

3rd Annual Gene Therapy Initiative Symposium

POSTER ABSTRACTS

WEDNESDAY, SEPTEMBER 16TH
4:30 PM TO 5:30 PM

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A novel RNA-based platform to rescue neurodevelopmental disorders caused by haploinsufficiency

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Scripps Research

ABSTRACT

- **Background:** Haploinsufficiency is a prevalent pathogenic mechanism in neurodevelopmental disorders (NDDs). Current therapeutic strategies, including gene therapy or antisense oligonucleotides (ASOs), are limited by safety concerns, off-target effects, and scarcity of target sequences. Even when the healthy allele is transcriptionally intact, microRNAs (miRNAs) binding to 3' untranslated regions (3' UTRs) further suppress translation, compounding protein deficits. Exploiting this regulatory axis has been hindered by the lack of scalable tools to map and functionally assess miRNA-target interactions (MTIs) in disease-relevant cell types.
- **Methods:** To address this gap, we developed a high-throughput platform integrating: (i) cell-type-specific MTI mapping via chimeric enhanced crosslinking immunoprecipitation (chim-eCLIP) to identify bona fide binding sites; and (ii) programmable MTI blockade using a catalytically inactive dCas13 (dCasRx), which sterically outcompetes miRNA binding. As a proof of concept, we focused on *Sptbn1* (encoding β II-Spectrin) haploinsufficiency. Heterozygous *Sptbn1*^{+/-} mice display cognitive deficits and axonal abnormalities that recapitulate patient phenotypes.
- **Results:** Using chim-eCLIP in cortical pyramidal neurons, we identified multiple brain-enriched miRNA binding sites, demonstrating redundant suppression of the healthy allele. Targeted dCasRx blockade of one miRNA site achieved ~50% de-repression of *Sptbn1*, restoring near-wildtype protein levels and rescuing complexity in haploinsufficient neurons in vitro. To advance toward clinical translation, we designed ASOs targeting one MTI within the *Sptbn1* 3' UTR. The lead ASO was validated by intracerebroventricular injection (ICV) in *Sptbn1*^{+/-} mice, robustly increasing *Sptbn1* protein levels in vivo and restoring corpus callosum thickness at p7, 14 and 25.
- **Conclusion:** This scalable, mutation-agnostic platform provides a generalizable strategy for identifying 3' UTR regulatory elements that can be therapeutically targeted to upregulate genes of interest, opening new avenues for RNA-based intervention across NDDs.

Tszuru: A Solar powered decentralize Biotechnology Platform for future bioprinting, organoids, and organ-on-a-chip applications

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ABSTRACT

- **Background:** Regenerative medicine technologies, including bioprinting, organoids, and organ-on-a-chip systems, are transforming disease modeling, precision therapeutics, and tissue engineering. However, these capabilities remain largely confined to centralized laboratories and specialized healthcare facilities. Tszuru is being developed as a decentralized biotechnology platform designed to support future deployment of regenerative medicine capabilities in remote, resource-limited, disaster-response, and isolated environments where conventional laboratory infrastructure is unavailable.
- **Methods:** Tszuru integrates autonomous mobility, telehealth infrastructure, digital health technologies, renewable energy systems, and portable additive manufacturing capabilities. To establish operational feasibility for future decentralized biotechnology applications, a solar-powered manufacturing platform was deployed and evaluated during six analog astronaut missions conducted in isolated, confined, and extreme environments. The system was utilized to fabricate mission-support components, life-support equipment, and medical tools on demand. Operational performance, portability, sustainability, and field usability were assessed to inform future integration of bioprinting technologies, organoid culture systems, and organ-on-a-chip workflows.
- **Results:** The platform successfully demonstrated portable manufacturing capabilities across six analog mission deployments. On-demand fabrication supported mission operations while reducing dependence on external supply chains and centralized resources. Operational testing established the feasibility of distributed manufacturing in austere environments and provided foundational infrastructure for future decentralized biotechnology applications. Based on these findings, ongoing development efforts are focused on integrating portable bioprinting capabilities and supporting future organoid and organ-on-a-chip research workflows.
- **Conclusion:** Operational validation of portable manufacturing infrastructure represents an important translational step toward distributed regenerative medicine systems. Tszuru provides a feasible platform for future integration of bioprinting, organoid, and organ-on-a-chip technologies that may enable regenerative medicine research, precision therapeutics, and advanced healthcare delivery in remote and resource-constrained environments.

Transcriptome-based Next Generation Sequencing (NGS) shows superiority compared to traditional biosafety testing strategies for the detection of viral adventitious agents in cell and gene therapies.

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PathoQuest & Charles River Laboratories

ABSTRACT

- **Background:** Recent revisions such as ICH Q5A (R2), 3Rs (replacement, reduction, and refinement), and FDA Modernization Act 2.0 and 3.0 have made great strides in encouraging the replacement of animal testing through “New Approach Methodologies” (NAMs). Next Generation Sequencing (NGS), as one of the NAMs, holds promise for biosafety testing by overcoming many shortfalls in animal-based testing, including limited susceptibility of test systems to viral agents and poor resolution/sensitivity, while aligning with regulatory efforts to phase out animal testing.
- **Methods:** Here, we assessed the use of the iDTECT® transcriptome-based assay as a direct replacement for hamster, mouse, and rat antibody production tests (HAP/MAP/RAP), 9 CFR bovine/porcine virus tests, and human/simian virus PCR panels. We evaluated the ability of NGS to detect virus families targeted by traditional tests using synthetic 600 bp RNA transcripts corresponding to closely and distantly related viral sequences. These were spiked at decreasing concentrations into representative cell lysates shortly after cell disruption and prior to nucleic acid purification. Libraries were prepared from each test sample and sequenced using an Illumina® NGS platform. Data were analyzed using our proprietary data analysis pipeline.
- **Results:** The results demonstrated a high level of assay sensitivity, capturing a total of 78 viruses covering a broad range of virus families and viral genome types. Transcriptome-based NGS provided a broad, agnostic approach capable of detecting both known and novel viral variants that traditional assays may miss.
- **Conclusion:** These findings support transcriptome-based NGS as a sensitive and comprehensive alternative to traditional animal-based and targeted viral biosafety assays, further supporting the adoption of NAMs and efforts to reduce and replace animal testing.

Brain-Targeted Hematopoietic Stem Cell Delivery in Alzheimer's Disease

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ABSTRACT

- **Background:** Alzheimer's disease (AD) is the most prevalent cause of dementia and the most common age-related neurodegenerative disorder, yet current therapies provide limited efficacy and do not adequately address the neuroinflammatory and vascular dysfunction associated with disease progression. Previous work from the Cherqui Lab demonstrated that transplantation of wild-type (WT) hematopoietic stem and progenitor cells (HSPCs), which give rise to blood and myeloid cells, generates donor-derived microglia-like cells in the brain and rescues cognitive decline, neuroinflammation, and vascular pathology in a mouse model of AD. However, systemic HSPC transplantation requires chemotherapy for myeloablation, limiting clinical translation due to treatment-associated toxicity.
- **Methods:** We investigated whether intracerebroventricular (ICV) delivery of WT-HSPCs could establish a therapeutic population of donor-derived cells in the brain and promote functional rescue.
- **Results:** Following ICV administration, donor-derived cells stably engrafted in the brain and adopted a microglia-like phenotype, demonstrating that local delivery is sufficient to establish a persistent brain-resident population. Importantly, AD mice receiving ICV-delivered WT-HSPCs in the presence of systemically delivered AD cells exhibited behavioral performance comparable to untreated WT mice across multiple tests, including novel object recognition, elevated plus maze, and light-dark box.
- **Conclusion:** These findings demonstrate that ICV delivery of WT-HSPCs achieves stable brain engraftment and is sufficient to promote robust behavioral rescue despite the presence of systemic AD-derived cells. Together, these results support brain-targeted HSPC delivery as a potential strategy and provide a foundation for HSPC based gene therapy for non-systemic CNS disorders.

Prioritizing Modifier Gene Therapy Targets for Hereditary Spastic Paraplegia Using Transcriptomic and Real-World Evidence

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ABSTRACT

- **Background:** Hereditary spastic paraplegia (HSP) comprises more than 80 genetic subtypes, making mutation-specific gene therapy difficult to scale. Although causative gene replacement can restore individual disease genes, progressive neurodegeneration suggests that shared downstream pathways may also represent therapeutic targets. We investigated whether convergent molecular pathways across HSP-associated genes could provide a framework for prioritizing modifier gene therapy targets applicable across multiple HSP subtypes.
- **Methods:** Sixty-four HSP-associated genes were analyzed using multi-tissue transcriptomic data from the GTEx project. Gene set enrichment analysis identified pathways recurrently enriched across cerebellar cortex, cerebral cortex, spinal cord, and tibial nerve. To provide clinical context, a nationwide Korean National Health Insurance Service cohort comprising 1,656 patients with HSP was analyzed to characterize long-term disease burden and healthcare utilization.
- **Results:** Four hallmark pathways-protein secretion, unfolded protein response, mTORC1 signaling, and oxidative phosphorylation-were consistently enriched across multiple HSP-associated genes in central nervous system tissues. These findings suggest that genetically diverse SPG gene defects converge on common biological pathways despite marked genetic heterogeneity. The recurrent enrichment of oxidative phosphorylation is consistent with accumulating evidence that mitochondrial dysfunction represents a shared downstream mechanism in HSP and highlights mitochondrial pathways as promising modifier therapeutic targets. Nationwide real-world data further demonstrated the substantial long-term clinical burden and unmet therapeutic needs of patients with HSP.
- **Conclusion:** Our findings provide a transcriptomic framework for prioritizing modifier gene therapy targets that could complement mutation-specific gene replacement strategies by restoring convergent disease pathways shared across HSP subtypes. Combined with causative gene replacement, modifier gene therapy may enhance functional recovery by targeting shared downstream mechanisms across genetically diverse forms of HSP. This framework may also support future combination strategies integrating rehabilitation and emerging repeat-dosable non-viral gene delivery platforms to promote functional recovery.

Revolutionizing RNA Therapeutics with Synthetic Optimized DNA (oDNA): A Cell-Free Template Platform for Enhanced In Vitro Transcription

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ARTIS & Afrigen

ABSTRACT

- **Background:** The rapid expansion of RNA therapeutics has intensified the need for DNA templates that improve manufacturing efficiency, product quality, scalability, and speed while minimizing limitations of conventional plasmid DNA. We evaluated a fully synthetic, cell-free optimized DNA (oDNA) platform as a next-generation template for in vitro transcription (IVT).
- **Methods:** oDNA templates were produced using an enzymatic, cell-free process and consisted exclusively of the gene of interest flanked by proprietary adaptor sequences designed to enhance RNA polymerase performance. Unlike plasmid DNA, oDNA contains no bacterial backbone and requires no plasmid linearization before IVT. Performance was evaluated across IVT workflows against linearized plasmid DNA by assessing RNA yield, transcript integrity, double-stranded RNA (dsRNA), and protein expression following cellular transfection. A Rift Valley Fever mRNA vaccine case study evaluated IVT performance and expression, including in vivo expression.
- **Results:** Across multiple IVT workflows, oDNA improved RNA production while maintaining high transcript integrity and low dsRNA. In the Rift Valley Fever case study, optimized DNA templates generated significantly higher IVT yields than linearized plasmid DNA while preserving transcript integrity and producing equivalent or superior protein expression following cellular transfection. Comparable in vivo expression demonstrated that IVT improvements translated into biological activity. Proprietary adaptor designs also enabled application-specific optimization of template architecture while maintaining compatibility with RNA production workflows.
- **Conclusions:** Synthetic oDNA represents a versatile alternative to plasmid DNA for IVT-based RNA manufacturing. Improved RNA yield, product quality, adaptable template architecture, rapid turnaround, and scalable GMP manufacturing may accelerate mRNA therapeutics and vaccines. Cell-free manufacturing also eliminates bacterial fermentation and plasmid linearization, offering opportunities to reduce development timelines, manufacturing complexity, and production costs.

Aptamer-Based Detection of Chronic Traumatic Encephalopathy Predictive Biomarkers Using Nonlinear Multiphoton Laser Wave-Mixing Spectroscopy

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ABSTRACT

- **Background:** Chronic Traumatic Encephalopathy (CTE) is a progressive neurodegenerative disease associated with repetitive head trauma, yet definitive diagnosis remains limited to postmortem neuropathological examination. This study aims to develop a highly sensitive, rapid, and minimally invasive analytical platform for detecting predictive CTE biomarkers using aptamer-based molecular recognition integrated with nonlinear multiphoton laser wave-mixing spectroscopy.
- **Methods:** DNA aptamers specific to CTE-associated biomarkers, including CCL11 and S100B, were employed as selective biorecognition elements. Biomarker-aptamer interactions were detected using nonlinear multiphoton laser wave-mixing spectroscopy, which provides coherent optical signal amplification with exceptionally low background noise. Assay performance was evaluated by monitoring wave-mixing signal changes over clinically relevant biomarker concentrations and assessing analytical sensitivity, selectivity, and reproducibility.
- **Results:** The nonlinear wave-mixing platform demonstrated rapid and sensitive detection of CTE predictive biomarkers with high specificity afforded by aptamer recognition. The coherent optical detection strategy significantly enhanced signal-to-noise ratio compared with conventional fluorescence approaches while requiring minimal sample volume. Preliminary findings indicate excellent reproducibility and the potential to detect low-abundance biomarkers suitable for early-stage disease assessment.
- **Conclusion:** This aptamer-assisted nonlinear multiphoton laser wave-mixing platform represents a promising approach for ultrasensitive detection of CTE-associated biomarkers. The technology has potential applications in early diagnosis, disease monitoring, and therapeutic evaluation for neurodegenerative disorders. Furthermore, the platform is readily adaptable for multiplexed detection of additional neurological biomarkers, providing a versatile analytical tool for precision medicine and translational neuroscience.

Receptor-guided AAV Tropism Engineering via MATCH

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ABSTRACT

- **Background:** Precise control over viral tropism remains a major limitation in gene delivery. We developed MATCH (Modulation of AAV Tropism through Conjugation to Homing proteins), a modular strategy for programmable, receptor-guided retargeting of adeno-associated viruses (AAVs).
- **Methods:** SpyTag peptides were site-specifically inserted into exposed capsid loops of AAV-DJ and AAV9 to enable covalent conjugation of SpyCatcher-linked targeting ligands. Mosaic capsids were generated to control ligand density. T cell targeting was evaluated in human PBMC cultures and transferrin receptor (TfR1) targeting was assessed both in vitro and in vivo using mouse and humanized mouse models. A one-pot "Mix-and-MATCH" production approach was also developed for co-expression of capsids and targeting proteins.
- **Results:** MATCH-AAVs conjugated to an anti-CD3 single-chain antibody efficiently activate and transduce resting human T cells within mixed PBMC populations in vitro, achieving transduction levels of up to ~58% of total PBMCs. Conjugation to transferrin receptor (TfR1)-binding proteins yielded enhanced brain transduction in vivo, with murine TfR1-targeted MATCH-AAV9 exhibiting up to an 84-fold increase in brain expression relative to wild-type AAV9. Human TfR1-targeted vectors similarly enabled robust, receptor-dependent transduction both in vitro and in humanized mouse models. Both TfR1-targeted vectors enabled widespread transduction of the parenchyma, consistent with TfR1-mediated crossing of the blood-brain barrier. The Mix-and-MATCH strategy produced functional targeted vectors at titers comparable to conventional methods.
- **Conclusion:** MATCH provides a simple, generalizable platform for modular AAV retargeting with tunable ligand display. This approach enables scalable, customizable vector design and expands the potential of AAVs for both research and therapeutic applications.

Single-cell multiomic evaluation of allelic zygosity, edit co-occurrence, and gene expression for analytical characterization of CRISPR edited multiplex-engineered cell therapies

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ABSTRACT

- **Background:** Multiplexed genome editing in cell and gene therapies (CGTs) introduces analytical challenges for defining Critical Quality Attributes (CQAs) under increasing regulatory scrutiny. Conventional bulk orthogonal assays obscure clonal heterogeneity, limiting the ability to resolve allelic zygosity, confirm co-occurrence of multiple edits, or distinguish therapeutically relevant cells from those harboring off-target events. To address this, we applied a single-cell multiomic workflow coupling targeted genotyping with gene expression profiling, directly correlating genomic edits at the PDCD1 and TRAC loci with transcriptional states in engineered T cells.
- **Methods:** Three engineered Jurkat T cell lines (PDCD1 knockout, TRAC knockout, and PDCD1/TRAC double knockout with a defined off-target edit) were spiked into healthy donor PBMCs to model heterogeneity following CRISPR editing. Targeted single-cell DNA and RNA profiling was performed on the Mission Bio Tapestry platform, with DNA panels detecting on- and off-target indels/SNVs and RNA panels quantifying PDCD1, TRAC, immune lineage markers, and pathway-relevant transcripts.
- **Results:** This approach resolved clonal heterogeneity by stratifying cells by on- and off-target genotype. Single-cell genotyping assigned allelic zygosity at PDCD1 and TRAC loci, distinguishing unedited, mono-allelic, and bi-allelic states. In the double-knockout sample, genotype calling quantified simultaneous PDCD1/TRAC edits and identified the off-target event, enabling segregation of edited subpopulations. Integrated genotype-transcriptome analysis linked zygosity to functional outcomes, with bi-allelic edits showing greater transcriptional perturbation than mono-allelic edits. Within the PBMC background, combined DNA/RNA signatures distinguished engineered Jurkat T cells from endogenous T, B, and NK subsets, and differential expression associated specific edit combinations with intended signaling versus off-target-associated effects.
- **Conclusion:** These results show single-cell multiomics enables high-resolution characterization of multiplex-edited T cells by resolving heterogeneity where bulk assays cannot - supporting definitive assessment of zygosity, edit co-occurrence, and genotype-expression relationships for robust CQA definition and regulatory-aligned risk assessment in CGT development.

Massively parallel analysis of coding mutations with CodonPrime

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ABSTRACT

- **Background:** Most coding mutations remain functionally uncharacterized, limiting their biological interpretation and therapeutic exploitation. Although prime editing enables precise genome modification, many disease-associated variants reside in sequence contexts that are poorly compatible with conventional prime-editing guide RNA (pegRNA) designs, restricting editing efficiency and large-scale functional studies.
- **Methods:** We developed CodonPrime, a prime-editing framework coupled with a transformer-based toolkit that exploits synonymous codon degeneracy to expand the design space of pegRNAs while preserving protein sequence. We applied CodonPrime to perform massively parallel functional screening of ~2,500 amino acid substitutions across 298 cancer-associated genes.
- **Results:** CodonPrime increased per-mutation editing efficiency 11.4-fold and expanded targetable substitutions by more than 50% compared with conventional pegRNA designs. Approximately 10% of screened variants enhanced cell growth, validating known oncogenic hotspots and identifying 30 previously uncharacterized mutations with oncogenic potential, including HNF1A R272C. Functional analysis further revealed dominant effects among paralogous genes (e.g., RHOTB1/2) and pathway-level organization of mutant phenotypes, including PI3K-mTOR signaling.
- **Conclusion:** CodonPrime substantially expands the editable landscape of coding variants and enables scalable, high-throughput functional interrogation of disease-associated mutations. By improving the efficiency and versatility of prime editing, CodonPrime provides a broadly applicable platform for variant interpretation, target discovery, disease modeling, and the development of precision genome-editing therapies.

Programmable gene insertion in human cells with CRISPR-free recombineering

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Scripps Research

ABSTRACT

- **Background:** Genes mutated in prevalent monogenic diseases harbor many distinct pathogenic mutations, limiting mutation-specific editing. Gene or cDNA integration offers a single therapeutic strategy for diverse patients, but current large-integration tools are inefficient, introduce unwanted indels, rely on cytotoxic double-strand breaks, or lack broad targetability. We developed Mammalian Recombineering Genome Editing (MRGE), a DSB- and CRISPR-free platform for programmable gene insertion, and evaluated it in a mouse model of X-linked Severe Combined Immunodeficiency (X-SCID).
- **Methods:** 30 single-strand annealing protein (SSAP) orthologs were screened for integration activity in HEK293T cells via flow cytometry reporter assays. SSAP-SSB fusions with varied linkers were tested to boost efficiency. Integration efficiency, fidelity, and stability were assessed by flow cytometry, ddPCR, Nanopore sequencing, and selection of edited cells. Single-AAV delivery of MRGE machinery was tested in HEK293T cells. Therapeutic potential was evaluated by MRGE-mediated Il2rg cDNA insertion at its endogenous locus in HSPCs from an X-SCID mouse model.
- **Results:** RecT alone mediated reprogrammable, homology-dependent integration without CRISPR machinery. Ortholog screening identified multiple active SSAPs, and linkage to cognate SSBs consistently enhanced efficiency. These constructs achieved gene insertions ranging from 0.5 to 10.4 kb across multiple loci, including disease-relevant sites such as SMPD1, GBA, and CFTR, single-AAV gene insertion in human cells, and insertion of Il2rg cDNA into its native locus in HSPCs from an X-SCID mouse model.
- **Conclusion:** MRGE, a compact (~1.6 kb) CRISPR-independent SSAP system, enables reprogrammable, kilobase-scale gene insertion, supports single-AAV delivery, and avoids DSB-associated safety concerns in stem cell engineering. Insertion of healthy Il2rg cDNA into its locus in X-SCID HSPCs supports MRGE as a mutation-agnostic curative platform for monogenic immunodeficiencies.

Transplantation of Friedreich's Ataxia (FRDA) hematopoietic stem and progenitor cells into healthy mice recapitulates features of FRDA disease pathology

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ABSTRACT

- **Background:** Friedreich's Ataxia (FRDA) is an inherited neurodegenerative disorder caused by a GAA trinucleotide repeat expansion mutation in the frataxin (FXN) gene, leading to reduced expression of the mitochondrial protein frataxin (FXN). Loss of FXN results in diminished mitochondrial energy production and an accumulation of reactive oxygen species (ROS), contributing to many of the disease symptoms including progressive loss of motor function, ataxia, and hypertrophic cardiomyopathy - the leading cause of death in FRDA patients. Previously we have shown that transplantation of wild type (WT), and gene-edited HSPCs results in engraftment of the transplanted cells as tissue-resident macrophages or microglia-like cells in the brain, leading to rescue of FRDA related motor deficits in the YG8JR murine model of FRDA. However, the exact mechanisms by which microglia contribute to neurodegeneration, and pathways by which gene-edited HSPC transplantation prevents this outcome remain unclear.
- **Methods:** In this study we assess the cell-autonomous contributions of FRDA microglia to FRDA neuropathology by transplanting healthy WT mice with FRDA HSPCs. At 6 months post-transplant we conducted behavioral analysis, and explanted tissues for immunohistochemical and transcriptomic analysis.
- **Results:** We show that introduction of FRDA microglia-like cells in the brain results in neurological deficits in mice, including FRDA specific phenotypes such as gait instability. We observe changes to the cerebellar architecture as evinced by immunohistochemical staining. Bulk RNA-Sequencing reveals shifts in the transcriptomic signatures of mice transplanted with FRDA HSPCs, namely downregulation of synaptic processes.
- **Conclusion:** These results posit FRDA microglia as key drivers of FRDA neuropathology, cementing the importance of their role in FRDA disease progression, and targeting them through a gene-edited HSPC transplantation approach.

A highly efficient AAV capsid engineering platform for generating novel capsids targeting therapeutically relevant tissues

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VectorBuilder

ABSTRACT

- **Background:** Adeno-associated virus (AAV) continues to be a key viral vector for cell and gene therapy development, with a broad range of serotypes that enable targeting of diverse cell and tissue types. Despite this, there is still a lack of AAV serotypes that efficiently and specifically target therapeutically relevant sites.
- **Methods:** To address this, we developed a highly efficient AAV capsid engineering platform capable of identifying novel AAV capsids with enhanced efficiency and specificity for various target tissues in mice and non-human primates. This platform offers several key advantages, including: 1) the ability to efficiently explore large sequence space, 2) high reproducibility of screening results, 3) the ability to target specific cell types, such as retinal photoreceptors, and 4) low cost.
- **Results:** Utilizing this platform, we identified a panel of novel AAV capsids with improved tropism for therapeutically relevant tissues compared to existing serotypes, enabling efficient transduction of the central nervous system following systemic injection, muscle following retro-orbital injection, and deep retinal layers following intravitreal injection.
- **Conclusion:** Overall, we highlight the potential of this approach to advance AAV-based next-generation cell and gene therapies.

Evolving from the Ground Up: Mammalian-Cell Directed Evolution of Wild-Type TadA

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ABSTRACT

- **Background:** Single-nucleotide variants cause a large fraction of genetic diseases, motivating precise tools such as adenine base editors (ABEs). Our goal is to create editors optimized specifically for mammalian cells, where they must function in research and therapeutic applications. Although most ABEs were evolved in bacteria or refined through short mammalian-cell campaigns, sustained evolution in mammalian cells may produce editors better suited to this environment.
- **Methods:** We established a mammalian-cell directed evolution platform beginning with wild-type TadA, an adenine deaminase with minimal DNA-editing activity. A single-mutant TadA library was screened using an episomal fluorescent reporter, followed by combinatorial testing of enriched mutations. Selected variants were evaluated at endogenous genomic sites and compared with bacterially evolved ABE benchmarks. A second round is underway using a genomically integrated reporter to select for editing of chromatinized mammalian DNA.
- **Results:** The first round identified mutations that increased DNA-editing efficiency. Single mutants improved activity, while combinations preserved narrow editing windows and produced further gains. At selected endogenous sites, several mammalian-evolved variants outperformed bacterially evolved benchmarks, achieving higher on-target editing with markedly less bystander editing. The integrated-reporter round will test whether genome-relevant selection further improves performance.
- **Conclusion:** Evolving editors directly in mammalian cells allows selection to reflect constraints poorly captured by bacterial systems, including protein expression and stability, nuclear access, cellular toxicity, and activity on chromatinized DNA. An integrated reporter further links enrichment to successful genomic editing. This strategy may produce editors with the activity, precision, and cellular compatibility needed for mammalian research and therapeutic development.

Investigating the genetic determinants of adenine base editing using a fluorescence-based CRISPRi screen

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ABSTRACT

- **Background:** Base editors are programmable genome editing tools which do not rely on the introduction of double-stranded breaks (DSBs) into the genome. Adenine base editors (ABEs) utilize an adenosine deaminase enzyme to convert target adenines into inosine intermediates. This ultimately facilitates the efficient incorporation of A•T to G•C single point mutations. ABEs use an intermediate consisting of a I•T non-canonical mismatch paired with a 5' nick from Cas9 on the T-containing strand. It is currently unknown how this unique DNA damage product is processed by cellular DNA repair factors to yield the desired G•C outcome. Base editors have far-reaching applications, most notably as therapeutics for genetic diseases or as tools for disease modeling in human cells, and a detailed understanding of the underlying genetic determinants governing base editing is necessary for therapeutic applications. Such information could also provide useful information for engineering more efficient and precise base editors.
- **Methods:** To accomplish this, we combined targeted gene knockdown via CRISPR interference (CRISPRi) with a fluorescent reporter for ABE editing. Using a library of CRISPRi gRNAs targeting 440 DNA repair genes, we performed a CRISPRi screen and identified both negative and positive regulators of adenine base editing. These genes were validated in a non-pooled setting with editing performed on the reporter as well as at endogenous loci.
- **Results:** Validation of genes revealed mismatch repair (MMR) proteins such as Replication Factor C (RFC) and the heterodimer MutSa are strong positive regulators of ABE editing, with their knockdown resulting in significant decreases in A•T to G•C editing. Conversely, base excision repair (BER) genes such as LIG3 are strong negative regulators of editing, with their knockdown causing significant increases in A•T to G•C editing.
- **Conclusion:** These data constitute the first mechanistic studies into A•T to G•C editing by ABE.

Redefining AAV Manufacturing Using Stable Producer Cell Lines as a New Paradigm

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Lonza

ABSTRACT

- **Background:** AAV-based gene therapies hold immense potential and have emerged as one of the fastest evolving therapeutic modalities. Yet, manufacturability of these next-generation therapeutics remains a challenge, with process scalability and the increasing cost of goods as limiting factors for making broader therapeutic access feasible.
- **Methods:** Our AAV producer cell line (PCL) technology is based on a proprietary approach, in which all of the AAV-producing components are stably integrated into a cell line and AAV production is triggered using a small molecule inducer. This platform has been evaluated across multiple AAV products, and at scales up to pilot-scale bioreactors, for various target product profiles such as genomic titers, full capsid ratio, infectivity and purity.
- **Results:** The established platform process enables the consistent generation of productive PCL clones across several serotypes and genes of interest while meeting or exceeding product quality attributes of traditional transient transfection methods, making it an excellent alternative to simplify and optimize AAV production efficiency, increase scalability, and improve batch consistency.
- **Conclusion:** This approach resulted in the development of a highly productive, scalable, helper virus-free stable cell line system capable of enabling large scale clinical and commercial manufacturing of AAV with reduced complexity and lowered costs of goods.

Highly efficient expression of DNA-peptide conjugates in growth-arrested cells

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ABSTRACT

- **Background:** Efficient delivery of DNA is a significant challenge for non-viral gene therapy. One major limitation is thought to be the translocation of DNA from the cytosol to the nucleus, particularly in non-dividing or slowly dividing cells, in which passive entry of DNA into the nucleus during mitosis is minimal. Minimizing DNA dose by maximizing delivery efficiency may reduce potential cytotoxic and immune response effects of DNA gene therapy.
- **Methods:** We developed PepTAG, a chemoenzymatic platform that leverages *E. coli* tRNA guanine transglycosylase to site-specifically install DBCO handles onto oligonucleotide stem-loops. These handles enable subsequent strain-promoted azide-alkyne cycloaddition to azido-functionalized nuclear localization sequence (NLS) peptides. Ligation of these peptide-oligo conjugates to genes creates a modular platform allowing the construction of user-defined peptide-gene conjugates. These conjugates were delivered to growth arrested HepG2, AC16, and HEK293T cells and gene expression was evaluated. Expression was quantified by flow cytometry, ELISA, and RT-qPCR.
- **Results:** The conjugation of NLS peptides to an eGFP gene cassette significantly increases the fraction of eGFP-positive growth arrested cells in a peptide-dependent manner. The increase in gene expression is specifically dependent on the enhanced translocation of the NLS-gene conjugate from the cytosol to the nucleus. The screening of distinct NLS peptide sequences reveals cell type specific preferences for NLS peptide sequence. The generality of the approach is demonstrated via the delivery of DNA payloads encoding both cytosolic and secreted genes, with sizes ranging from 1.3 to 7 kbp.
- **Conclusion:** These findings support the potential of DNA-NLS conjugates as a viable strategy for non-viral gene therapy by enhancing the nuclear delivery of therapeutic genes, minimizing the required DNA dose.

Mapping High-Risk Hepatic Targets: A Receptor-Focused Approach to Understanding Liver Toxicity Liabilities

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ABSTRACT

- **Background:** Liver toxicity remains a critical challenge in the development of advanced therapeutics, including gene based modalities. As new platforms increasingly interact with hepatocyte and non parenchymal cell pathways, understanding receptor level vulnerabilities associated with drug induced liver injury (DILI) is essential. Identifying molecular targets disproportionately represented among high risk compounds can guide safer therapeutic design and inform early risk mitigation strategies.
- **Methods:** We conducted a receptor target enrichment analysis using publicly available DILI datasets, evaluating odds ratios, false discovery rates, and external evidence support. Targets were categorized by statistical strength and biological relevance to hepatic function, including xenobiotic metabolism, bile acid transport, inflammatory signaling, and nuclear receptor activity. The analysis focused on identifying receptor families and metabolic enzymes with elevated association to high severity liver injury.
- **Results:** Multiple targets demonstrated strong associations with high DILI risk, including bile acid transporters, cytochrome P450 enzymes, inflammatory mediators, and select nuclear receptors. Several targets exhibited extremely elevated odds ratios, with some reaching statistical significance and others supported by independent evidence sources. These findings highlight mechanistic nodes where perturbation may exacerbate hepatocellular stress, disrupt bile acid homeostasis, or amplify inflammatory injury. The observed patterns suggest distinct clusters of high risk biology relevant to therapeutic payload design, vector tropism, and off target engagement.
- **Conclusion:** This analysis provides a receptor level framework for anticipating liver toxicity liabilities in emerging therapeutic modalities. By identifying molecular targets disproportionately associated with high DILI risk, the work supports safer design principles, improved preclinical screening, and more informed risk assessment. These insights contribute to the broader goal of advancing gene therapy platforms with enhanced hepatic safety profiles.

In Vitro Optimization of Trans-Splicing Group I Introns for Targeted Therapy of JAK2V617F-Driven Myeloproliferative Neoplasms

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ABSTRACT

- **Background:** Trans-splicing group I intron ribozymes are catalytic RNAs that can be engineered to reprogram disease-associated mRNAs, making them promising tools for targeted RNA-based therapy. Trans-splicing efficiency depends critically on the external guide sequence (EGS) appended to the ribozyme's 5' terminus, but no high-throughput method previously existed to identify optimal EGSs from large randomized libraries, since successful EGSs are not themselves incorporated into the final splice product.
- **Methods:** We developed RABADOCS, a double combinatorial selection strategy that indirectly identifies high-efficiency EGSs from a library of $\sim 3 \times 10^7$ randomized sequences via high-throughput sequencing. We applied this platform to optimize ribozymes targeting the G1849U mutation site in JAK2 V617F mRNA, the most common driver mutation in classical BCR-ABL1-negative myeloproliferative neoplasms (MPNs).
- **Results:** RABADOCS selected 23 candidate EGSs from the library, of which 7 showed high in vitro splicing efficiency (20-35%) and 9 showed intermediate activity. In contrast, 6 randomly chosen EGSs showed no detectable splicing activity, confirming that the selection strategy successfully enriches for functional, high-efficiency sequences over random background.
- **Conclusion:** RABADOCS enables efficient, high-throughput identification of high-performing EGSs from large randomized libraries, overcoming a key bottleneck in trans-splicing ribozyme engineering. This platform provides a generalizable strategy for optimizing trans-splicing efficiency and expanding the therapeutic utility of group I intron ribozymes for MPN and other mutation-targeted applications.

Systemic neuronal Caveolin-1 overexpression delays motor neuron and neuromuscular degeneration in a TDP-43 mouse model of ALS

Jerica Ju, Vinh Ta, Kate Kurata, Michael Van, Elinor Wong, Kenneth Ohgi, Shanshan Wang & Brian P. Head

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ABSTRACT

- **Background:** TDP-43 proteinopathy is observed in over 95% of ALS cases, yet effective disease-modifying therapies remain elusive. We previously showed that neuron-specific overexpression of caveolin-1 via SynCav1 extended survival and delayed neurodegeneration in an SOD1 mouse model of ALS. Here, we further assessed the effect of systemic delivery of AAV-PHP.eB-SynCav1 in the TDP-43A315T mouse model of ALS.
- **Methods:** Synapsin-driven Cav-1 (SynCav1) was used to overexpress neuronal Cav-1. AAV-PHP.eB-SynCav1 or AAV-PHP.eB-SynNull (3×10^{11} v.g./mouse) was systemically delivered via retro-orbital injection into 10-week-old TDP-43A315T female mice. At 24 weeks and 32 weeks, spinal cord and muscle tissue were collected for biochemical and histological analysis. To assess lower motor neuron (LMN) morphology and neuromuscular degeneration, immunofluorescence was performed on spinal cord and gastrocnemius muscle, respectively. RT-qPCR analysis was used to quantify transcription of various neurotrophic signaling markers. Immunoblotting was used to assess membrane lipid raft-localized GluA2 protein levels.
- **Results:** At 24 weeks, SynCav1-treated mice showed preserved motor neuron (MN) morphology, maintained neuromuscular junction (NMJ) innervation, and normalized pathologically driven neurotrophic signaling in the spinal cord (SC). Immunoblotting revealed that SynCav1 increased calcium-impermeable GluA2 in membrane lipid raft of lumbar SC. However, these effects diminished with disease progression: by 32 weeks, lumbar MN loss and NMJ denervation were no longer significantly different between TDP-43-SynNull and TDP-43-SynCav1 mice, although partial protection was observed in the cervical spinal cord.
- **Conclusion:** These findings establish SynCav1 as an effective early-phase neuroprotective strategy and highlight the need for combinatorial approaches to achieve sustained protection.

In Vivo Selective Gene Editing of Hematopoietic Stem Cells Using CD117-Targeted Lipid Nanoparticles

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ABSTRACT

- **Background:** Nitto has pioneered targeted lipid nanoparticle (LNP) technologies and extended our technology to in vivo gene editing of hematopoietic stem cells (HSCs), offering a potential alternative to complex and costly ex vivo HSC therapies.
- **Methods:** Anti-CD117 VHH-conjugated LNPs were developed using EGFP mRNA delivery in CD34⁺ hu-NSG mice. Lead formulations were screened for HSC delivery, biodistribution, germline exposure, and cytokine responses. In vivo gene editing was assessed using adenine base editor 8e. (ABE8e)-mediated PCSK9, B2M or HBG1/2 base editing. To suppress hepatic editing, miR-122 target sequences were incorporated into the editor mRNAs.
- **Results:** Among human-reactive anti-CD117 VHHs, one candidate showed the highest EGFP expression in HSCs and hematopoietic stem and progenitor cells (HSPCs) in CD34⁺ hu-NSG mice and was selected for further study. Importantly, it preserved stem cell factor (SCF) signaling, with no effect on SCF-dependent proliferation or CD117 phosphorylation. Screening of ionizable lipids identified several formulations achieving up to 80% EGFP-positive HSPCs in vivo, from which a lead LNP was selected. Biodistribution studies showed predominant liver accumulation, no detectable RNA delivery to oocytes, and minimal cytokine induction. The lead LNP achieved 50% B2M deletion and 50% PCSK9 and 25% HBG1/2 base editing in HSPCs. Incorporation of miR-122 target sequences into the ABE8e mRNA reduced hepatic editing by approximately 90%, while maintaining robust editing in HSPCs, demonstrating effective liver-specific suppression of gene editing activity.
- **Conclusion:** We establish a highly specific in vivo HSC gene-editing platform integrating SCF-neutral CD117 targeting, HSC-optimized LNP chemistry, and liver-selective miRNA dependent silencing. The platform enables delivery of diverse editing payloads, providing a versatile, scalable, and clinically translatable alternative to ex vivo HSC gene therapy.

Ligand-targeted lipid nanoparticles for replicon RNA delivery to lymphatic endothelium in head and neck squamous cell carcinoma

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ABSTRACT

- **Background:** Head and neck squamous cell carcinoma (HNSCC) responds poorly to checkpoint blockade, in part because CD8 T cell priming is inadequate and effector infiltration is sparse. Priming occurs in the sentinel and tumor-draining lymph nodes, where lymphatic endothelial cells (LECs) and fibroblastic reticular cells produce the CCR7 ligands Ccl19 and Ccl21 to organize the T cell zone, recruit naive and central memory T cells alongside mature dendritic cells, and govern lymphocyte entry and egress. Sustained local production of these chemokines could enlarge the pool of tumor-reactive T cells and redirect their trafficking to the tumor. Self-amplifying replicon RNA (RepRNA) sustains expression from low doses, but lipid nanoparticles (LNPs) have no intrinsic tropism for lymphatic endothelium. The cyclic RGD peptide (cRGD) is a well-established ligand for integrins on vascular endothelium and has not previously been evaluated against lymphatic endothelium.
- **Methods:** Primary mouse LECs (pMLECs) were isolated and verified by Prox1 and podoplanin (PDPN) expression. RepRNA encoding NanoLuc luciferase was formulated into LNPs and surface-functionalized by bioorthogonal conjugation with either cRGD or a PDPN-binding aptamer. Targeted and untargeted formulations were compared by luminescence.
- **Results:** cRGD-targeted RepRNA-LNPs produced a 34-fold increase in NanoLuc signal over untargeted particles, identifying cRGD as a potent ligand for lymphatic endothelium despite its established association with blood vasculature. The PDPN-directed aptamer produced a 5-fold increase, indicating that receptor specificity alone does not predict delivery efficiency.
- **Conclusion:** cRGD substantially enhances RepRNA-LNP delivery to lymphatic endothelium in vitro. In vivo studies are underway to assess cRGD and aptamer targeting after sentinel node and peritumoral injection, and to determine whether sustained Ccl19/Ccl21 expression from node-resident cells increases dendritic cell recruitment, T cell priming, and CD8 infiltration in orthotopic 4MOSC2 tumors.

Testing Geroscience Therapeutics with an Isogenic Brain on a Chip Model

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ABSTRACT

- **Background:** Aging is the strongest risk factor for central nervous system diseases, yet 2D cultures and animal models incompletely reproduce the cellular complexity, vascular interface, and cell-type-specific vulnerabilities of the aging human brain. Geroscience drug development is further constrained by a lack of human-relevant systems assessing neuroinflammation, barrier function, and treatment response.
- **Methods:** We used Emulate's microfluidic Brain-on-a-chip (BoC), incorporating isogenic iPSC-derived neurons, microglia, astrocytes, and pericytes with iPSC-derived brain microvascular endothelial cells under perfusion to mimic a blood-brain barrier (BBB). To model an aged, inflammatory-primed state, the system was challenged with cytokines and candidate geroscience drugs. Cells were collected for single-cell RNA sequencing (scRNA-seq) and immunofluorescence imaging, with BBB permeability assessed in parallel. Because transposable element (TE) derepression is a hallmark of aging and driver of sterile inflammation, we analyzed TE expression as a vulnerability readout.
- **Results:** The fully isogenic, five-cell Brain-Chip maintained distinct neural and vascular populations and established a functional blood-brain barrier. Inflammatory challenge with 100 ng/mL TNF produced coordinated neurovascular responses, including altered barrier permeability and cell-type-specific transcriptional activation. scRNA-seq identified astrocytes as the most transcriptionally responsive, whereas mature neurons showed the greatest TE dysregulation. Followed by TNF, 2-day treatments with a reverse transcriptase inhibitor differentially attenuated these inflammatory and TE signatures, with effects varying across cell populations and corresponding barrier changes. **Conclusion:** This geroscience-focused isogenic brain-on-chip reproduces key multicellular and vascular features of human brain and identifies distinct cellular vulnerabilities to inflammatory and TE dysregulation. By integrating single-cell profiling with functional barrier measurements, the platform provides a scalable system for evaluating geroscience interventions and prioritizing therapies for translational development.

Keeping Pace with Emerging FDA Expectations Using a Comprehensive Genomic Safety Assessment Platform

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ABSTRACT

- **Background:** Gene editing technologies, including CRISPR-Cas nucleases and base editors, have emerged as powerful therapeutic modalities capable of introducing targeted genomic modifications. Despite their promise, unintended off-target activity remains a critical safety concern that can impact therapeutic development. As gene editing platforms continue to evolve, comprehensive approaches are needed to assess genomic safety and characterize potential off-target effects.
- **Methods:** Here, we demonstrate the SeQure™ platform as a regulatory-aligned genomic safety assessment framework. The framework consists of three stages: (1) off-target site nomination using orthogonal [CL3.1] approaches, including in silico, biochemical and cellular nominations; (2) confirmation of editing activity at nominated off-target sites; and (3) detection of structural variations, including translocations, deletions, and other rearrangements.
- **Results:** By integrating orthogonal analyses of nomination, confirmation, and structural variation, this framework provides a comprehensive approach to off-target risk assessment for CRISPR-based therapies.
- **Conclusion:** This framework aligns with recent FDA guidance and supports IND-enabling therapeutic development.

Long-term effects of systemic delivery SynCav1 on TDP-43-induced neurodegeneration and memory loss in an FTD mouse model

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ABSTRACT

- **Background:** Dysregulation of TDP-43, an RNA/DNA-binding protein, occurs in half of frontotemporal dementia (FTD) cases and is strongly implicated in cognitive dysfunction and neurodegeneration. Caveolin-1 (Cav-1), a membrane lipid raft scaffolding protein, has been shown to support neuronal signaling and cellular resistance in vitro and in vivo. We previously showed that SynCav1 (5×10^{11} vg/mouse) prevents early memory loss in TDP43A315T mice. However, significant anxiety-like behavior was observed in the SynCav1-treated animals. Thus, the current study investigated whether lower doses of SynCav1 can eliminate this off-target effect while still maintaining neuroprotection at a late disease stage.
- **Methods:** AAV-PHP.eB-SynCav1 (2×10^{11} vg/mouse) or AAV-PHP.eB-SynNull was delivered systemically to 10-week-old female TDP-43A315T mice. Transgene-negative littermates were used as controls (TgN). Anxiety and memory were assessed at 32 weeks by open field and fear conditioning test, respectively. Immunoblotting was used to assess Cav-1 and GluN2A expression in the hippocampus and prefrontal cortex. Nissl staining was used to quantify the lateral ventricle size.
- **Results:** Sustained Cav-1 overexpression was observed in the frontal cortex and hippocampus of TDP-43-SynCav1 mice at 22 weeks post-delivery. Lower-dose SynCav1 did not increase anxiety-like behaviors. Fear conditioning tests revealed impaired contextual memory in TDP-43-SynNull mice compared to TgN, whereas TDP-43-SynCav1 mice exhibited preserved memory compared to TDP-43-SynNull. Immunoblotting revealed higher hippocampal GluN2A expression in TDP-43-SynCav1 mice compared to TDP-43-SynNull mice (1.06 vs 0.86, $p = 0.015$). TDP-43-SynNull mice showed enlarged ventricular area compared with controls, whereas SynCav1 preserved ventricular area, suggesting reduced brain atrophy.
- **Conclusion:** Lower-dose SynCav1 confers durable neuroprotection in late-stage TDP-43A315T mice by preserving contextual memory, reducing ventricular enlargement, and maintaining hippocampal GluN2A expression.

Hematopoietic stem cell gene therapy for Mucopolysaccharidosis type IIIC

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ABSTRACT

- **Background:** Mucopolysaccharidosis type IIIC (MPSIIIC) is a neurodegenerative lysosomal storage disorder (LSD) caused by mutations in HGSNAT, a lysosomal transmembrane protein. The disease is characterized by heparan sulfate (HS) accumulation, neuroinflammation, developmental delay, and neuronal cell loss leading to premature death. Our group demonstrated that gene-modified hematopoietic stem and progenitor cell (HSPC) transplantation rescues cystinosis, another LSD also caused by mutation in a lysosomal transmembrane protein. Therefore, we hypothesized that this could be translated to treat MPSIIIC.
- **Methods:** We generated an MPSIIIC mouse model that recapitulates key disease phenotypes, including HS accumulation, splenomegaly, neurological defects, and the presence of disease-specific non-reducing end carbohydrates (NREs). In proof-of-concept studies, wild-type HSPCs transplanted into MPSIIIC mice differentiated into microglia-like cells in the central nervous system and macrophages in peripheral tissues, restoring HGSNAT activity and reducing HS, inflammation, and locomotor defects.
- **Results:** For clinical translation, we developed PPL-004, an autologous *ex-vivo* HSPC gene therapy using a lentiviral vector encoding human HGSNAT. Following the transplantation of PPL-004 in our mice, we confirmed engraftment and observed a recovery in enzymatic activity across tissues. Treatment resulted in significant reduction of HS and disease-specific NREs in the brain. Furthermore, treatment ameliorated locomotor defects, and reduced macrophage activation in tissues. RNA-seq analysis, also showed that the upregulation of inflammatory gene expression was corrected.
- **Conclusion:** To advance this promising therapy into the clinic, we held a successful Pre-IND meeting with the FDA in June 2026 in which the agency agreed with our proposed regulatory, manufacturing, and preclinical strategies. We are now moving forward with the final IND-enabling studies with the ultimate goal of delivering an autologous HSPC gene therapy to patients with MPS IIIC.

High-Resolution Capillary Electrophoresis Workflows for Quality Assessment of sgRNA and Cas9 mRNA in CRISPR Therapeutic Development

Jane Luo, Peter Holper, Marcia Santos & Tingting Li

SCIEX

ABSTRACT

- **Background:** As CRISPR-based therapeutics progress toward clinical development, robust analytical methods are needed to characterize the purity, integrity, and composition of critical RNA components. Co-formulation of sgRNA and Cas9 mRNA is a common strategy for CRISPR-Cas9 therapeutics; however, the nearly 45-fold difference in RNA length between sgRNA (~100 nt) and Cas9 mRNA (~4.5 kb) presents significant challenges for analytical characterization, ratio determination, and quality control. This study evaluated capillary electrophoresis (CE)-based approaches for characterizing the sgRNA and Cas9 mRNA.
- **Methods:** High-resolution capillary gel electrophoresis, with laser-induced fluorescence (CGE-LIF) and UV detection (CGE-UV), was used to characterize the purity and integrity of sgRNA and Cas9 mRNA individually and in mixtures prepared at different sgRNA-to-Cas9 mRNA ratios.
- **Results:** The CGE-LIF method demonstrated strong repeatability and robust performance in the purity analysis of a Cas9 mRNA sample, with %RSD of 0.4% for migration time and 1.0% for corrected peak area percentage. Purity differences between two Cas9 mRNA lots were also detected, revealing insights about lot-to-lot variation. A high-resolution CGE-UV method enabled differentiation of full-length sgRNA from a single-nucleotide truncated variant and other impurities, supporting quantitative assessment of low-level impurities. Simultaneous analysis of sgRNA and Cas9 mRNA mixtures by CGE-UV demonstrated excellent agreement between theoretical and measured component ratios, yielding an R^2 value of 0.9997 across the tested range.
- **Conclusion:** These results demonstrate the utility of CE for characterization of multiple critical quality attributes of CRISPR-derived therapeutic materials, including sgRNA integrity, impurity profiling, Cas9 mRNA quality, and quantitative assessment of sgRNA-to-Cas9 mRNA formulation ratios. The workflows provide high-resolution analytical support for process development, quality assessment, and comparability studies of next-generation genome-editing therapeutics.

Nanopore sequencing unravels complexities in integration site analysis in recombinant cell lines

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ABSTRACT

- **Background:** Recent emergence of a wide range of novel modalities such as Cell and Gene Therapy (CGT) and mechanisms for modifying genomes has created a paradigm shift for biologics manufacturing. Despite this changing landscape, for decades the tests required to characterize these engineered cell lines, as required by global health authorities and regulatory agencies, have consisted of a complex collection of aging, low-resolution assays. These include Sanger sequencing, Southern blotting, FISH or SKY analysis, and quantitative PCR.
- This desire for simplified testing is often complicated by the varying nature of testing requirements outlined in global guidance documents, including ICH Q5B, EU Phar Chapter 5.14, and FDA Draft Guidance for Allogenic Cell Therapy and Gene Therapy. Despite these disparities, the intent of these documents is clear: utilize testing strategies that provide the highest resolution of characterization to ensure not only product safety but also safety to the patient. One technology fully capable of achieving the desired level of resolution in a rapid and simplified format is Next Generation Sequencing (NGS).
- **Methods:** Here, we showcase two studies using engineered cells to demonstrate the power and flexibility of NGS to address and potentially simplify current testing requirements.
- **Results:** N/A
- **Conclusion:** The two studies aim to demonstrate the potential of NGS to revolutionize testing requirements for the characterization of engineered cell lines used in emerging modalities such as cell and gene therapy.

Raising the good copy: cortical organoids as a discovery system for dosage-restoring antisense oligonucleotides

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ABSTRACT

- **Background:** Severe neurodevelopmental disorders are commonly caused by insufficient dosage of a dose-sensitive transcription factor, leaving patients with an intact but under-expressed copy. Oligonucleotide drugs have transformed spinal muscular atrophy, Dravet and Angelman syndromes, yet each program is built one gene at a time. We asked whether patient-derived cortical organoids can work as a human discovery system for oligonucleotides that raise a chosen transcription factor.
- **Methods:** We built an iPSC cortical-organoid pipeline spanning healthy donors and three FOXG1-syndrome mutation classes. Oligonucleotides were screened first in neurotypic organoids by qPCR, a wild-type-first triage that discards inactive candidates before patient material is spent. Leads advanced to patient-derived organoids, profiled by transcriptomics, allele-resolved sequencing, imaging and bioenergetics, and then to Foxg1-mutant mice receiving a single neonatal intracerebroventricular injection, read out by transcriptome, protein markers, brain morphometry and behavior.
- **Results:** Screening in neurotypic organoids rapidly nominated a single lead, ASO4, that raised endogenous FOXG1 by ~55% where other candidates did not. In patient organoids ASO4 amplified FOXG1 per cell and re-engaged forebrain-identity and mitochondrial programs. A single neonatal injection in Foxg1-mutant mice raised Foxg1, corrected the mitochondrial deficit genotype-specifically, normalized altered molecular paths, and partially rescued brain size. The molecular target is confined to forebrain across adult, fetal and organoid systems, and upregulation occurred only in actively transcribing cortical cells, two constraints against off-target overexpression.
- **Conclusion:** Neurotypic-organoid screening followed by patient and in vivo validation nominated and de-risked an upregulating oligonucleotide for a dose-sensitive transcription factor, providing a human route from screen to in vivo rescue.



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