne) patients. SGT-003 utilizes a novel muscle-tropic capsid, AAV-SLB101, which in non-clinical studies has been shown to result in multiple-fold higher levels of biodistribution and

expression in muscle tissues and decreased biodistribution to the liver. SGT-003 microdystrophin is a unique construct that includes the neuronal nitric oxide synthase (nNOS) binding domain, responsible for protection against ischemia-induced muscle injury by enabling nitric oxide (NO) signaling at the muscle sarcolemma.

As of a data cut of March 13, 2025, 7 study participants have received SGT-003 at the single study dose level of 1E14 vg/kg. A prophylactic immunomodulatory regimen consisting only of increased glucocorticoids was used in the period surrounding dosing. These participants have been monitored in follow-up periods spanning up to approximately 6 months post-infusion.

As of the data cut, SGT-003 has been well tolerated and no serious adverse events (SAEs) have been reported in the study. Participants generally experienced adverse events (AEs) that are common for a gene therapy in the days following infusion, which resolved within the first weeks post-dosing. Importantly, there have been no reports of hepatotoxicity, myocarditis, myositis, thrombotic microangiopathy, or any severe symptom that may result from a gene therapy. Preliminary biomarker data from the first 3 participants that have completed the Day 90 visit will be presented.

These initial results suggest a positive safety profile of SGT-003, using only glucocorticoids for immunomodulation.

2013 AAV.Olig001-Mediated Transdifferentiation: Harnessing Oligodendrocytes for Neural Reprogramming in Neurodegenerative Diseases

Christina Chatzi[1], Liujiang Song[2], Lester Suarez[3], Abhinandan Pattanayak[4], Hiroyuki Nakai[1], Sheila Mikhail[1], Josh Grieger[1], Weidong Xiao[5], Weiming Wang[6], Anna Tretiakova[1], R. Jude Samulski[1]*
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Neurodegenerative diseases (NDDs) are a leading cause of disability, with no curative treatments. Current therapies, such as Levodopa for Parkinson's disease (PD) and cholinesterase inhibitors for Alzheimer's disease (AD), provide symptomatic relief without addressing the underlying neuronal degeneration driving disease progression. As the global burden of NDDs continues to rise, there is an urgent need for innovative therapeutic strategies that not only prevent further neuronal loss but also restore lost functions. Recent advances suggest that in vivo reprogramming of endogenous cell populations may be a viable alternative for neuron replacement.

We previously demonstrated that AAV.Olig001 capsid selectively transduces oligodendrocytes across species and that downregulation of Polypyrimidine Tract Binding Protein 1 (PTBP1) promotes effective transdifferentiation of resident oligodendrocytes into neurons in rats, establishing this approach as a strategy for neuronal regeneration. We have now refined the AAV.Olig001 cassette for both rodent and human applications; additionally, we incorporated Glial Cell-Derived Neurotrophic Factor (GDNF) as a second therapeutic modality to enhance integration of reprogrammed neurons into existing circuits and support neuronal survival. To fully evaluate this dual-modality approach, we developed a series of AAV.Olig001 expression cassettes: a control cassette expressing GDNF with a scrambled miRNA, a second control lacking GDNF expression but incorporating anti-PTBP1 miRNA to isolate the effects of either GDNF or PTBP1 downregulation alone, and therapeutic cassette co-expressing GDNF and anti-PTBP1 miRNA to promote both, neuronal survival and oligodendrocyte transdifferentiation. Two distinct anti-PTBP1 miRNAs were designed—one for rodents and one for human PTBP1—ensuring precise knockdown across species for robust clinical translation.

To validate this strategy, we first assessed the efficacy of the transgenes/therapeutic cassettes using transient transfections in vitro. Our optimized cassettes effectively downregulated PTBP1 and induced GDNF expression in both HEK293 and N2A cells. In addition, in primary rat oligodendrocytes, we observed robust PTBP1 knockdown accompanied by GDNF secretion, demonstrating vector functional activity in relevant cells. Immunocytochemistry revealed an increase in expression of neuronal marker MAP2 and a decrease in expression of oligo marker PLP, suggesting a shift toward a neuronal phenotype. Investigations are ongoing measuring the effects of PTBP1

knockdown and GDNF expression in vivo to further characterize the effects of the dual mechanism of action on neuronal reprogramming, transdifferentiation, and functional integration. Our findings support AAV-mediated oligodendrocyte-to-neuron transdifferentiation as a promising therapeutic approach for NDDs. By optimizing our vectors for both rodent and human applications, we aim to ensure clinical translation. Future studies will focus on assessing therapeutic efficacy of our dual approach in mouse models of NDDs in parallel with human iPSC-derived oligodendrocytes.

This work represents a step toward regenerative therapies for NDDs, offering a strategy that not only halts disease progression but also replaces lost neurons while promoting neuronal survival providing a foundation for further research into endogenous cell reprogramming as a therapeutic avenue.

Poster Presentations

2014 Countdown to Package: Molecular Insights into the Rep-mediated Adeno-associated-virus Packaging Machinery

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Recombinant adeno-associated viral (rAAV) vectors are among the most widely used gene therapy vectors. At present, developmental advancements have outpaced platform development where challenges such as product heterogeneity due to the variations in quality and efficiency of payload packaging; and difficulty in separation of heterogeneous particles in the downstream purification leads to high cost and low yield. Furthermore, the presence of empty capsids heightens the overall immune response without therapeutic benefit, contributing to the high dosage required for AAV-based therapies which lowers its safety profile. Here, we address this challenge by understanding the molecular mechanism of payload packaging mediated by the AAV Replication protein (Rep). Replication proteins are viral proteins responsible for AAV genome replication and packaging where the four isoforms coordinate the replication, strand-separation and encapsidation of AAV genome into pre-assembled empty capsids. Here, we utilize cryoEM, mass photometry and molecular dynamics (MD) simulations to study how the minimal helicase domain Rep, Rep40, interacts with the inverted-terminal-repeat (ITR) hairpin DNA and the AAV capsid. Notably, we observe different Rep40 oligomeric states depending on bound-substrates including heptamer and hexamers representing the state of Rep bound on the dsDNA and ssDNA of ITR respectively; and pentameric Rep bound on the five-fold-axis pore of the AAV capsids. This novel data elucidates a mechanism where a pool of Reps are able to dynamically form different oligomeric states and orchestrate ITR hairpin recognition, unwinding and subsequent capsid association through homomeric interactions. Preliminary MD simulations of hexameric Rep40 bound to ssDNA and ATP suggests a potential mechanism of ATP-binding triggered base distortion of the bound ssDNA which allows a directional translocation with prevention of back-sliding. Taken together, this data sheds light on the mechanism of Parvoviral DNA packaging and highlights potential targets for optimization of the payload packaging process during rAAV production.

2015 Extracellular Vesicle-Encapsulated AAV Enhances Gene Delivery to Human Chondrocytes and Evades Neutralizing Antibodies in Human Synovial Fluid

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Gene therapy holds great promise for the treatment of arthritis by delivering therapeutic genes directly to the joints. Our laboratory has developed adeno-associated virus (AAV)-based gene delivery technologies and completed a Phase I clinical trial in which AAV encoding the interleukin-1 receptor antagonist (IL-1Ra) is injected into the knee joints of nine osteoarthritis (OA) patients (ClinicalTrials.gov Identifier: NCT02790723).[1] While the trial has successfully confirmed safety and identified a clinically useful dose, it has also revealed the production of high titers

of neutralizing antibodies (Nab) to the vector capsid; moreover, one patient had pre-existing Nab of moderate titer.1 Nabs would present a barrier to the successful repeated dosing of patients. Extracellular vesicles (EVs) are lipid-bound vesicles that mediate intercellular communication via paracrine signaling. EVs can also serve as natural carriers for AAV delivery, forming EV-AAV complexes that have demonstrated enhanced transduction efficiency, reduced immunogenicity, prolonged transgene expression, and most importantly the ability to evade neutralization of antibody (Nab), which is a major limitation for repeated AAV dosing.[2] This study explores the potential of EV-AAV complexes as a strategy to overcome NAb-mediated immune responses while enhancing transduction efficiency for intra-articular gene delivery. Naked AAV2 or EV-AAV2 carrying GFP or luciferase-encoding genes were prepared according to previously described methods[3] and characterized using TEM. HEK293 cells and human chondrocytes were transduced with naked AAV2-GFP, EV-AAV2-GFP, naked AAV2-Luciferase, and EV-AAV2-Luciferase. GFP expression was visualized and quantified with a fluorescence reader, while luciferase expression was measured using a luciferase assay kit. To assess Nab resistance, synovial fluid from a patient previously treated with AAV was used for Nab testing.[4] HEK293 cells were incubated with serial dilutions of the synovial fluid (1:10,000; 1:50,000; 1:100,000), and transduction efficiency was evaluated by measuring GFP and luciferase signals in the presence of Nab. TEM analysis confirmed the structural integrity of EVs and successful encapsulation of AAV2 within EVs. HEK293 cells transduced with EV-AAV2-GFP and EV-AAV2-Luciferase exhibited significantly higher transduction efficiency compared to naked AAV2. Similarly, human chondrocytes treated with EV-AAV2 demonstrated increased GFP and luciferase expression. Most importantly, EV-AAV2 complexes maintained robust transduction efficiency in HEK293 cells despite the presence of Nab in patient synovial fluid, demonstrating significantly higher GFP and luciferase expression compared to naked AAV2 vectors. Our findings demonstrate the potential of EV-AAV as an improved gene delivery platform compared to conventional AAV vectors. Human chondrocytes transduced with EV-AAV2 exhibited significantly enhanced GFP and luciferase expression compared to naked AAV2, suggesting that EVs facilitate more efficient cellular uptake and gene transfer. Additionally, EV-AAV2 complexes effectively circumvented Nab interference, addressing a critical challenge in AAV-based gene therapy. These findings highlight the potential of EV-AAV complexes in enabling repeated AAV administration. Future studies will focus on intra-articular injection of EV-AAV-luciferase complexes to evaluate their immunogenicity and tissue distribution within the joint.

Acknowledgements: This work was funded in part by a career development award from the ASGCT-Cystic Fibrosis Foundation to Dr. Atasoy-Zeybek. Dr. Evans's research is funded, in part, by the John and Posy Krehbiel Professorship in Orthopedics.

References:

1. Evans CH et al. PMID: 36508307.
2. Witwer KW et al. PMID: 36117545.
3. Molina E et al. PMID: 39559560.
4. Abdul TY et al. PMID: 35260797.

2016 Development and Scale-up of a Novel Adenovirus Production Process

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Virus-like particles (VLPs) are non-replicating therapeutic vectors devoid of viral genes. VLPS are created by transfection of plasmid DNA containing a therapeutic-related expression cassette (eg transgene, gene editor, etc; flanked by ITRs and an adenovirus packaging signal) into an engineered production cell line. DNA transfection is followed by infection of the production cell line with a "helper" virus (HV) to co-locate both genomes in the nucleus. The HV provides adenovirus genes in trans to replicate the VLP genome and create capsid particles. The production cell line then precisely edits the helper viral genome to remove the packaging signal in a highly efficient process (Figure 1). With no viral genes required, the entire VLP genome capacity – 35 kb – is available for therapeutic designs that are more than 7x AAV and 4x Lenti viral or LNP capacities.

VLP production in an academic setting utilized an adherent engineered production cell line with minimal process optimization. Ensoma's process development strategy focused on adaptation of this adherent cell line to serum-free, suspension cell culture. Suspension processing is ideal to achieve cell culture consistency and ability to scale the process. The adherent cell line was adapted by stepwise removal of serum into chemically defined media followed by adaptation to suspension culture. The adapted suspension cell line was compared to the adherent cell line to ensure product quality was not altered in the VLP product. With a suspension cell line in hand, the next stage of development focused on bioreactor parameter screening at small scale in both AMBR15 and 2L bioreactors. In bioreactors, VLPs are produced by co-infection of the production cell line with existing "helper" and VLP viral stocks. A DOE strategy in AMBR15 and 2L bioreactors identified key parameters to further optimize in subsequent experiments. The finalized small scale process was scaled to 20L bioreactors internally for process confirmation and then transferred to a CDMO for a clinical scale production.

At the clinical scale, cell expansion operations required changes that differed from small scale, including cell culture expansion through a rocker reactor. The first clinical scale production saw an increase in host cell protein (HCP) impurities not observed at small scale. Experiments were performed that focused on a deeper understanding of key cell culture parameters to reduce HCPs. When these parameters were implemented in subsequent clinical scale batches, final HCPs impurities values fell back into an acceptable range.

In summary, VLP process development utilized a DOE strategy to quickly develop a clinical scale production process. This strategy identified cell expansion and production parameters that demonstrated linear scalability from AMBR15 to 20L to clinical scales, which should allow for consistent scaling beyond the clinical scale. The timeline from successfully adapted production cell line to a clinical scale batch was <6 months and was aided by both DOE and QbD principles.

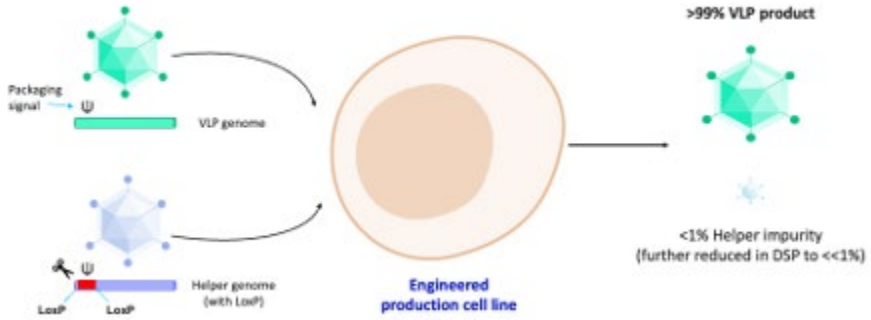

Figure 1. Schematic of VLP production, including precise VLP packaging

2017 FcεRIγ-Negative Natural Killer (g-NK) Cells Potentiate Cetuximab in Treating EGFR-Positive Head and Neck Cancer SCC-4 Lung Metastases Mouse Xenografts

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Purpose: FcεRIγ-negative natural killer (g-NK) cells are a subset of naturally occurring NK cells that lack the FcεRIγ adapter protein through epigenetic reprogramming resulting in a multifold increase in antibody-dependent effector function (ADCC) as compared to conventional NK cells. Allogeneic g-NK cell therapy, is currently in early-phase clinical trials for the treatment of hematological malignancies and autoimmune disease alone and in combination with therapeutic monoclonal antibodies. Cetuximab is an anti-Epidermal Growth Factor Receptor (EGFR) monoclonal antibody with a primary mechanism of action (MOA) mediated through competitive inhibition of EGFR phosphorylation and downstream signaling. Cetuximab contains an IgG1 Fc domain and, therefore, can bind CD16 and facilitate ADCC of EGFR+ tumor cells. The present study aimed to evaluate the efficacy of g-NK cells alone or in combination with the anti-EGFR monoclonal antibody cetuximab in a preclinical mouse model of EGFR-positive head and neck squamous cell carcinoma (HNSCC).

Methods: Immune-deficient NSG mice were intravenously inoculated with 5×10^5 SCC-4-luc tumor cells to model HNSCC lung metastasis. Mice were randomized into cohorts receiving weekly treatments for six doses of vehicle, cetuximab (60 μg/mouse, IP), cryopreserved g-NK cells (1x107/mouse, IV), or cetuximab + g-NK cells of same dose. IL-15 (2 μg/mouse, IP) was administered every 3 days to support NK cell engraftment and persistence. Tumor progression was monitored via bioluminescent imaging (BLI).

Results: Single agent cetuximab or g-NK cells significantly delayed the tumor progression in the mouse lung compared to control mice on day 45 post therapy. However, combination of g-NK cells and cetuximab elicited the greatest reduction in tumor burden as compared to controls, evidenced by significantly lower BLI signals in the lung over 6 weeks of therapy. Combination treatment led to complete elimination of tumor burden in lung in 4 of 7 mice during day 8-40 post therapy while all 7 control mice developed lung metastases. The antibody-dependent cytotoxicity mediated by activated g-NK cells is involved in the enhanced tumor cytotoxicity mechanism.

Conclusion: FcεRIγ-negative, g-NK cells and cetuximab combination therapy showed potential in treating preclinical solid tumor HNSCC metastases. These data demonstrate the potential complimentary MOAs of antibody-based competitive inhibitors and cell-based ADCC to effectively target solid tumors. g-NK cells are currently being evaluated in hematologic-based diseases and these data suggest their potential therapeutic application in solid tumors when combined with monoclonal antibodies.

2018 Paeoniflorin Combined with Neural Stem Cell Transplantation for Parkinson's Disease: Synergistic Effects of Cell Therapy and Inflammation Regulation

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Objective: To investigate the feasibility and efficacy of paeoniflorin (PF) combined with neural stem cell (NSC) transplantation in treating Parkinson's disease (PD).

Background: Parkinson's disease is a common chronic progressive neurodegenerative disorder characterized by irreversible loss of dopaminergic neurons in the substantia nigra. Current pharmacological and surgical interventions alleviate symptoms but fail to halt disease progression. NSC transplantation, which replaces lost dopaminergic neurons, represents a potential curative approach. However, challenges such as low cell survival rates, impaired differentiation, and maturation due to transplantation-related inflammation and inflammatory microenvironments remain unresolved. Paeoniflorin, an active compound from traditional Chinese medicine, exhibits anti-inflammatory, antioxidant, and neuroprotective properties. However, whether it can regulate transplantation-related inflammation and synergize with neural stem cell transplantation to treat Parkinson's disease remains unclear.

Methods: NSCs were isolated from the midbrain of embryonic day 14 Sprague-Dawley rats, and their purity was confirmed via immunofluorescence. Differentiation medium was used to induce maturation into midbrain dopaminergic neurons (mDA). CCK-8 assays and Calcein-AM/PI double staining evaluated PF's effects on mDA and microglial cell viability. An in vitro inflammatory model was established using lipopolysaccharide (LPS)-stimulated microglia/mDA co-culture systems. Pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were quantified via ELISA, while inflammatory pathway proteins were analyzed by Western blot (WB). A PD rat model was generated via stereotaxic 6-hydroxydopamine (6-OHDA) injection into the substantia nigra, validated by apomorphine-induced unilateral rotations (>7 rotations/minute). mDA cells with or without PF were transplanted into the striatum, followed by tyrosine hydroxylase (TH) immunofluorescence and WB to assess dopaminergic neuron survival and inflammatory protein expression.

Results: Primary NSCs formed high-purity undifferentiated neurospheres (Nestin-positive) and differentiated into mature dopaminergic neurons (TH-positive). PF pretreatment showed no toxicity to NSCs or microglia but protected mDA from LPS-induced inflammatory damage and enhanced functional maturation. In co-culture models, PF significantly reduced IL-1β, IL-6, and TNF-α levels while suppressing TLR4/MYD88/NF-κB signaling and NLRP3 inflammasome activation. In PD rats, 6-OHDA-induced TH+ neuron loss and apomorphine-triggered rotations

confirmed model validity. The PF + mDA transplantation group exhibited significantly higher striatal TH+ neuron survival rates. PF effectively inhibited transplantation-associated inflammation and promoted mDA survival, differentiation, and maturation.

Conclusion: Paeoniflorin mitigates inflammatory damage to transplanted cells, enhances survival and differentiation, and synergizes with NSC therapy to improve outcomes in PD. Compared to mDA transplantation alone, PF combination therapy significantly elevates cell survival and differentiation, offering a novel strategy for cell-based PD treatment.

2019 Precision RNA Delivery via Bispecific Antibody-Targeted Lipid Nanoparticles

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Lipid nanoparticles (LNP) are the most clinically advanced non-viral gene delivery system. While progress has been made for enhancing delivery, cell-specific targeting remains a challenge. Targeting moieties such as antibodies can be chemically-conjugated to LNPs however, this approach is complex and challenging for scale up. We developed an approach to generate antibody-conjugated LNPs that utilizes a bispecific antibody (bsAb) as the targeting bridge. As a dock for the bsAb, we add a short epitope (HA) to the LNP surface (LNPHA). We generated bsAb in which one domain binds HA and the other binds different cell surface proteins, including PD-L1, CD4, CD5, DEC205 and SunTag. Non-chemical-conjugation of the bsAb and LNP resulted in a major increase in efficiency and specificity of target cell transfection. LNP/bsAb mediated transfection of 90% of quiescent primary human T cells ex vivo, and in vivo, mediated 4-fold increase in targeting of PD-L1-positive cancer cells, and >90% transfection of germinal center B cells. Additionally, we created a universal bsAb recognizing HA and anti-rat IgG2, enabling LNP tethering to off-the-shelf antibodies such as CD4, CD8, CD20, CD45, and CD3. By utilizing a molecular dock and bsAb, these studies demonstrate a straightforward and effective strategy to generate antibody-conjugated LNPs, enabling precise and efficient mRNA delivery.

2020 Next-Generation Hemogenetic Tools for Brain-Wide Functional Imaging of Cells and Circuits to Advance Neurotherapeutics

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Precise modulation and comprehensive imaging of specific neural populations are critical for understanding brain function and developing targeted treatments for neurological disorders. Existing neuromodulation methods, such as ultrasound, provide broad coverage but lack cellular-level specificity, whereas traditional functional imaging methods often lack the spatial and temporal resolution to investigate complex neural circuits. To overcome these limitations, we previously introduced a family of genetic probes, called NOSTICs, which allow noninvasive monitoring of cellular-level activity in intact living animals using functional magnetic resonance imaging (fMRI). NOSTICs are engineered enzymes that transduce the cytosolic calcium dynamics of the probe-expressing cells into localized hemodynamic responses. Our initial studies establish NOSTICs as a tool for studying information flow in neural circuitry during rewarding or sensory stimulation, in an approach we refer to as hemogenetic imaging. Here, we describe the development of next-generation NOSTICs with significantly improved sensitivity, specificity, and versatility for targeting precise cellular populations and neural circuits. Our advancements based on three major innovations: First, we developed a high-throughput engineering method, using artificial intelligence-based protein structure prediction models to optimize probe sensitivity while minimizing endogenous background signals. Second, we established a diverse set of viral vectors suitable for delivering and controlling NOSTIC genes, including Cre-dependent constructs, vectors co-expressing NOSTICs and optical reporters, and compact adeno-associated vectors

(AAVs) that leverage cutting-edge viral technology for delivery and circuit labeling. Lastly, we created and validated novel drug-induced, activatable hemogenetic probes, termed diaNOSTICs, which can be switched on by small molecules to reveal expression profiles or facilitate probe multiplexing. Collectively, our novel NOSTIC probes represent a transformative advance in hemogenetic imaging, enabling comprehensive, noninvasive functional analysis of neural circuits at cellular resolution. These groundbreaking tools will significantly enhance our ability to decode complex neural networks, advancing neuroscience research and facilitating novel therapeutic strategies for neurological disorders.

2021 Unveiling the Molecular Mechanism of Polydatin in Ameliorating Parkinson's Disease via the DRD2/CRYAB/NF-κB Axis in Astrocytes and Exploring Cell Therapy Strategies

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Objective: To elucidate the mechanism by which polydatin alleviates Parkinson's disease (PD) through modulating astrocyte-mediated neuroinflammation and to validate a synergistic cell transplantation therapeutic strategy based on this mechanism.

Background: As the second most common neurodegenerative disorder, PD is pathologically characterized by progressive loss of dopaminergic neurons in the substantia nigra, with current therapies lacking disease-modifying efficacy. Aberrant astrocyte activation and subsequent neuroinflammation drive PD progression. Although polydatin, a natural bioactive compound, exhibits neuroprotective potential, its precise molecular mechanisms in regulating astrocytic inflammation and therapeutic applications remain undefined.

Methods: Network pharmacology utilizing multi-source databases (CTD/TargetNet/SwissTargetPrediction/ChEMBL) identified polydatin targets. PD-related targets were screened through differential gene analysis (DESeq2) and weighted gene co-expression network analysis (WGCNA) of the PD transcriptome dataset GSE7621 (9 controls vs. 17 PD patients). Molecular docking (AutoDock Vina) confirmed target interactions. Integration of substantia nigra single-cell sequencing data (GSE184950) via Seurat clustering and cell-type enrichment analysis localized DRD2 specificity to astrocytes. In vitro, lentivirus-mediated DRD2-knockout primary astrocytes were subjected to RNA-seq and KEGG pathway analysis post-polydatin treatment. Astrocyte-specific DRD2 conditional knockout mice were established and MPTP-induced PD models were analyzed using Western blot, ELISA, and immunofluorescence to assess CRYAB/NF-κB regulation, cytokine suppression, and dopaminergic neuroprotection. Innovatively, DRD2-edited embryonic stem cell-derived dopaminergic progenitors (TH+/FOXA2+) were transplanted into AAV-GFAP-Cre-mediated astrocyte-specific DRD2-knockout PD rats for systematic evaluation of graft viability, maturation, and inflammatory microenvironment modulation.

Results: Network pharmacology identified 404 polydatin targets, with DRD2 exhibiting the strongest binding affinity (-7.61 kcal/mol) among PD-related targets. Single-cell analysis revealed DRD2 enrichment in activated astrocytes (GFAP+/S100β+) from PD patients ($p<0.01$). Mechanistically, polydatin suppressed NF-κB phosphorylation and upregulated CRYAB via DRD2 activation, effects abolished upon DRD2 knockout. In vivo, polydatin increased dopaminergic neuron survival by 55% (TH+ cell count, $p<0.01$) and reduced IL-1β/IL-6/TNF-α levels by 70% ($p<0.001$) in PD mice. Co-transplantation with polydatin enhanced graft survival 2.2-fold ($p<0.01$), concomitant with attenuated astrogliosis and microglial infiltration, confirming dual therapeutic efficacy through CRYAB/NF-κB inhibition.

Conclusion: This study pioneers the demonstration that polydatin exerts neuroprotection in PD via the astrocytic DRD2/CRYAB/NF-κB signaling axis. The innovative dual-modality strategy combining cell transplantation with polydatin intervention provides critical theoretical foundations for developing disease-modifying therapies for PD.

2022 IMANs: A Transformative Solution for Overcoming Solid Tumour Immunotherapy Challenges and Rejuvenating Innate Immunity, with Broad Implications for Oncology, Longevity, Autoimmunity, and Neuroinflammation

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Immunotherapies for cancer treatment have predominantly focused on fixed-targets and lymphocytes such as autologous αβ T cells, including CAR-T cell therapies. While these approaches have shown promise, their success in solid tumours remains limited due to challenges such as antigen heterogeneity, physical barriers, and an immunosuppressive tumour microenvironment (TME). Effective immunotherapy for solid tumours requires both adaptive and innate immune competence to be restored for active remodelling of the immunosuppressive TME and direct tumour cell killing to drive sustained anti-tumour immune responses.

At LIfT Biosciences, we have developed Immunomodulatory Alpha Neutrophils (IMANs), a novel allogeneic immuno-cell therapy derived from CD34+ hematopoietic stem cells (with an iPSC-derived version also in development). Using a proprietary GMP-compliant manufacturing process, IMANs are uniquely differentiated along a newly described neutrophil lineage whose presence in man has been clinically linked to tumour clearance. Through active remodelling of the TME and direct cytotoxic activity, IMANs provide durable anti-tumour immunity and restore immunocompetence in cancer patients. These off-the-shelf innate immune cells offer a cost-effective, allogeneic approach for treating multiple solid tumour types.

IMANs demonstrate a dual mechanism of action, combining immune modulation with direct cytotoxicity. In humanised NCG mice bearing A549 alveolar adenocarcinoma tumours, IMANs successfully infiltrated the tumour site following IV administration. Within days, IMANs were detected in key immunologically relevant sites, including the lungs, spleen, liver, and brain. IMAN infiltration was still observed in tumours at the end of the study three weeks post-administration, which is consistent with data showing that IMANs persist for four weeks in vitro. Intratumoral IMAN infiltration was rapidly followed by mass T-cell infiltration in this model. Importantly, histopathological analysis revealed no safety concerns.

Advanced in vitro models, such as PDX-O and tumour-on-a-chip, further support the dual modality of IMANs. PDX-O models demonstrated antigen-independent cytotoxicity across various solid tumour types. Tumour-on-a-chip data showed that IMANs infiltrated tumours and recruited other immune cells, leading to enhanced proliferation of cytotoxic lymphocytes. Co-culture experiments with allogeneic PBMCs showed upregulation of key activation markers, including OX40, 4-1BB, CD25, and CD69, as well as increased secretion of TME-modulating factors like CXCL10 and IFN-γ.

IMANs have also been uniquely differentiated to express an Fc receptor profile, enhancing their ability to target and directly kill tumour cells. These data suggest that IMANs can reshape the TME as a monotherapy, with potential for combination use with other immunotherapies, such as checkpoint inhibitors, vaccines, and bispecific antibodies. IMANs represent an exciting new approach for overcoming the challenges of solid tumour immunotherapy and could offer significant benefits in the treatment of autoimmune disorders, neuroinflammation, antimicrobial resistance and longevity.

2023 SKG1108: Optogenetic Gene Therapy with AAV-BWLP for Visual Restoration in Retinitis Pigmentosa via a Single Intravitreal Injection

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Retinitis Pigmentosa (RP) is a hereditary retinal dystrophy caused by mutations in over 60 genes, leading to photoreceptor degeneration, night blindness, and legal blindness eventually. Current treatments aim to alleviate symptoms but do not halt disease progression, highlighting a desperately unmet medical need. Optogenetics, utilizing light-sensing proteins to restore light perception and acuity, offers a gene-agnostic treatment approach, benefiting a broader patient population.

SKG1108* is an optogenetic AAV gene therapy featuring a novel capsid and an engineered broad wave-length light-sensing protein (BWLP) comprising opsins from different sources, which is aimed at improving the vision of RP patients with photoreceptor degeneration. The BWLP displayed strong responses to light stimulation in in vitro electrophysiological studies. SKG1108 vector also exhibited superior efficacy and safety in multiple functional tests in rd1 mice via a single intravitreal injection, including visual pathway electrophysiological recordings, behavior tests, and biodistribution analysis.

SKG1108 significantly improved visual functions, mediated visually evoked potential (VEP) signals in the visual cortex, and exhibited an excellent safety profile in rd1 mice. Data from multi-electrode array (MEA) analysis in retinal slices and excitatory post-synaptic current (EPSC) recording in lateral geniculate nucleus (LGN) neurons of brain slices indicated that SKG1108 effectively mediated optogenetic activity in retinal neurons (Figure 1) with substantial projections to the LGN, which was confirmed by immunofluorescence staining results in the AAV-treated mouse retina and brain. Dose-range finding (DRF) study revealed dose-dependent visual improvements through electroretinogram (ERG) recording, with significant enhancements in visual perception (assessed by dark-light box test, DLT) and acuity (measured by optomotor response, OMR) at doses ≥8E8 vg/eye. Long-term studies revealed sustained improvements in vision for up to 26 weeks, supported by increased VEP amplitudes (Figure 2) and behavioral improvement in treated rd1 mice.

In conclusion, by mediating the expression of never-before-used light-sensing protein, SKG1108 represents a promising optogenetic gene therapy, which has a great therapeutic penitential to offer long-lasting visual improvements for RP patients.

* SKG1108 has received orphan drug designation from the US Food and Drug Administration (FDA), and SKG1108 is currently planning for GLP studies of toxicology and biodistribution in NHPs.

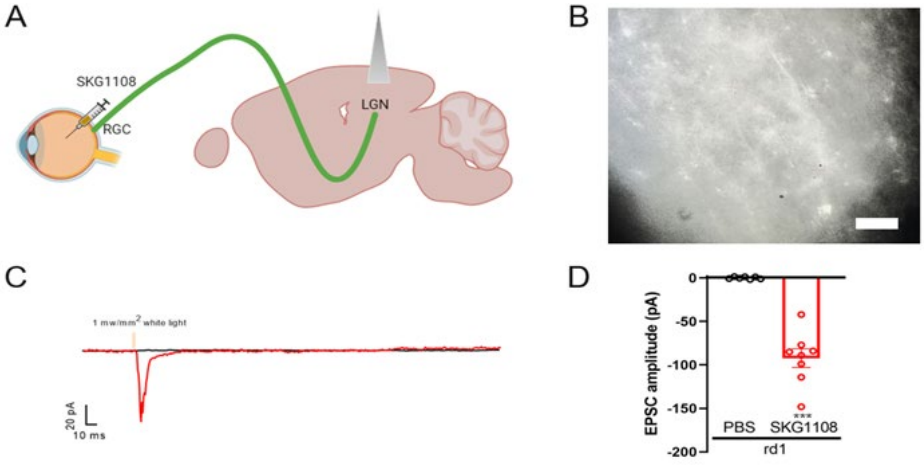

Figure 1: Light-induced EPSCs in LGN neurons after SKG1108 IVT treatment. SKG1108 (1E10 vg/eye) was intravitreally delivered into rd1 mouse eyes. After eight weeks, whole cell recordings of LGN neurons were performed on acute brain slices. (A) Schematic diagram of whole cell recordings in LGN neurons. (B) LGN region with observed dTomato fluorescent signals from SKG1108-treated rd1 mouse brain. (C) Representative EPSC traces of LGN neurons from untreated (black) or SKG1108-treated (red) brain slice preparations elicited by light stimulation (5 ms). Scale bars, 20 pA and 10 ms. (D) Analysis of EPSC amplitude from recorded LGN neurons. ***$p<0.001$, compared to PBS (rd1) group. Data are shown as mean ± SEM. Statistical analysis was performed using a two-tailed, unpaired Student's t test.

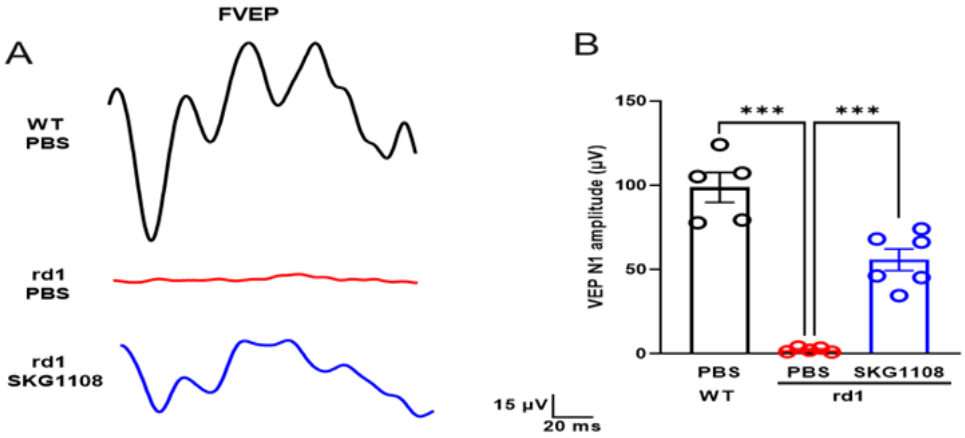

Figure 2: SKG1108 treatment produced VEP responses in rd1 mice. SKG1108 (4E9 vg/eye) was intravitreally delivered into rd1 mouse eyes. Electrodes were implanted into the visual cortex and VEP signals were recorded at least one week after the surgery. (A) Representative flash VEP waveforms under the light intensity of 3 cd·s/m2. Scale bars, 15 μV and 20 ms. (B) The analysis of VEP N1 amplitudes from different groups. ***p<0.001, compared to PBS (rd1) group. Data are shown as mean ± SEM. Statistical analysis was performed using one-way ANOVA with Turkey's post-hoc test.

2024 Identification of a heart tropism AAV novel capsid enabling effective systemic heart transduction while liver de-targeting in non-human primates

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In our previous study, we have developed a platform for AAV capsid directed evolution in development of tissue specific viral vector. By 2 rounds screening in non-human primates (NHP), 150 novel capsid candidates were selected for single serotype validation in a pooled experiment in 2 NHPs. ID-31, which from a DNA shuffling library, is one of the top candidates which showed high heart tropism and avoid liver distribution by the same time. The mRNA transcription level of ID-31 in heart is 3.3-fold increasing, while in liver represented 22.7-fold decreasing of wtAAV9 in this pooled study, which indicated that a superior heart-to-liver ratio of 75-fold increasing than AAV9. Encourage by this data, a head-to-head experiment of ID-31 and AAV9 with same dose in NHP has been studied. The doses of each AAV vector are 2E12 vg/kg and 1.5~2E13 vg/kg in different groups of NHPs. As a result, in the dose of 1.5~2E13 vg/kg, ID-31 represents 9-fold increasing of mRNA transcription in heart vs. AAV9, while only 5% mRNA transcription level of AAV9 in liver. The DNA distributions of ID-31 and AAV9 also kept consistency with mRNA results (Fig. 1). Therefore, ID-31 showed an extremely high heart-to-liver ratio in NHP in mRNA transcription level as 180-fold which indicates a potential vector for heart specific delivery. Moreover, the GOI expression in heart and liver were also analyzed by IHC staining. The result clearly showed that ID-31 gave more GOI expression in heart compared with AAV9 in a dose range of 1.5-2E13 vg/kg. After quantification by software or by pathologist, around 30-50% cardiomyocytes were infected by ID-31 but only 10-20% were infected by AAV9. In contrast, ID-31 represented negative staining in liver while AAV9 showed over 95% hepatocyte infection (Fig. 2).

In conclusion, our data clearly showed that ID-31 behaved super high heart transduction while liver de-targeting as well in NHPs. The high heart-to-liver ratio of mRNA in NHP make it one of the best-in-class heart tropism AAV novel capsid to date. The mechanism of this novel capsid will be studied later on.

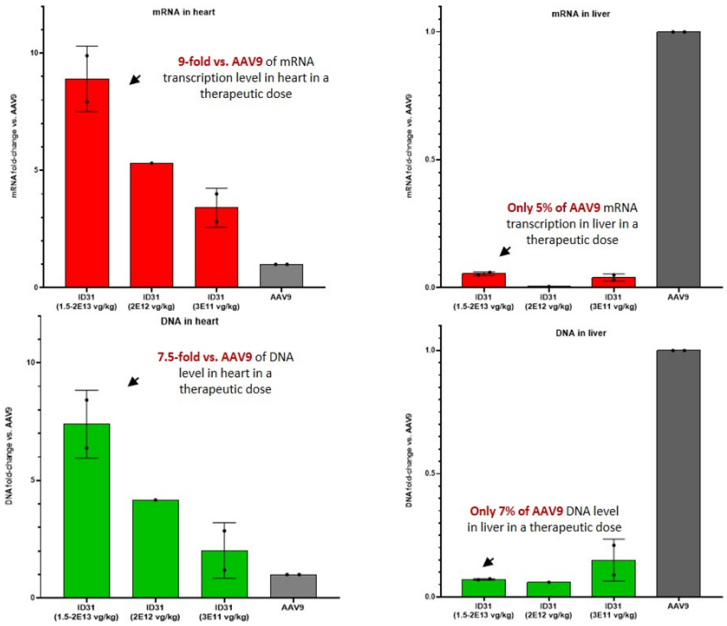

Figure 1. The mRNA and DNA level of ID-31 in compare with AAV9. Head-to-head experiment of ID-31 and AAV9 in doses of 1.5-2E13 and 2E12 vg/kg, mRNA of GOI was normalized to GAPDH first, followed by calculation of ID-31/AAV9 ratio. Pooled experiment in dose of 3E11 vg/kg compare with AAV9, NGS for barcode was monitored.

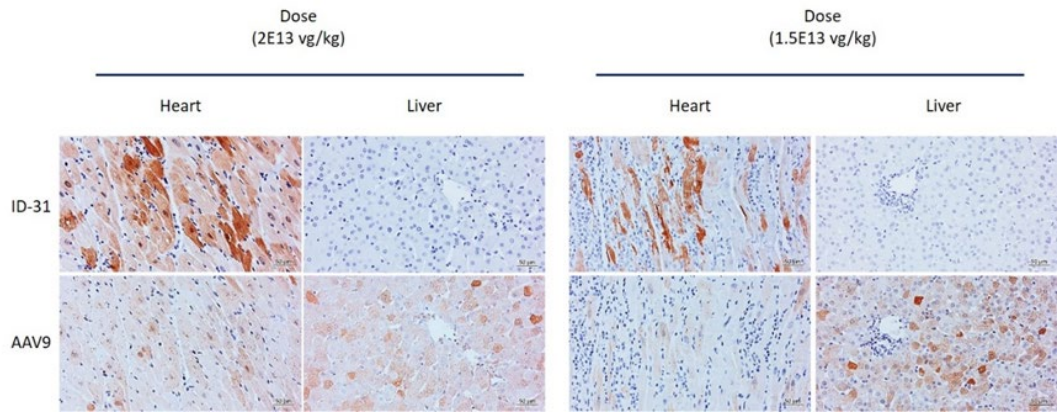

Figure 2. GOI expression (IHC) test of ID-31 vs. AAV9 in heart and liver in NHPs.

2025 An Allele-Selective CRISPR Correction of Huntington's Disease via Novel Delivery

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Huntington's Disease (HD) is a heritable neurodegenerative disorder characterized by neuronal loss and cognitive deficits. HD is caused by a gain of function mutation that produces a polyglutamine expansion in exon 1 of the HTT gene. This expansion encodes a mutant protein that is cleaved into toxic N-terminal fragments that aggregate in the nucleus, promoting apoptosis. Recent clinical trials have failed due to non-selective reduction of both mutant and wild type HTT (i.e. GENERATION HD1). Prior studies have found that wild type HTT is essential in the brain, making a mutant allele selective approach ideal for treatment.

CRISPR-based strategies have selectively targeted the mutant allele via disease-linked single nucleotide polymorphisms (SNPs) that create a protospacer adjacent motif (PAM) required for Cas9 cleavage. When paired with a nonselective cut, this strategy produces mutant allele selective excisions. One study using this approach reduced mutant HTT (mHTT) expression by up to 60% in vivo. We have further advanced this strategy, identifying a novel pair of target sites that significantly improves excision efficiency. The method is compatible with an aggressive murine model of HD (R6/2) as well as HD patient-derived cells.

Editing using CRISPR ribonucleoprotein (RNP) enzymes in human iPSCs homozygous for several candidate SNPs identified a promising approach with an editing efficiency over 90%, which compares favorably to the ~50% observed in a prior study. We are currently performing CHANGE-seq to evaluate the off-target profile of our guide RNAs. Work is underway to engineer this line to contain the NGN2 cassette, facilitating uniform and rapid neuronal differentiation. In R6/2-derived neural cells, mHTT expression was reduced by up to 60%, with efficient excisions observed when genomic DNA was analyzed.

Based on promising results obtained via non-viral RNP delivery in vivo, and the clinical precedent of AAV therapies, the same editing strategy is being co-developed and evaluated in parallel using two platforms: engineered RNPs and next-generation AAV. This study establishes a ground-breaking, versatile method of treating HD with substantial promise for future clinical trials.

2026 First in human Clinical Trial of AAV-Based Gene Therapy for RDH12-Associated Retinopathy: Safety, Tolerability, and Preliminary Efficacy

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Background: RDH12-associated retinopathy is a severe inherited retinal disorder caused by mutations in the RDH12 gene, leading to progressive vision loss and blindness. Currently, there are no approved treatments for this condition. Since the retina is severely degenerated, subretinal injection for patients with RDH12 mutation might be challenging. The IVB107, AAV.IVT18-RDH12 was developed, based on a new developed intravitreal injection vector to treat RDH12-associated retinopathy. This First in human trial of IVB107 evaluates the safety, tolerability, and preliminary efficacy of an adeno-associated virus (AAV)-mediated gene therapy designed to deliver a functional copy of the RDH12 gene to retinal cells.

Methods: This is an open-label, dose-escalation study enrolled two dose cohorts with 5 patients in each group. Participants received a single intravitreal injection of the AAV-RDH12 vector in one eye. Primary endpoints included safety and tolerability, assessed by the incidence and severity of adverse events (AEs) over 12 months. Secondary endpoints included changes in visual function (best-corrected visual acuity (BCVA), low luminescence visual acuity(LLVA), and full field stimulus test(FST)) and quality of life measures.

Results: Until end of February, 10 patients were enrolled and 6 patients finished 8-week visit. Preliminary data from the trial demonstrated that the AAV-RDH12 gene therapy was well-tolerated, with no serious AEs related to the treatment. Mild to moderate ocular AEs, such as transient inflammation and eye pressure increase, were observed and resolved with standard management. At 8-week post injection, BCVA increase was observed in all study eye, with an average of 8.7 letters, ranging from 5-23 letters. LLVA was evaluated in 5 patients, with an average increase of 11.6 letters, ranging from 0-33 letters. FST and quality of life measures were planned to be evaluated at 12-week visit.

Conclusion: IVB107, AAV.IVT18-RDH12 is safe and potentially efficaious in patients with RDH12-associated retinopahty.

2027 TurboPRANCE: A Fully Autonomous, High-Throughput Platform for Multi-Class Continuous Evolution of Therapeutic Biomolecules

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Directed evolution is a powerful tool for engineering therapeutic biomolecules, but existing methods remain labor-intensive, inflexible, and low-throughput. Phage-Assisted Continuous Evolution (PACE) has been used to evolve Cas9, base editors, and polymerases, yet it frequently fails, requires constant oversight, and lacks real-time optimization. Phage-Assisted Non-Continuous Evolution (PANCE) has been widely attempted but is constrained to one evolution cycle per day, leading to metabolite depletion and mutagenesis breakdown, making it unsuitable for complex biomolecules.

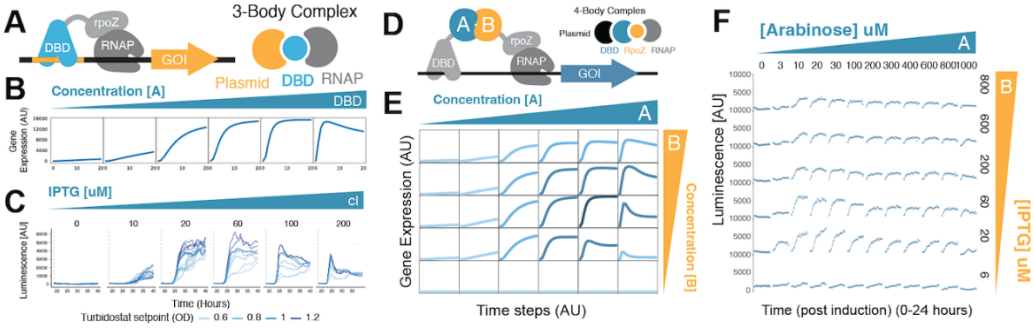
Previously, we developed PRANCE (Phage and Robotics-Assisted Near-Continuous Evolution), improving reliability and selection flexibility. However, PRANCE was limited to evolving many variants of a single biomolecule per experiment (e.g., 100 polymerases at a time). Here, we introduce TurboPRANCE, the first fully automated, multi-class parallel continuous evolution system, capable of 100+ simultaneous evolution experiments across distinct biomolecule classes (e.g., Cas9, proteases, and PPIs).

Methods: TurboPRANCE integrates on-deck culturing, allowing for fully continuous strain maintenance without manual intervention. Unlike standard liquid-handling robots constrained to batch processing, TurboPRANCE leverages our open-source automation framework, enabling asynchronous, fully parallel execution of continuous evolution experiments on a Hamilton STARlet platform with real-time evolution tracking. The system dynamically optimizes bacterial strain allocation, selection stringency, and evolutionary conditions, maintaining stable long-term experiments. Critically, the system is a fully hands-off, end-to-end system, continuously queuing and executing experiments without shutdown, enabling round-the-clock evolution with no downtime.

Results: Using TurboPRANCE, we have uncovered previously unrecognized challenges in continuous evolution, particularly in DNA-binding domain (DBD) and protein-protein interaction (PPI) evolution. Prior methods failed to account for gene expression dynamics, kinetic effects of binding, and competitive inhibition in non-equilibrium selection environments. These overlooked factors can cause evolutionary trajectories to plateau prematurely or, in some cases, even reduce biomolecule activity rather than improve it. We have revealed that past CRISPR and PPI evolution experiments likely stalled due to unintended competitive interactions or kinetic bottlenecks, meaning previous efforts may have unknowingly selected for suboptimal variants. These findings suggest that previously "failed" or plateaued directed evolution campaigns could be revisited and optimized with precisely tuned selection conditions.

Conclusion: TurboPRANCE is a transformative advance in continuous evolution, enabling massively parallel, multi-class evolution at an unprecedented scale. By integrating real-time adaptive selection, autonomous strain management, and high-throughput parallelization, TurboPRANCE enables fully autonomous, uninterrupted evolution with no downtime. Built on PyHamilton, an open-source automation framework, TurboPRANCE is fully accessible and reproducible, ensuring rapid adoption. For gene therapy companies reliant on PACE-evolved biomolecules, TurboPRANCE represents the next leap—unlocking the evolution of more complex gene-editing

circuits, optimizing previously intractable biomolecules, and overcoming hidden kinetic barriers that may have limited past efforts.



TurboPRANCE reveals unrecognized kinetic limitations in biomolecule evolution. (A) Schematic of a 3-body complex (e.g., Cas9 direct fusions), where DNA-binding domain (DBD), RNA polymerase (RNAP), and gene of interest (GOI) interact. These systems exhibit hook effects, requiring multi-dose optimization. **(B)** Simulated ODE solutions showing 3-body binding dynamics approaching equilibrium. **(C)**Experimental data for a direct fusion system at varying IPTG concentrations, confirming predicted non-monotonic responses due to hook effects. **(D)**Schematic of a 4-body complex (e.g., protein-protein interactions), previously believed to behave as a 3-body system, requiring only single-variable optimization. **(E)** Simulated ODE solutions for 4-body systems, revealing emergent kinetic behaviors necessitating multi-variable optimization. **(F)** Experimental luminescence data validating 4-body model predictions. Responses across arabinose and IPTG gradients suggest past evolution efforts may have been constrained by incorrect kinetic assumptions.

2028 Cell-Based Potency Assay for ECUR-506: A Groundbreaking Investigational Therapy for the Treatment of Neonatal Ornithine Transcarbamylase (OTC) Deficiency

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Background: OTC deficiency is a serious, rare genetic disease, and the most common urea cycle disorder. Newborns with neonatal onset OTC deficiency experience symptoms of hyperammonemia shortly after birth, including lethargy, poor suck and vomiting, that if left untreated can quickly escalate to seizures, brain damage, coma and eventual death. In severe cases, liver transplantation is the only known curative option. iECURE, Inc., a clinical stage gene editing company focused on the development of mutation-agnostic *in vivo* gene insertion therapies, recently reported preliminary findings showcasing a complete clinical response as demonstrated by the removal of standard of care ammonia scavenging medicines, followed by absence of hyperammonemic crises and normalization of protein intake in the first infant dosed with its *in vivo* gene editing candidate ECUR-506 in an ongoing phase 1/2 clinical trial in OTC deficiency.

Unlike traditional AAV treatments, ECUR-506 comprises two AAV vectors: ECUR-506A, which expresses ARCUS nuclease to target the PCSK9 locus, and ECUR-506D, which is designed to integrate the functional OTC gene within the genome. In collaboration with iECURE, the Pharmaron team is developing two complementary in-vitro cell-based assays to assess the efficacy of ECUR-506: (1) A functional potency assay to measure OTC enzyme activity and (2) a PCR-based assay to confirm the successful integration of the OTC gene at the PCSK9 locus.

Methods: Huh7 cells were transduced with a combination of ECUR-506A and ECUR-506D at varying MOIs, and the rate of conversion of d7-ornithine to d7-citrulline was quantified using LC-MS/MS for quantitative OTC enzyme activity. For OTC gene integration analysis, genomic DNA was extracted from transduced cells, followed by PCR amplification using PCSK9/OTC-specific primers and agarose gel electrophoresis. Sanger sequencing was performed to confirm successful OTC gene integration into the PCSK9 locus.

Results: A dose-dependent increase in OTC enzyme activity was observed with increasing drug product, indicating functional restoration of OTC activity. PCR analysis yielded single bands exclusively in samples transduced with ECUR-506, indicating successful OTC gene integration into the PCSK9 locus. Sanger sequencing further confirmed the precise integration of the OTC transgene, with consensus sequences matching the reference sequence.

Conclusion: Validating the physiological activity of the drug product via cell-based potency assay is critical in demonstrating the assay is accurate and reliable in its ability to ensure manufactured vector lots are comparable, and the assay is representative of the mechanism of action. Although evaluating gene activity *in vitro* can pose difficulties, managing a drug involving double AAV and integration issues may prove even more complex. The developed assays provide robust methods to assess the functional potency and genomic integration of iECURE's gene insertion therapy for OTC deficiency. We aim to work with iECURE to validate these methods and facilitate BLA submission.

2029 Apernavec: First mRNA Combined Therapy for the Treatment of Secondary Lymphedema

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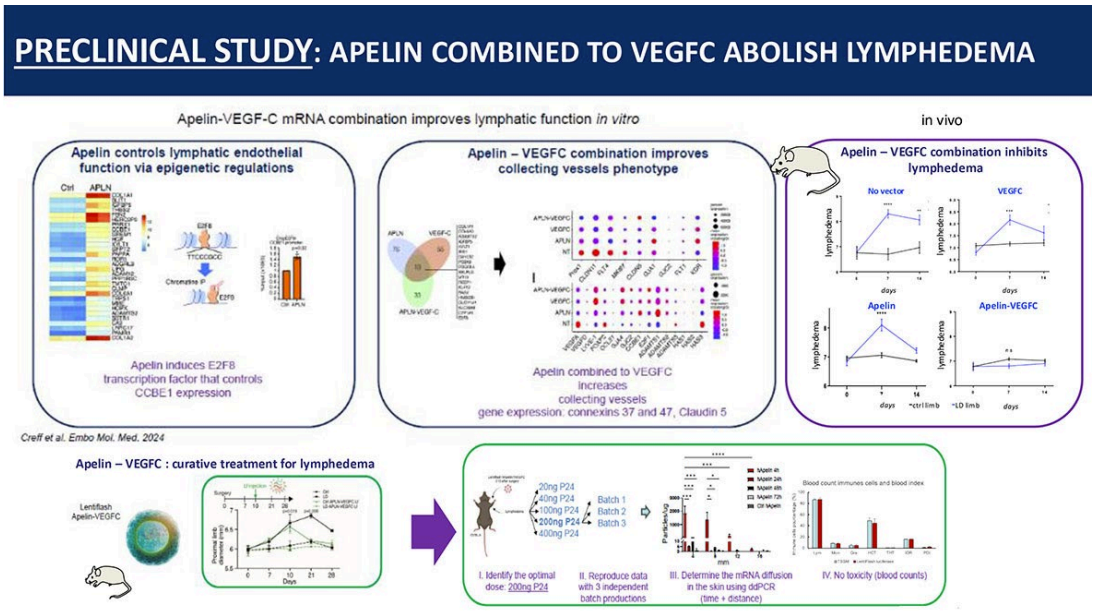
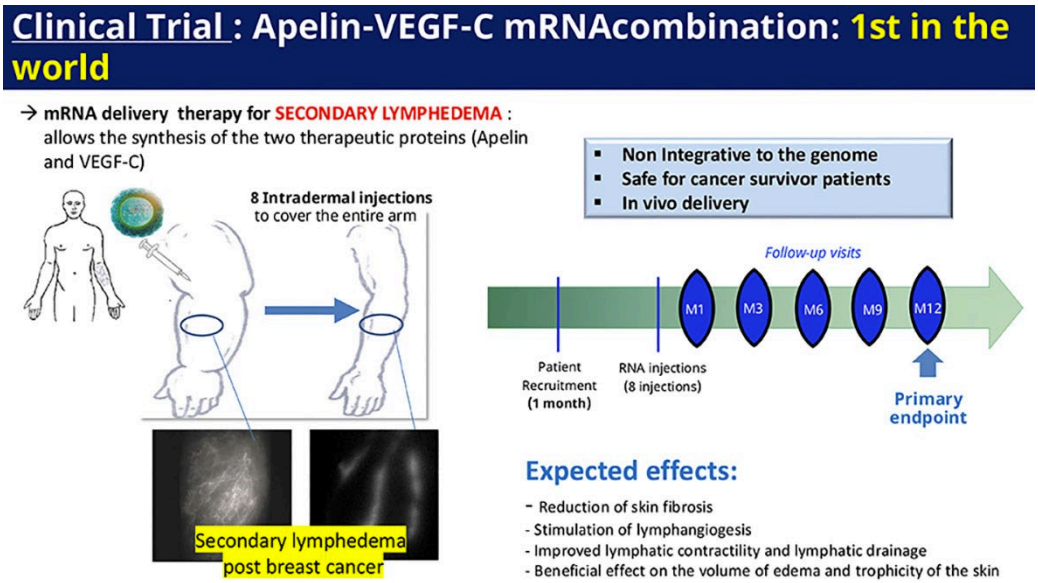
Secondary lymphedema is an unmet medical need that corresponds to a severe lymphatic dysfunction leading to the accumulation of fluid and fibrotic adipose tissue in a limb. In western countries, it develops after cancer treatments, raising an important ethical issue in treating cancer survivor patients without reactivating the tumor with pro-lymphangiogenic therapy. Therefore, we used a biological RNA delivery approach called FlashRNA®, based on a novel class of chimeric lentiviral platform, that allows the delivery of transient multiple biological mRNA molecules.

Recently, VEGF-C, the major lymphangiogenic growth factor, was found to be not sufficient to restore the lymphatic function in lymphedema. As lymphedema is a multifactorial pathology with lymphatic dysfunction, adipose tissue accumulation, and fibrosis, a multiple therapy appears to be the solution to cure this harmful condition.

By performing gene expression analysis of dermolipectomies from women who developed secondary lymphedema after breast cancer, we identified a significant decrease in apelin expression. The effect of the lack of apelin in aggravating lymphedema was confirmed in apelin-KO mice. In a mouse model of lymphedema, apelin improves lymphatic pumping function and reduces tissue fibrosis. In lymphatic endothelial cells, apelin controls the expression of genes involved in extracellular matrix remodeling and valve maintenance.

When combined apelin to VEGF-C, double mRNA delivery abolished lymphedema and restored the lymphatic flow compared to single mRNA delivery. Therefore, we proposed to use the APLN-VEGF-C mRNA delivery vector for a phase I/II gene therapy clinical trial that will be launched in 2025 Toulouse University Hospital.





2030 Tunable Gene Control via RNA Splicing with Clinically Approved Small Molecule

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Precise regulation of gene expression is crucial in gene therapy and gene editing, where uncontrolled or prolonged expression can cause serious side effects like off-target activity and immune responses. Existing inducible systems often require co-expression of regulatory proteins—adding to vector size and potential immunogenicity—or utilize small molecules not approved for clinical use, limiting their utility, especially in adeno-associated virus (AAV)-based therapies.

We have developed a novel small molecule–inducible gene expression system leveraging the clinically approved, orally bioavailable splice modifier risdiplam. Risdiplam modulates splicing of the SMN2 gene by promoting inclusion of exon 7. By incorporating risdiplam-responsive splice sequences into the gene of interest, our system enables precise control of transgene expression solely through endogenous splicing machinery and the presence of risdiplam, eliminating the need for exogenous regulatory proteins.

Through randomized mutagenesis screens and sequence modifications, we optimized the system for robust inducibility and minimal background expression. We demonstrated precise gene expression control over reporter genes across multiple cell lines, confirmed at RNA, protein, and functional levels. In proof-of-concept studies, we successfully integrated our system with Cas9, highlighting its potential for safe and controlled gene editing applications. Finally, in an in vivo study, we showcased the dynamics of the inducible gene expression system, demonstrating its ability to be activated and to remain inactive when risdiplam application is discontinued.

Our inducible system is fully modular and compatible with any promoter, regulatory sequences, and expression cargos, allowing for fine-tuning and tissue-specific expression. Its small genetic footprint makes it ideal for incorporation into AAV vectors. The use of risdiplam, already approved for human use and capable of reaching both peripheral tissues and the brain further enhances its translational potential.

This system provides a versatile and clinically feasible tool for controlled transgene expression without the drawbacks of existing systems. Its application could improve the safety and efficacy of gene therapies and gene editing approaches by offering dose flexibility, reversibility, and a reduced risk of adverse effects.

2031 GNTI-932: A Gut Specific CAR-Engineered T Regulatory Cell Therapy to Treat Inflammatory Bowel Disease.

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Regulatory T cells (Tregs) play a vital role in natural immune regulation and are an appealing strategy to treat autoimmune and inflammatory diseases. Polyclonal Tregs have demonstrated safety, tolerability, and some clinical benefit in trials for refractory inflammatory bowel disease (IBD). However, limited efficacy has been observed, potentially due to challenges in Treg persistence and target specificity. Pre-clinical studies have demonstrated higher potency of antigen-specific Tregs compared to polyclonal Tregs at reducing inflammation. Chimeric antigen receptor (CAR) Treg therapies have emerged as a promising treatment option for IBD.

Here, we report for the first time GNTI-932, a development candidate stage engineered Treg product (EngTreg) expressing a CAR specific to a gut antigen designed to treat IBD. These CAR-EngTregs are generated from human bulk CD4 T cells by RNA-guided nuclease (RGN)-mediated genome engineering comprised of these 3 major pillars: i) Constitutive expression of FOXP3 to ensure stability of the Treg phenotype; ii) a rapamycin-activated chemically induced IL-2 signaling complex (CISC) for persistence; and iii) a CAR to support EngTreg localization to the gut and boost antigen-specific suppressive activity at the site of inflammation.

A variety of integral membrane proteins with expression restricted to the human intestine were identified as promising CAR targets. The top 3 candidates and their murine orthologs were selected for antibody discovery campaigns. A multi-phase screening strategy in human CD4 T cells and EngTregs was implemented to assess the activity and functionality of binders when expressed in two distinct CAR backbones. CARs exhibiting a range of activity, low tonic signaling, and minimal polyreactivity were selected for functional evaluation in CAR-EngTregs.

Human CAR-EngTregs displayed characteristic Treg markers (CD25, CTLA4, EOS, CD39, CD27, TNFRII, low CD127, low CD70, elevated LAP and GARP) and suppressed effector T cell (Teff) proliferation in a CAR activation-dependent manner. Additionally, these CAR-EngTregs demonstrated functional stability under pro-inflammatory cytokine (TNFα, IL-6, IL1-β, IL-23) challenge and exposure to prednisolone, the standard-of-care drug for patients with IBD during flare ups.

Murine CAR-EngTreg (CAR-mEngTreg) surrogates, designed to bind the human ortholog of our target, showed preferential gut localization and proliferation with reduced off-tissue localization in both naïve and colitis models (Acute Dextran-sodium-sulfate and CD45RBhi adoptive transfer). Notably, these CAR-mEngTregs demonstrated significant efficacy in the CD45RBhi colitis model, characterized by improved survival and histopathological scores compared to disease control in addition to reduced Teff IFNγ production. These preclinical findings in addition to the comprehensive characterization of GNTI-932 indicate that CAR-EngTregs targeting tissue-specific antigens may enhance the therapeutic potential of Treg therapies for IBD.

2032 Enhanced CNS Transduction and Safety of S01coAAV2 Following Intra-Cerebrospinal Fluid (CSF) Administration in Cynomolgus Macaques

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Adeno-associated virus (AAV) gene therapy for central nervous system (CNS) disorders holds great therapeutic promise. Intra-CSF delivery offers the potential for minimally invasive and local brain delivery, yet the limited brain penetration and off-target toxicity of current vectors after intra-CSF delivery limits their broad applications.

To address these limitations, we utilized our ALIGATER™ conjugation platform to develop a library of conjugated AAV2 (coAAV2) vectors designed for enhanced CNS targeting following intra-CSF administration. Leveraging AAV2's natural neuronal tropism, we conjugated ligands to improve its brain penetrance and transduction efficiency, reducing extracellular matrix interactions and protecting the capsid against proteasomal degradation.

Following a single round of screening in non-human primates (NHPs), we identified S01coAAV2 as one of the top-performing capsids with notable increased brain penetrance and liver and dorsal root ganglia (DRG) detargeting compared to AAV9. S01coAAV2 was then further characterized in two cynomolgus macaque validation studies. In the first study, S01coAAV2 and AAV9 expressing a reporter gene were administered intra-cisterna magna (ICM) at 1e13 vg total (n=2/group). In the second study, vectors expressed a therapeutic transgene for a brain indication and were delivered via intra-cerebroventricular (ICV) injection. S01coAAV2 was injected at a 5-fold lower dose (1e13 vg) compared to AAV9 (5e13 vg) (n=3/group). Vector biodistribution (DNA and RNA) and safety were evaluated at 60 days post-injection.

In both validation studies, S01coAAV2 demonstrated widespread CNS distribution, with transgene expression in all assessed regions, including cortex, putamen, hippocampus, thalamus, cerebellum, medulla, and substantia nigra. Following ICM administration, S01coAAV2 exhibited a significant improvement over AAV9 at the same dose, with, for example, up to a 100-fold increase in mRNA levels in the cortex and a 10,000-fold increase in the hippocampus. In parallel, a strong detargeting from DRG and liver was observed. In the ICV study, S01coAAV2 achieved equivalent transgene expression in the brain and spinal cord at one-fifth the dose of AAV9. Notably, S01coAAV2 demonstrated an improved safety profile, with strong detargeting of peripheral organs such as liver, heart, and spleen, and an absence of nerve conduction deficits, in contrast to AAV9-treated animals, where DRG toxicity and nerve fiber degeneration were observed.

These findings position S01coAAV2 as a promising candidate for CNS gene therapy via intra-CSF delivery. By enhancing AAV2's natural tropism, conjugation improved brain penetration, reduced off-target transduction, enabled dose reduction, and improved safety. The benefits of intra-CSF administration, including reduced doses and systemic exposure, further underscore its potential for treating neurodegenerative and neurodevelopmental disorders.

2033 ABCB11 mRNA therapy for the treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2)

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Progressive familial intrahepatic cholestasis type 2 (PFIC2) is a rare autosomal recessive liver disease caused by mutations in the gene of ATP-binding cassette subfamily B member 11 (*ABCB11*), which encodes the bile salt export pump (BSEP) responsible for canalicular transport of bile acids from hepatocytes to bile duct. Deficiency in *ABCB11* results in intrahepatic bile acid accumulation, triggering a cascade of pathological consequences including jaundice, pruritus, elevated serum bile acids, malabsorption and failure to thrive, and ultimately liver failure at ages ranging from infancy to adolescence.

Current treatment options for PFIC2, including the newly approved small molecule ileal bile acid transporter (IBAT) inhibitors such as Odevixibat and Maralixibat, have limited efficacy and are associated with numerous side effects. The only curative treatment option for PFIC2 patients is liver transplantation, which has high risk and often presents significant morbidity. Traditional protein replacement strategies are not feasible due to challenges in drug delivery, while viral-mediated gene therapies have yet to be successful owing to inherent limitations in adeno-associated viruses (AAV) packaging capacity, pre-existing neutralizing antibodies, and potential genotoxic risks.

To develop novel therapies for PFIC2, we engineered chemically-modified and codon optimized mRNA encoding human ABCB11 (hABCB11) encapsulated in lipid nanoparticles (LNPs). Our studies demonstrate the therapeutic efficacy of hABCB11-mRNA-LNP in cultured human cells and in a genetic mouse model of PFIC2 (*Abcb11-/-*). Robust protein expression of human BSEP was observed in transfected cells, along with a significant increase in bile acid transporting activity. Intravenous administration of hABCB11-mRNA-LNP in the *Abcb11-/-* resulted in robust and sustained hepatic expression of the hABCB11 protein and significant improvement in disease biomarker such as serum bile acid levels. In addition, repeat doses of ABCB11-mRNA-LNP caused a sustained improvement in serum bile acid and liver enzyme levels in the *Abcb11-/-* mice fed with diet containing 0.1% cholic acids, accompanied by a significant improvement in liver pathology. Furthermore, administration of ABCB11-mRNA-LNP also improved the conditions and extended the survival of the *Abcb11-/-* mice challenged by diet enriched in cholic acids. Collectively, our data provides strong preclinical proof-of-concept for systemic mRNA-LNP as a potential disease-modifying therapy for patients with PFIC2.

2034 Highly Efficient iPSC Reprogramming from Human Vδ1T Cells for Development of the 'off-the-shelf" Allogenic Cell Therapy

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Chimeric antigen receptor-T cell (CAR-T) has demonstrated remarkable success in treating hematologic and lymphatic tumors. However, significant gap remains in the high-cost and lengthy manufacture process associated with autologous CAR-αβT cells. In addition, solid tumors still present a major challenge, such as insufficient tumor infiltration of CAR-T cells, suppressive tumor microenvironment (TME), and T cell exhaustion and reduced persistence due to repeated antigen stimulation. To address this challenges, substantial efforts have been made to develop "off-the-shelf" allogenic cell therapy using alternative immune effector cells such as healthy donor-derived αβT cells, natural killer (NK) cells, γδT cells, macrophages, etc. Among these cell types, γδT cells especially Vδ1T

subtype has received a lot of attention recently due to its unique features in low GvHD risk, preferential tissue infiltration, and innate receptor-mediated cytotoxicity in addition to CAR-dependent tumor killing. Nevertheless, Vδ1T cells only constitute a small population of PBMC, which poses a significant challenge for in vitro expansion to generate sufficient doses for treatment. In that regard, reprogramming of Vδ1T cells into induced pluripotent stem cells (iPSCs), which can be expanded indefinitely and further re-differentiated into Vδ1T could potentially provide unlimited supply for cell therapy. However, iPSCs reprogramming from T cells especially Vδ1T cells is technically challenging and is a very inefficient process. In this study, thorough extensive optimization, we have established a method supporting highly efficient iPSC reprogramming from Vδ1T cells (~ 1.5% reprogramming efficiency). Using this method, we successfully established 12 Vδ1T-iPSC lines. These Vδ1T-iPSCs could be expanded, and differentiated into Vδ1T cells, expressing characteristic markers similar to primary Vδ1 T cells. Most importantly, these iPSC-derived Vδ1T cells exhibited potent in vitro cytotoxicity against multiple tumor cell lines through CAR-independent innate killing. Overall, we have developed an efficient iPSC reprogramming method from Vδ1T cells, and to the best of our knowledge, it is the first time such highly efficient iPSC reprogramming from Vδ1T cells has been achieved. Established Vδ1T-iPSCs could further be differentiated into Vδ1T cells, paving the way for a robust "off-the-shelf" allogeneic cell therapy product with significant potential for clinical application.

2035 Regulated Expression of Human Coagulation Factor IX (hFIX) Using an AAV-encoded RNAi-based Gene Circuit

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A challenge of "once-and-done" AAV-based gene therapy is the lack of control over the therapeutic payload expression levels after vector administration. The endogenous RNA interference (RNAi) pathway constitutes a clinically proven modality for regulating gene expression *in vivo*. In this work, we evaluated Alnylam's RNAi-based strategy for regulating the magnitude and timing of expression from a liver-targeted therapeutic transgene post-AAV dosing. First, we designed multiple RNAi-responsive human coagulation Factor IX (hFIX) cassettes containing siRNA binding sites (siRNA-BS) in the 3' UTR and shRNA cassettes that co-express the cognate siRNA for RNAi-mediated self-repression (AAV_{rr}). Separately, GalNAc-modified oligos called REVERSIRs matching the sequence of the siRNA-BS were chemically synthesized and tested for their ability to induce transgene expression *in vivo*. Each REVERSIR is designed to specifically bind a complementary siRNA-loaded RNA-induced silencing complex (RISC) and block its RNAi activity. The AAV cassettes utilized a liver-specific promoter and were packaged in a hepato-selective proprietary AAV capsid (Spark100).

During an eight-week run-in period following administration of our liver-directed AAVs, plasma concentration of hFIX was successfully attenuated in AAV_{rr} dosed animals compared to those dosed with the non-self-repressing AAV. *In vitro* and *in vivo* data measuring hFIX expression levels showed varying degrees of self-regulation of the 'off state' depending on the sequence of the shRNA/siRNA-BS pair. A single subcutaneous dose of 1.0 mg/kg REVERSIR in AAV_{rr}-transduced mice (Day 0) led to a significant up to 20-fold increase in plasma hFIX by Day 3, comparable to the magnitude of plasma hFIX expression in animals similarly transduced with a non-self-repressing AAV. Plasma hFIX remained elevated in REVERSIR-treated animals until study terminus 12 weeks later. Terminal mean liver transgene mRNA was significantly higher in REVERSIR-treated versus mismatch-treated AAV_{rr}-transduced animals. Administration of a mismatched control oligo had no significant effect on plasma hFIX levels or liver transgene mRNA in AAV_{rr}-transduced animals.

In summary, the liver-directed AAV_{rr} + REVERSIR approach constitutes a specific OFF/ON switch, potent (>1-log), and durable (≥12 weeks) regulation of antigen hFIX expression. Additional studies are needed to further evaluate the potential of REVERSIR in gene therapy – including adjustable durability, dose-response, alternative tissue tropism, and tolerability.

2036 Microglia-targeted Gene Therapy Using Receptor-based rAAV Delivery

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Microglia, as the immune cells of the central nervous system (CNS), play dynamic roles in maintaining neuronal health and contributing to various neurological conditions. However, it has challenging to explore the molecular mechanisms that drive microglia function because traditional viral approaches such as Adeno-Associated Virus (rAAV) are not compatible with microglia. Conventional AAV-based microglia targeting, whether through microglia-specific promoters or AAV capsid engineered expressing transgene in vitro and in vivo, often results in low transduction efficiency and unstable transgene expression. This limitation arises from a lack of understanding of the underlying mechanisms that reduce AAV microglia transduction, which, if addressed, could improve transduction efficiency. The circumvent this limitation, we develop a AAV based microglial compatible gene therapy approach. We first screen rAAV capsids that can target the microglial receptor protein (MRP). We will identify efficient rAAV variants from an AAV library using naturally expressed or genetically modified microglial engineered to express MRP in vitro and in vivo. To this end, we will initially screen for the most effective MRP, either P2Y12 or TMEM119, in mouse microglial cell lines BV2 and SIM-A9. This will be achieved through immunohistochemistry, size analysis, and protein homology matching with human protein sequences to facilitate future clinical applications. Finally, the most efficient MRP will be overexpressed in HEK cells to pull down rAAV variants from the AAV library, which will then be further characterized using deep DNA sequencing. The top-performing capsid variants will be used for AAV packaging to express transgenes or GFP in vivo in the mouse brain. This design strategy enhances the precision and efficacy of gene therapy applications to microglia, facilitating AAV-mediated microglial targeting for therapeutic interventions in neurological disorders.

2037 KLS-3021: Innovative Oncolytic Virotherapy for the Treatment of Advanced and Metastatic Cutaneous Squamous Cell Carcinoma

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Background: Cutaneous squamous cell carcinoma (cSCC) is the second most prevalent form of non-melanoma skin cancer, with an increasing incidence and significant disease burden. While early-stage cSCC can be managed effectively with surgical excision and radiation therapy, advanced and metastatic cases present substantial therapeutic challenges due to high recurrence rates and limited treatment options. Despite advancements in treatment modalities, including targeted therapies and immunotherapies, the prognosis for patients with advanced and metastatic cSCC remains poor, highlighting the urgent need for novel therapeutic approaches. Oncolytic virotherapy has emerged as a promising strategy, leveraging the ability of engineered viruses to selectively infect and lyse cancer cells while stimulating systemic anti-tumor immune responses. KLS-3021, an innovative oncolytic virotherapy, is designed to enhance viral dissemination, immune cell infiltration, and anti-tumor activity through the expression of PH-20, IL-12, and sPD1-Fc. This study aims to evaluate the therapeutic potential of KLS-3021 in preclinical models of advanced and metastatic cSCC.

Methods: *In vitro* cytotoxicity and viral productivity were evaluated in human cSCC cell lines and primary normal keratinocytes. An orthotopic cSCC model was established by intradermally injecting human A-431 cells into the flanks of BALB/c nude mice. KLS-3021 was administered via a single intratumoral (IT) injection once. A metastatic cSCC model was created by intradermally injecting luciferase-labeled human A-431 cells into the dorsum of the foot to induce popliteal lymph node metastasis. Mice with PLN metastases, detected via *In Vivo* Imaging System (IVIS) imaging, received a single IT injection of KLS-3021 into the primary tumor. In both models, tumor growth was monitored, and histological analyses were conducted to assess viral spread, extracellular matrix (ECM) remodeling, and immune cell infiltration.

Results: KLS-3021 demonstrated selective cytotoxicity and robust replication in cSCC cell lines compared to normal keratinocytes. In the orthotopic model, treatment with KLS-3021 resulted in a significant reduction in tumor volume and achieved complete regression in all KLS-3021-treated mice. Notably, in the metastatic model, a single IT injection at the primary tumor site led to effective viral dissemination into both the primary tumor and tumor-draining lymph nodes, ultimately eradicating lesions at both sites. Histological evaluation revealed pronounced degradation of hyaluronan, a major component of the ECM, and enhanced viral spread. Additionally, immune cell infiltration was observed in tumors at the KLS-3021 administration site, characterized by a predominant M1 macrophage milieu with anti-tumor properties.

Conclusion: Preclinical evaluation of KLS-3021 demonstrates its potential as a potent oncolytic virotherapy for advanced and metastatic cSCC. KLS-3021 showed selective cytotoxicity and robust replication in cSCC cell lines, leading to significant tumor regression and effective eradication of metastatic lesions. The observed hyaluronan degradation, enhanced viral dissemination, and favorable immune cell infiltration highlight its multifaceted mechanism. These findings support further clinical development of KLS-3021 as a promising therapeutic strategy for advanced and metastatic cSCC, addressing the need for novel treatments.

2038 Reversibly Engineered CRISPR RNPs for Improved Editing in the Brain

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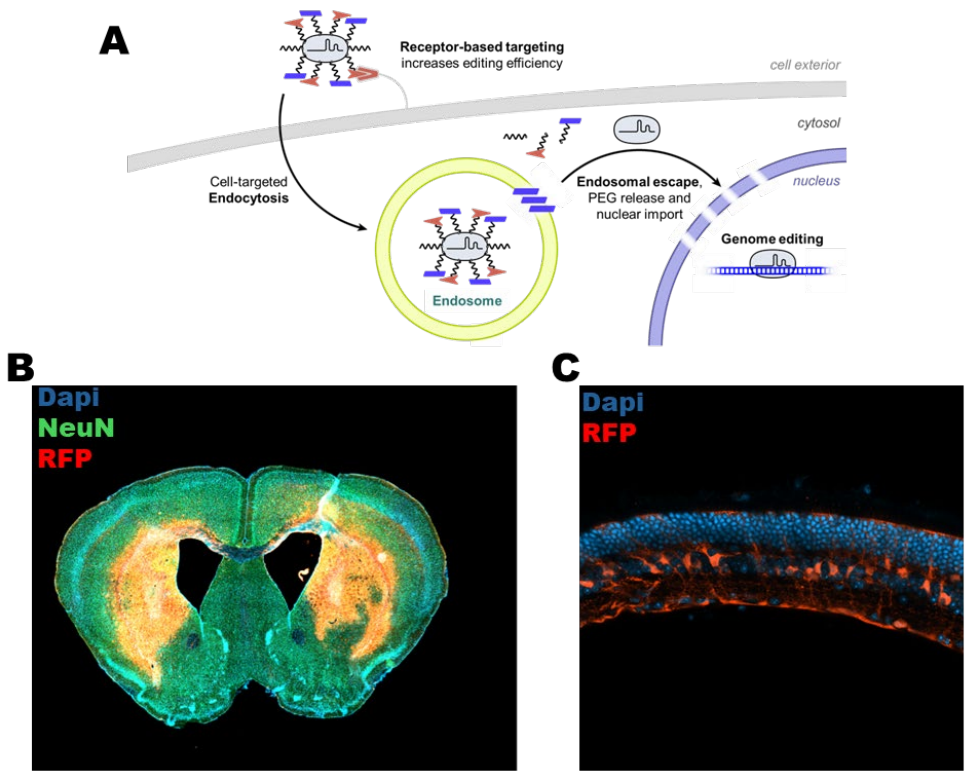
There is a substantial need for improved targeted delivery of CRISPR effectors to the brain parenchyma and other tissues. Viral vectors (e.g., AAV) are challenging to manufacture, and persistent cargo expression can increase the risk of genotoxicity and immunogenicity. Lipid nanoparticles (LNPs) carrying CRISPR mRNA offer an appealing non-viral option; however, their large particle size limits effective distribution in the brain. In contrast, CRISPR ribonucleoprotein (RNP) complexes are readily manufactured, non-viral, and their small size supports improved tissue distribution, however, efficient cellular uptake remains a key hurdle.

To improve RNP delivery after local delivery to the striatum, we engineered a traceless and reversible chemically conjugated platform for pegylation and peptidylation of CRISPR RNPs. This method covalently attaches bio-functional targeting moieties to RNPs, allowing for cell-specific self-delivery and enhanced tissue distribution. When these chemically modified RNPs are covalently conjugated to amphiphilic delivery peptides, and neuron targeting ligands, neuronal editing is significantly boosted.

After in-brain administration of Cas9 RNPs tethered to both a delivery peptide and a neuron-targeting peptide, we observed editing in 75% of the murine striatum and modification of 66% of striatal neurons within the edited region. These outcomes represent a substantial improvement over our initial RNP + peptide non-covalent mixtures and roughly a twofold enhancement compared to other non-viral brain editing methods reported in the literature. Additional studies demonstrated efficient editing in the thalamus via convection-enhanced delivery, in the murine retina (with implications for age-related macular degeneration), and in colon organoids. In an R6/2 Huntington's disease model, our platform achieved the highest level of editing yet reported in the murine striatum, with 10% of mutant HTT alleles modified as confirmed by NGS, after local CED administration, alongside microscopy evidence of reduced mutant HTT protein. This is the highest reported in vivo editing of a Huntington model mouse striatum yet reported in the literature.

These findings establish our engineered CRISPR RNP platform as a promising approach for genome editing of the brain, charting a course toward accessible, non-viral in vivo genome engineering therapies for neurological disorders and beyond.



Reversibly Engineered RNPs for Improved Delivery: A. Schematic of the nuclear delivery of chemically modified RNPs, demonstrating cell specific uptake and the shedding of peptide and PEG in the cell cytoplasm. **B.** Ai9 ('RFP on') murine brain after convection enhanced delivery of engineered CRISPR RNPs, demonstrating enhanced delivery and editing of the murine striatum, red tissue is indicative of editing. **C.** Ai9 murine retina after local injection of engineered CRISPR RNPs demonstrating robust editing of muller glia cells in the retina.

2039 Development of a Delivery Strategy for Neural Microtissues in Parkinson's Disease Cell Therapy

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With the rapid expansion of the field of 3D cultures and organoids, interest in their therapeutic use is growing. However, these innovative formats pose unique challenges for clinical translation as their physical and biological properties substantially differ from those of conventional single-cell-based products. Their handling imposes new constraints, such as much faster sedimentation, which must be addressed from the fill-and-finish stage to the final delivery procedure in the target region to ensure accurate dosing and precise cell placement. In this study, we present the development of a strategy to maintain homogeneity throughout the entire downstream process for the delivery of 3D neural microtissues as a cell therapy for Parkinson's disease. This includes the development of a custom-made delivery solution. Various delivery methods were compared using in vitro tests. The final selected strategy was validated through in-use testing and led to successful engraftment in a non-human primate, with the presence of dopaminergic (DA) neurons observed 1 month after transplantation. The development approach described here holds potential for broader applications in other diseases and supports using next-generation cell therapies employing 3D formats.

2040 A Unique Model for Accelerating Drug Development and Clinical Trials towards Drug Approvals in Rare Pediatric CNS disorders

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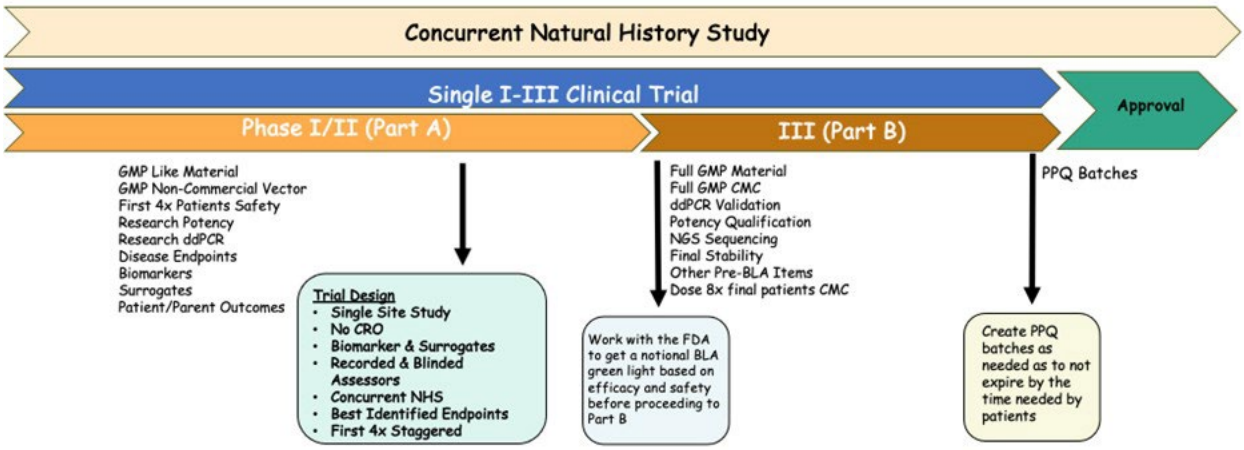
Background: Elpida Therapeutics is a social purpose company pioneering an innovative approach to drug development that focuses on expediting the timeline and reducing costs from preclinical discovery to regulatory approval for rare diseases. Given the significant challenges to delivering therapies to the CNS, a new model designed to address these challenges is developed, as traditional pharmaceutical development where lengthy timelines often delay the availability of life-saving therapies. By strategically redesigning the clinical trial process and using strategic partnerships and a data-driven approach, our model can reshape the landscape of drug development and clinical trials.

Methods: Elpida's unique model integrates several key strategies that collectively shorten development timelines. Elpida incorporates adaptive clinical trial designs, initiating single studies Phase 1-3 trials, enabling real-time modifications based on interim data. This reduces the need for extensive trial phases and facilitates quicker regulatory submissions. In addition, Elpida maintains close collaborations with regulatory agencies, streamlining approval processes and gaining early feedback on drug development strategies. A key feature of Elpida's model is its focus on patient-centric trial designs. By using digital health tools and real-time data monitoring, ensuring that clinical trials are more efficient and better aligned with patient needs and the ability to capture Real World Data.

Results: Our approach has significantly reduced time and costs for several drug candidates. Our lead clinical-stage program for SPG50 disease has progressed through clinical phases in half the time of traditional pipelines, and our lead product MELPIDA currently at Phase 3 trials. We share regulatory interactions and methods to navigate this process specifically for rare and ultra rare pediatric disorders and lessons learnt. Furthermore, adaptive trial designs have led to faster recruitment and higher retention rates, improving overall trial efficiency.

Conclusions: Our strategy at Elpida Therapeutics exemplifies a novel and highly effective approach to drug development in rare CNS disorders where there is a limited patient population and a significant unmet medical need. This model not only promises to shorten timelines but also aims to make the drug development process more patient-centric, cost-effective, and scalable for the future of precision medicine in rare diseases.



2041 Effective and Broad Biodistribution of mRNA Expressed Reporter in Microglia Following Single ICM Administration of Lipid Nanoparticles (LNPs) in Cynomolgus Macaques

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Objectives: Microglial cells in the brain represent an important therapeutic target for treatment of severe neurodegenerative diseases such as Alzheimer's disease and many others. To date, efforts to safely deliver therapeutic nucleic acid cargo via non-viral means in non-human primates has been largely ineffective. The aim of this study was to identify a LNP that targets microglia specifically, without inducing an inflammatory response, and to evaluate whole brain and cell-specific biodistribution and expression of a reporter mRNA encapsulated in the LNP upon direct injection to the cerebrospinal fluid (CSF) of non-human primates (NHPs).

Methods: Fifty ReCode SORT LNPs were screened in Ai14 reporter mice and wild-type rats using Cre and tdTomato mRNA. Lead candidates demonstrating microglial uptake following intrathecal (IT) and intracisterna magna (ICM) injection were evaluated for efficacy, tolerability, and the impact of Dexamethasone pretreatment. A specialized dissociation protocol and FACS workflow with multiple markers for different cell types was applied to identify formulations for preferential uptake by microglial cells. Macaques (n=3) received a single ICM injection of tdTomato-mRNA LNP. Brain sections from different regions were analyzed at 24h post dosing (p.d.) via FACS, single-cell RNA sequencing (scRNA-seq), RNA in situ hybridization (RISH), and immunohistochemistry (IHC) to confirm microglial targeting and biodistribution. Cytokine levels from CSF samples were measured at 4h and 24h p.d.

Results: Rodent screening identified several lead SORT LNPs, with traditional 4-component LNP control (DLin-MC3-DMA) showing negligible reporter gene expression in microglia. A lead was selected for dosing in NHPs, with a high and low dose administered via single ICM injection. Both doses were well tolerated, and animals recovered without any observable clinical symptoms. FACS analysis of brain sections revealed high levels of tdTomato positive cells co-localizing with microglial cells (CD11b+). Microglial transfection efficiency was dose-dependent, with 5% at low dose (1mg/kg of brain weight) and 23% at high dose (3mg/kg). No uptake was detected in endothelial cells (CD31+). ScRNA-seq identified transfected microglial subtypes, with no evidence of inflammatory responses or peripheral immune cell infiltration. RISH confirmed exclusive microglial targeting. scRNA-seq in rats indicated initial microglial activation post-LNP uptake, followed by a return to a homeostatic state suggesting that the changes are transient. Dexamethasone pretreatment reduced perivascular macrophage reporter expression by >80% without affecting microglial transfection levels. Lastly, increasing lipid to mRNA ratio did not change the tolerability profile but increased microglial reporter expression. No adverse cytokine production was observed following treatment.

Conclusions: We show that systematic screening of unique SORT LNPs in rodents enabled the identification of potent and safe formulations that are preferentially targeted to microglia. The NHP data recapitulates data in rodents, and represents a significant advance for the application of efficient and safe non-viral vehicles for delivering nucleic acid cargos to microglia at therapeutically efficacious levels throughout the brain. Future applications include the ability to deliver therapeutic RNAs, such as gene editing cargos, for applications where transient activity in microglia is needed.

2042 Development of OL-280: An Allogeneic CD19-Targeting γδ CAR-T Cell Therapy with Pro-Survival Signaling Armoring Enhancing Persistence and Potency for Treating Autoimmune Diseases and Cancers

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Autologous αβ CAR-T therapies have shown success in treating autoimmune diseases and cancers but face limitations like costly patient-specific manufacturing and severe toxicity. Allogeneic αβ CAR-T therapies improve accessibility but require TCR/HLA editing to prevent GvHD and rejection. γδ-T cells offer a promising alternative with HLA-independent targeting, avoiding GvHD without gene editing risks. Additionally, γδ CAR-Ts can be engineered to express cytokines, enhancing potency and persistence, and providing a safer, scalable off-the-shelf therapy.

Here we describe the development of OL-280, an anti-CD19 allogeneic γδ CAR-T product with cytokine armoring designed to illicit potent and durable cytotoxicity. First, we established a scalable, donor-agnostic manufacturing process for γδ T cells by developing a robust, GMP-compatible protocol for large-scale expansion from healthy donor PBMCs. This method reliably produced high purity γδ T cell products (>99% γδ T cells) with minimal αβ T cell contamination (<0.1%), balanced Vδ1/Vδ2 subtype ratios, and favorable differentiation profiles. The protocol demonstrated consistent scalability across donors of diverse gender and racial backgrounds. Second, we engineered fully human CD19-targeting CARs with systematically optimized structural components—including hinges, transmembrane domains, and co-stimulatory motifs—to maximize effector potency. Through iterative, multi-parametric screening of parameters such as expansion kinetics, CAR expression, transduction efficiency, cytokine secretion, cytotoxicity, and persistence, we identified CAR configurations that synergistically enhanced tumor cell killing and cytokine production in Nalm6 leukemia models. Third, we integrated cytokine-armored signaling modules into the optimal CAR backbone to enhance durability and reduce reliance on exogenous cytokines, ultimately selecting the final candidate for OL-280. These modifications improved γδ T cell survival in situ while maintaining strict target-specific activation, thereby addressing the limited persistence often observed in allogeneic cell therapies.

In vitro, cytokine-armored OL-280 cells demonstrated sustained persistence and superior cytotoxicity compared to non-armored counterparts, maintaining full potency through three sequential rounds of Nalm6 tumor challenges. The armored cells exhibited over 100-fold higher effector cell counts after repeated antigen exposure, alongside enhanced tumor-killing capacity without functional exhaustion. In vivo, a single dose of armored γδ CAR-T cells achieved sustained complete remission (over 45 days) of disseminated Nalm6 tumors in NSG mice without exogenous cytokine support. These cells displayed robust cellular kinetics with potent bone marrow homing and durable engraftment (>10,000 CAR transgene copies/μg genomic DNA for ≥28 days in both peripheral blood and bone marrow). Finally, the treatment elicited no adverse safety signals: animals maintained stable body weight and temperature, and showed no systemic cytokine release or histopathological evidence of inflammation/graft-versus-host disease (GvHD) in vital organs.

In conclusion, we developed a scalable γδ T cell expansion platform and advanced OL-280, an allogeneic γδ CAR-T candidate. OL-280 demonstrated sustained efficacy and a favorable safety profile, positioning it as a promising off-the-shelf therapy for B cell-associated autoimmune diseases.

2043 ABBIE Gene Editing: A Breakthrough in Precise, Replicable and Traceable Genomic Integration

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The advent of CRISPR-Cas9 technology has revolutionized the field of genome editing by enabling precise modifications in living organisms. However, traditional CRISPR systems often lack the ability to efficiently integrate donor DNA, particularly large DNA sequences, into specific genomic locations while also providing traceability and ensuring safety. In this study, we introduce ABBIE (A Binding Based Integrase Enzyme), a novel

fusion protein comprising the integrase domain of HIV (Human Immunodeficiency Virus) and the dead Cas9 (dCas9) protein. ABBIE is designed to drive targeted integration of large donor DNA sequences (up to ~8 kb in size based on current data) into the genome, thus, enhancing the efficiency and safety of gene editing applications.

Our experimental model utilized ABBIE to integrate a donor DNA cassette of approximately 4.2 kb encoding for green fluorescent protein (GFP) expression and neomycin resistance (NeoR) into the genomes of cultured HEK cells. This was also observed in other cell types. The integration process was guided by a specific guide RNA (gRNA) designed to direct ABBIE to genomic DNA. Following the addition of ABBIE, gRNA, and donor DNA to the cell cultures, we observed significant GFP expression as compared to non-transfected, donor only or no gRNA controls, indicating successful integration of the donor DNA. Furthermore, cells that had undergone successful editing were subjected to selection based on neomycin resistance, allowing for the enrichment of modified cells.

To validate the integration events, we collected genomic DNA from the selected neomycin-resistant cells. We developed specific DNA probes targeting the GFP/NeoR donor sequence to isolate genomic regions containing the integrated donor DNA. Subsequent sequencing of the probe-selected genomic DNA revealed precise integration at genomic sites, with a notable frequency of common integration sites across independent replicates. This consistency underscores the efficiency and specificity of the ABBIE editing system when combined with the designed gRNA used in the study. Our findings demonstrate that ABBIE effectively enhances the integration of donor DNA into the genome by leveraging the targeted binding capabilities of dCas9 and the integration properties of HIV integrase. This innovative approach not only facilitates robust gene expression through the incorporation of GFP but also establishes a reliable method for selecting edited cells via neomycin resistance. The identification of common integration sites across replicates further suggests that ABBIE-mediated editing can achieve good reproducibility and predictability in genomic integrations. This reproducibility highlights ABBIE's ability to achieve consistent and predictable genomic modifications—a critical advantage over traditional CRISPR-based integration methods. Surprisingly, the system does not target in the expected fashion of CRISPR/Cas9 but in a new and unique way.

In conclusion, the ABBIE system represents a significant advancement in genome editing technology, combining the precision of CRISPR with the integration capabilities of viral integrases. By enabling precise, replicable and traceable genomic integration, ABBIE opens new frontiers in gene therapy, synthetic biology, and functional genomics. This platform has potential applications in gene therapy, synthetic biology, and functional genomics, paving the way for more refined and effective strategies in the manipulation of genetic material in diverse biological contexts. Future studies will explore ABBIE's adaptability across diverse cell types and its potential for targeting additional genomic loci, broadening the scope of gene editing possibilities.

2044 Induced Pluripotent Stem Cell-derived CAR MAIT Cells for Cancer Immunotherapy

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Allogeneic chimeric antigen receptor (CAR)-T cells hold potential for more accessible and affordable cancer treatments, but challenges such as limited donor cells, manufacturing variability, and multiplex gene engineering persist. iPSCs provide a promising solution due to their unlimited self-renewal properties and the ability for single-instance genetic modifications. However, efficiently differentiating iPSCs into mature CAR-T cells remains a hurdle, particularly as CAR engineering may arrest T cell development from stem cells, and iPSC-derived CAR-T cells often lack in vivo persistence and clonal expansion needed for long-term tumor suppression.

Mucosal-associated invariant T (MAIT) cells, a subset of αβ T cells that recognize MR1 and do not cause graft-versus-host disease in allogeneic settings, have shown unique properties in tissue homing and tumor infiltration. Yet, their limited numbers in humans hinder their full therapeutic potential.

We have developed a feeder-free and serum-free ex vivo culture method capable of generating 1013 CAR MAIT cells from 106 iPSCs. Our culture method allows iPSCs to differentiate into mature CAR MAIT cells without the need of TRAC knockin, TCR knockout, or engineering iPSCs with CAR that contains compromised signaling domain. These iPSC-derived CAR MAIT cells, can be either single positive (SP)-CD4 or SP-CD8, exhibit potent antitumor capabilities and robust clonal expansion in vivo.

As a proof-of-concept for this innovative platform, we employed a BCMA-targeting CAR (BCAR) to treat BCMA+ multiple myeloma, and a mesothelin-targeting CAR (MCAR) to treat mesothelin+ mesothelioma.

2045 Induction of Immune Tolerance in AAV Gene Therapy Through Oral Administration of N-Acetylgalactosamine-6-sulfate Sulfatase Enzyme and its Epitopic Peptides

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Background: Mucopolysaccharidosis IVA (MPS IVA) is one of the lysosomal storage diseases caused by the accumulation of undegraded glycosaminoglycans, keratan sulfate (KS) and chondroitin-6-sulfate, in the absence of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). Different degrees of phenotypic symptoms appear in skeletal abnormalities, short stature, corneal clouding, narrowing trachea, laxity of joints, hip dysplasia, and knocked knees. Gene therapy (GT) is potentially a one-time treatment for this disease, in which the enzyme is continuously produced, circulated, and delivered to target tissues. However, immune responses to gene products and viral vectors can diminish therapeutic efficacy. The main objective of this study was to evaluate the effect of oral immunotolerance on AAV gene therapy in the MPS IVA mouse models. To develop a new strategy to induce immune tolerance, we hypothesized that oral delivery of GALNS or its peptides induces immune tolerance to GALNS in MPS IVA mouse models, leading to increased therapeutic efficacy.

Method and Materials: Within 72 hrs of birth, mice were treated orally with a combination of three T-cell/B-cell epitope peptides or GALNS enzyme on alternate days for 20 days with either 2.5 μg/g or 5 μg/g body weight. At 30 days old, AAV9-CAG vectors encoding recombinant human GALNS were introduced through the tail vein. We followed the mice until 32 weeks. **Results:** Anti-GALNS antibodies in plasma were not detectable in groups treated with oral administration of peptides, in contrast to the non-immunotolerant control group. GALNS enzyme activities in plasma and tissues were higher in the orally treated groups than in the control group. KS levels in plasma, liver, and bone were normalized in the orally treated groups. In pathology, complete correction for heart

vacuolization was achieved in peptide-treated groups, and substantial correction for bone pathology was observed in all Gene Therapy-treated groups.

Conclusion: The results of our study support the concept of combining antigen-specific oral tolerance with gene therapy to prevent unwanted immune responses against therapeutic transgene products. The induction of oral immune tolerance with AAV9 gene therapy provides a sustainable enzyme supply that improves bone pathology in MPS IVA mice without the elevation of anti-GALNS antibodies. Early diagnosis of MPS IVA is achievable through newborn screening programs in various countries. Therefore, this oral treatment will be applied to the affected newborns. A novel approach to *in vivo* GT using oral immunogenic peptides will provide a new paradigm of therapeutic options for MPS IVA patients. It will have a wide range of applications for other types of MPS, lysosomal storage diseases, and genetic diseases.

2046 Tour de Tumor: scRNA Uncovers CD4+ Cycling State & CD8+ Finish Line Linked to Enhanced Anti-Tumor Efficacy and Decreased Dysfunction in Solid Tumors

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Chimeric Antigen Receptor (CAR) T cell therapies face significant challenges in targeting solid tumors due to the immunosuppressive tumor microenvironment. In this study, we engineered CAR T cells to express the interleukin-9 receptor (IL-9R), enabling IL-9 signaling, and analyzed their functionality using single-cell RNA sequencing (scRNA-seq) and trajectory analyses under antigen stress conditions. Our findings reveal that IL-9 signaling profoundly alters CAR T cell differentiation, steering CD8+ T cells toward memory and effector states while promoting the cycling of CD4+ T cells.

Trajectory and RNA velocity analyses demonstrated enhanced multipotency in IL-9-signaling CAR T cells, avoiding dysfunctional fates and favoring central memory T cell phenotypes. Differential transcription factor activation identified STAT1 and STAT4 as key drivers of these transitions. Additionally, single-cell transcriptomics highlighted the upregulation of chemokine receptors (CXCR3, CCR5) and cytotoxic effectors (IFNG, PRF1), reinforcing the enhanced antitumor potential of IL-9-signaling CAR T cells.

These insights into the molecular mechanisms underlying IL-9-mediated CAR T cell reprogramming showcase its promise in enhancing the efficacy and persistence of CAR T cell therapies for solid tumor treatment.