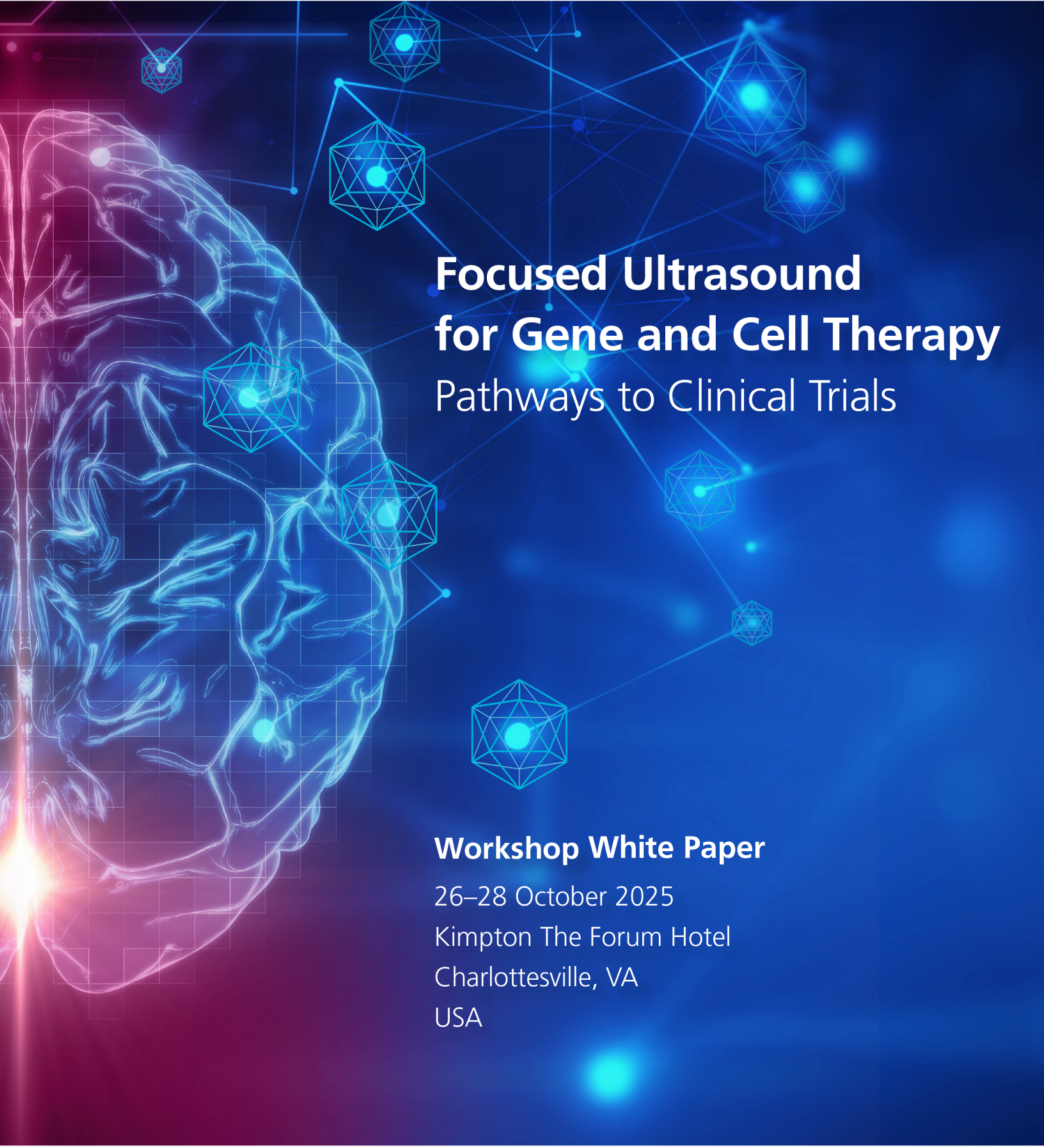

Focused Ultrasound for Gene and Cell Therapy

Pathways to Clinical Trials

Workshop White Paper

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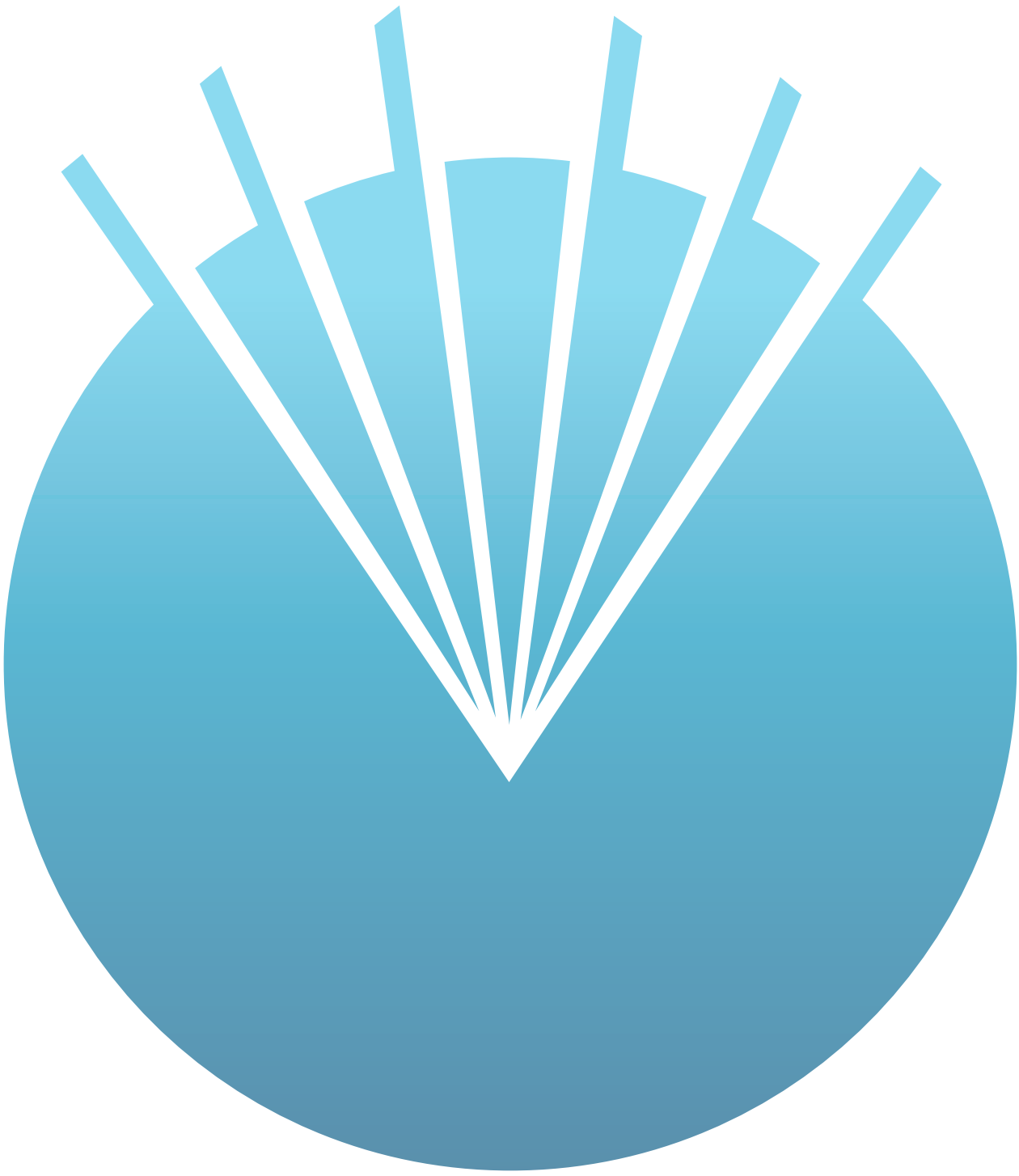

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Executive Summary

The Focused Ultrasound Foundation hosted the second workshop on focused ultrasound (FUS) for gene and cell therapy on October 27-28, 2025, in Charlottesville, VA. This workshop assembled a diverse group of experts from academia, industry, government, and the not-for-profit sector.

Significant preclinical progress was demonstrated across multiple applications. FUS-mediated blood-brain barrier (BBB) opening enables increases in adeno-associated virus (AAV) delivery to primate brains while reducing systemic doses, thereby addressing critical safety concerns. Novel engineered capsids further enhanced neuronal targeting and reduced liver accumulation. Beyond the brain, FUS improved gene delivery to the heart and liver, achieving functional rescue in disease models. Innovative approaches using thermally controlled chimeric antigen receptor (CAR) T-cells demonstrated enhanced tumor trafficking and immune modulation, with clinical trials in sight.

Participants identified critical barriers to clinical translation. A substantial gap exists between proof-of-concept studies and the investigational new drug (IND)-enabling work. Species differences in AAV receptor expression limit the predictive value of rodent studies. While infrastructure limitations, particularly the scarcity of FUS equipment at primate research centers, constrain necessary validation studies.

The group reached several strategic consensus points:

- Indication Selection: Prioritize diseases with established biomarkers and unmet needs, if possible monogenic, such as Huntington's disease and some forms of Parkinson's disease, over starting entirely from scratch. Leveraging already-approved therapies, such as onasemnogene abeparvovec (Zolgensma), may de-risk FUS components.
- Routes of Administration: Abandon one-size-fits-all approaches. Intravenous delivery remains a target but still faces significant limitations for brain applications. Intra-cerebral spinal fluid (CSF) routes combined with FUS targeting to deep structures show promise. Additionally, disease-specific optimization is essential.
- Device and Trial Design: Early trials could employ spoke-and-hub models with centralized expertise rather than distributed sites. Use natural history controls rather than placebo arms where appropriate for rare diseases with severe outcomes.

Path Forward

The FUS Foundation proposed establishing a FUS Gene Therapy Translation Consortium (FGTTC) modeled after the Bespoke Gene Therapy Consortium, creating a shared playbook to eliminate redundant work. Immediate priorities include generating additional non-human primate (NHP) safety and efficacy data, share resources such as standardized protocols across institutions, and supporting early academic-led clinical trials that can demonstrate proof-of-concept and attract industry investment.

Opening Remarks

Focused Ultrasound Foundation

Frédéric Padilla, PhD, welcomed participants to the workshop. He also thanked the scientific advisory board of the gene and cell therapy program: **Isabelle Aubert**, PhD; **Catherine Bollard**, MBChB, MD; **Elisa Konofagou**, PhD; **Jonathan Lindner**, MD; **Mikhail Shapiro**, PhD; **Bob Smith**, MBA; **Ramasamy Paulmurugan**, PhD and **Michael Werner**. This workshop was to expand the focus of FUS and gene therapy to large organs and cell therapies for brain tumors.

Focused Ultrasound Foundations (FUSF) Perspective: A Framework for Program Selection

Alec Batts, PhD, reported on the FUS Foundation effort to produce a framework analysis to identify pathways for a first-in-human trial of FUS and gene therapy for the brain. There has been extensive preclinical research, gene therapy is inherently complex, and adding a FUS device will increase regulatory complexity. Current methods for delivering therapy to the brain are quite complicated and include intraparenchymal (IP), delivery to the cerebrospinal fluid—including intracerebroventricular (ICV), intracisterna magna (ICM) and intrathecal (IT) — and intravenous (IV). When selecting an indication, consider leveraging already-developed gene therapy constructs to accelerate clinical trials while balancing scientific readiness, operational feasibility, and commercial viability (Figure 1). Considerations for adding FUS to the trial include whether there is a need for localized FUS-mediated delivery, preclinical validation for a FUS-BBB opening paradigm for this capsid and route administration, whether a systemic dose reduction is expected with FUS-BBB opening, whether there is a need for a local delivery boost with engineered brain-penetrating capsids, and how to evaluate optimized FUS treatment parameters and devices suited for a particular indication or AAVs. Based on these considerations, some of the priority indications include Parkinson's disease, SMA, Huntington's disease, and Duchenne muscular dystrophy.

Figure 1: A framework for indication selection

The current landscape analysis includes a lot of preclinical data with a large gap between proof-of-concept work with FUS delivery of AAV and INitial Targeted Engagement for Regulatory Advice on CBER/CDER ProducTs (INTERACT)- or IND-enabling studies. In designing first-in-human studies, it might be helpful to start with a highly prevalent disease with an unmet need or to establish a predicate in rare-disease populations. The FUSF also suggested a few recommendations for action. For example, the Foundation for the National Institutes of Health (FNIH) Bespoke Gene Therapy Consortium (BGTC) can serve as an instructive model by creating a roadmap for translating FUS and gene therapy to the clinic. This model helps to eliminate redundant work across individual programs. A proposition is to establish a similar consortium model, a Focused Ultrasound Gene Therapy Translation Consortium (FGTTC), to create a playbook for the community. As part of this, there would be an emphasis on middle-ground preclinical studies that would lead to an Interact meeting. Dr. Batts provided a preview of how such "playbook" could be structured as an online reference repository on the suitability of different indications and gene therapy strategies for translation to the first-in-human clinical trial with FUS and gene therapy.

Questions

Q: What criteria and strategic considerations should guide the prioritization of clinical indications for gene therapy trials, and how should the necessary expertise be organized to execute these trials successfully?

Dr. Batts recommended that gene therapy trial prioritization should focus on indications with established biomarkers and ongoing trials, particularly monogenic disorders that offer regulatory advantages like Food and Drug Administration (FDA) rare-disease designations. The selection criteria should emphasize "gene therapy readiness," choosing conditions where scientific foundations are already well-established for successful first-in-human trials. Equally important is assembling the right team by identifying clinical experts in the target disease areas and operational experts capable of managing complex trial coordination. Organizing this collaborative network of specialists should be a key focus, as successful gene therapy trials require seamless integration of scientific expertise and operational excellence.

Q: While technical and regulatory frameworks are essential for establishing an FGTTC consortium, the financial reality cannot be overlooked. The BGTC consortium required approximately $100 million to advance just 8 to 9 programs from preclinical to clinical stages, highlighting the substantial resources needed for both initiation and long-term sustainability.

Dr. Padilla replied that the FUS Foundation plans to hold a public comment period on their website as the initial step for gathering input. Rather than solely supporting the entire effort, they aim to identify and kickstart a few key initiatives through partnerships and collaborations, using public feedback to guide their approach.

Q: What are the major impediments and pinch points in gene therapy development; are they primarily related to manufacturing challenges, toxicology and biodistribution issues, pharmacology concerns, or other specific areas?

Dr. Batts said that the gene therapy field is currently experiencing significant preclinical momentum. Yet researchers find themselves at a pivotal moment were identifying and understanding future "pinch points" has become crucial for continued progress. This is an optimal time to analyze the barriers, whether they stem from manufacturing complexities, toxicology and biodistribution concerns, pharmacology issues, or other technical barriers.

Q: When does private investment and venture capital become interested?

The gene therapy investment landscape presents a severe "valley of death" where securing capital is extremely difficult until companies progress beyond phase I trials. Even companies with superior technology face challenges because good manufacturing practices are expensive, and market entry barriers are high. While raising capital becomes much easier after successful phase I-II trials, reaching that point requires navigating expensive early development with limited financial support, creating a fundamental funding gap that impedes promising therapies from advancing to clinical stages.

Session 1: Overview Presentations

Large Organ Gene Therapy: Challenges/Opportunities, Recent FUS Data

Jonathan Lindner, MD, discusses advanced strategies for improving gene therapy delivery to large organs like the heart, kidneys, and skeletal muscle. Systemic administration faces significant challenges in achieving both efficacy and safety. The fundamental trade-offs between viral vectors (such as AAVs) and non-viral vectors (such as lipid nanoparticles, or LNPs) underscore that successful gene therapy for large tissues requires effective systemic delivery while maintaining safety.

Dr. Lindner mentioned a promising approach using FUS and more broad-field ultrasound (US) cavitation to enhance gene delivery. Combining LNPs with microbubbles and US improved both efficiency and duration of gene transduction. Surprisingly, simply co-administering LNPs with microbubbles (without physically attaching them) works equally well, making the approach more clinically viable. This technique not only increases delivery but also extends the duration of gene expression, which is particularly valuable for CRISPR applications where persistent transduction is crucial.

A major focus is addressing the safety concerns with AAV vectors, particularly the dose-dependent adverse events that have led to multiple clinical trial shutdowns. Achieving therapeutic efficacy with AAV9 in cardiac applications requires dangerously high doses that cause thrombo-inflammatory complications. One solution could be to use contrast ultrasound cavitation to improve AAV delivery efficiency, potentially enabling lower, safer doses while maintaining therapeutic benefit. Preclinical work using reporter genes (luciferase, Td-tomatoe, and sodium iodide reporter) combined with non-invasive imaging (optical, PET, SPECT) at different doses of AAV9 found that cavitation increased AAV9 transduction to the heart. This approach uses FDA-approved ultrasound settings and clinically available contrast agents, making translation to human trials more feasible.

Ultrasound-enhanced delivery works through multiple mechanisms beyond opening blood vessels; it also increases tissue blood flow by activating shear-dependent pathways that involve red blood cells, which release nitric oxide and ATP. A mouse model of hypertrophic cardiomyopathy was created by inserting an aggressive human gene variant via CRISPR. At 6 weeks of age, researchers used ultrasound cavitation to deliver a rescue gene (human myosin-binding protein C3) to 40% to 60% of the heart, resulting in significant improvements in cardiac function, including reduced dilation, lower left ventricular mass, and better ejection fraction.

Additionally, Dr. Lindner established a NHP multimodality imaging center, which includes advanced imaging equipment (PET, SPECT, computed tomography (CT), MR, ultrasound, and angiography) and is available to researchers with National Institutes of Health (NIH) funding at a lower cost than using a contract research organization (CRO).

Questions

Q: What are your key takeaways about ultrasound-mediated gene delivery across different organs and vascular systems?

Dr. Lindner replied that acoustic conditions for cavitation-mediated gene delivery must be tailored to each organ's unique vasculature, with the liver requiring only about half the acoustic pressure of other organs due to its fenestrated blood vessels. However, this area of research is still in the preliminary stages.

Q: What is the optimal timing window between nanoparticle and microbubble administration, and does this differ between AAVs and liposomes?"

Dr. Lindner responded that they attempted to attach AAV to microbubbles but abandoned this approach in favor of sequential administration. For safety-monitoring purposes, they injected AAV9 first, confirmed stability, then administered microbubbles and applied FUS. Liposomes were co-administered with microbubbles, with the microbubble charge carefully tuned to prevent problematic clustering while allowing the components to work together effectively.

FUS for Brain Gene Therapy: Challenges, Opportunities, and Recent FUS Data

Eliza Konofagou, PhD, provided a review of FUS for brain gene therapy. She explained that her own lab focuses on Parkinson's disease because gene therapy for dopaminergic neurons offers a comprehensive way to assess both expression and functionality. The BBB represents an obstacle for gene therapy and drug delivery. Unlike blood vessels in other organs, such as the heart, brain vessels contain tight junctions that make delivery extremely difficult. However, by circulating microbubbles in combination with FUS, the BBB can be temporarily relaxed, enabling drug delivery and gene therapy. FUS offers a unique combination of non-invasiveness and targeted delivery, unlike other delivery methods that are either invasive and targeted (e.g., direct injections) or non-invasive but untargeted (e.g., intranasal delivery or chemical approaches).

Microbubbles act as engineered resonators that respond to FUS. When activated, they create two regimes: stable cavitation and inertial cavitation. The acoustic pressure level determines the size of the barrier opening: lower pressure allows smaller molecules through, while higher pressure allows larger molecules to pass. For gene therapy, large vectors need to be delivered, which the team successfully demonstrated using inertial cavitation safely.[1]

Recent preclinical research showed that after injection into the CSF (ICM), FUS can improve the delivery of AAVs to the striatum.[2] An AAV library optimized for FUS delivery has also been created that expresses at higher levels in the brain than in the liver.[3] Additionally, PET-traceable AAVs for preclinical models were also developed.[4]

In Dr Konofagou's lab, using a rodent model of Parkinson's disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), both neuroprotection and restoration of dopaminergic neurons were demonstrated after FUS-mediated delivery of AAVs.5 When they delivered AAV9 expressing GDNF (glial-derived neurotrophic factor) after MPTP administration, they observed significant neuronal survival in the substantia nigra and functional improvement.[5]

The lab has developed a method to use standard diagnostic ultrasound transducers for both imaging and BBB opening.[6] This approach enables cavitation mapping, allowing visualization in real time where cavitation occurs throughout the brain. In their studies with diagnostic ultrasound and AAV9 delivery in MPTP mice, they achieved up to a 76% increase in dendritic fiber networks in the substantia nigra and an increase in terminal density in the caudate putamen. In a mouse model, a safe and effective gene-editing technique using FUS to transiently open the BBB for transport of intravenously delivered CRISPR/Cas9 machinery to the brain was demonstrated.[7]

In non-human primates, a 50-fold increase in vector DNA with transduction of dopaminergic neurons in the substantia nigra compared with the contralateral hemisphere has been reported.[8] In a separate study, an increase of 200-fold in vector DNA was found after FUS BBBo in the striatum, with expression in both neurons and astrocytes. FUS-BBBo post ICM

injection of AAV (AAV2-HBKO) in NHP resulted in enhanced transduction in the striatum, the area targeted by FUS, with expression generally more robust in the putamen than in the caudate. Different AAV capsids show varying performance characteristics.[9] While AAV9 showed good brain transduction, it also caused significant peripheral transduction in the liver. AAV5 spared the liver and showed greater neuronal expression but had lower vector RNA levels despite higher vector DNA.[9]

For clinical translation, Dr. Konofagou described a portable FUS system with cavitation monitoring for BBB permeability. In a recent publication, they reported that cavitation dose correlated with amyloid reduction on PET imaging in patients with Alzheimer's disease, suggesting that cavitation monitoring could serve as a predictive tool for gene therapy dosing.[10]

Key opportunities in the field include engineering AAV capsids specifically optimized for FUS-BBB opening using stable cavitation dose to guide and predict gene copy numbers, reducing systemic dosing requirements, and demonstrating neuronal functionality restoration (not just vector delivery and expression). Major challenges include engineering better promoters and reporters for cell-type-specific expression (neurons versus astrocytes), leveraging PET and ultrasound imaging capabilities, detargeting the liver and peripheral organs to reduce off-target effects (where FUS can increase brain delivery while reducing peripheral exposure), and investigating multiple routes of administration.

Q & A Session with Dr. Konofagou and Dr. Lindner

Q: What is meant by the term 'whole brain'?

Dr. Konofagou acknowledged that calling the technology "focused" ultrasound is somewhat limiting, as the technology can be engineered to open larger brain regions or even the entire brain simultaneously by adjusting frequency and transducer geometry. However, safety remains a primary concern, as some clinicians worry about exposing the whole brain to potentially toxic substances at once, preferring quadrant-by-quadrant approaches even though diseases like Alzheimer's affect the entire brain.

Q: What is the status of developing chimeric AAV vectors that can target both astrocytes and neurons, similar to engineered adenoviral serotypes?

Dr. Konofagou replied that understanding AAV serotype specificity is still in the preliminary stages. If there is an AAV with reliable cell-type-specific expression, her lab would be interested in testing whether it translates to in vivo disease models.

Dr. Aubert added that viral tropism is challenging to predict across species, and transfecting astrocytes poses immune response risks that cell-type-specific promoters cannot fully mitigate. The broader topic of promoters and regulatory elements is complex enough to warrant its own symposium.

Q: Do you always need to co-administer microbubbles with the nanoparticles for this ultrasound approach to work, or is the targeting achievable without them?

Microbubbles are necessary for cardiac delivery but likely not for liver targeting. The key challenge is delivering LNPs to target tissues during peak concentration before they're cleared by the reticuloendothelial system (RES). Even with targeting moieties, LNPs struggle to exit the vasculature efficiently, which is why ultrasound-mediated disruption at peak LNP concentration is crucial for effective tissue uptake before systemic clearance.

Q: Can you target the microbubble?

With proper pulsing intervals, all microbubbles in the field can be cavitated based on vascular flow patterns. Targeting microbubbles is generally unnecessary, except in specific cases, such as treating arterial plaques, where high shear forces keep bubbles centered in large vessels. In these scenarios, targeting endothelial or plaque epitopes helps bubbles escape the flow stream and attach to the desired tissue.

Dr. Konofagou explained that contrast imaging can be pushed further to achieve higher resolution, potentially allowing visualization of individual microbubbles. The fields of contrast imaging and contrast-enhanced therapy are beginning to converge, opening possibilities for super-resolution imaging techniques.

Q: When you apply ultrasound-mediated delivery, do you see differences in transfection efficiency between different cell types, for example, between cardiomyocytes and non-cardiac cells, or between neurons and non-neuronal cells?

Dr. Konofagou answered that the neurovascular unit comprises multiple cell types beyond tight junctions. Expression patterns vary by cell type and promoter. CAG promoters show high expression in astrocytes, while synapsin promoters drive primarily neuronal expression.

Dr. Lindner also mentioned that ultrasound parameters, contrast agents, and pulse types must be specifically tuned for different target tissues.

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Session 2: Presentations

Thermogenic Control of CAR T Cells to Potentiate Brain Tumor Therapy

Lena Gamboa, PhD, discussed the development of thermally controlled CAR T-cell therapy for treating solid tumors, particularly brain cancers. While CAR T-cells have been transformative for liquid tumors, expanding this success to solid tumors remains challenging. A key limitation is the inability to spatially control T-cells once administered, as systemic delivery of CAR T-cells and their supporting drugs leads to dose-limiting toxicities.

To address this challenge, the team developed a "thermal bioswitch," a heat-activated gene-expression system triggered noninvasively via FUS.[11] The system uses mild hyperthermia (41-42°C, just above high fever temperature) to activate therapeutic genes within T-cells with millimeter precision. Notably, the bioswitch has very low background activity and is not activated by normal fever conditions. The system is tunable, allows for repeated activation cycles, and produces transient expression that remains localized to the heated tissue, preventing off-target toxicity.

This research demonstrates promising applications for brain tumors, which face major challenges including antigen heterogeneity, immunosuppressive microenvironments, and T-cell exhaustion. In preclinical models, the team successfully used thermally activated bispecific T-cell engagers to address antigen escape (targeting HER2-negative tumor populations missed by HER2 CAR T cells) and to counteract immunosuppression by eliminating myeloid-derived suppressor cells expressing NKG2D ligands.[12] These approaches enhanced the durability of therapeutic responses. Dr. Gamboa encouraged early FDA feedback, noting that heat is considered a safe trigger and that CAR T-cell therapies do not require large-animal models for preclinical evaluation, potentially accelerating clinical translation.

Questions

Q: How does the engineered CAR T cell work after activation, and how is it designed to express its therapeutic components?

Dr. Gamboa explained that the engineered CAR T cells are always active with continuous CAR expression, but they are enhanced with bispecific T-cell engagers that are only transiently expressed. While systemically delivered CAR T cells may not efficiently reach tumor sites, they use local delivery methods such as intrathecal or intraventricular administration. This positions the cells directly near the tumor, where they can be activated with additional biologics to enhance their therapeutic response at the targeted location.

Q: Have you measured the distribution and number of engineered CAR T cells at the tumor site before and after activation?

The effects depend on what is activated. IL-15 activation promotes CAR T-cell proliferation, while bispecific T-cell engagers targeting CD3 and NKG2D increase the number of anti-tumor effectors by recruiting bystander T cells, enhancing overall immune infiltration and kick-starting anti-tumor immunity.

Q: Have you been able to look at the persistence of CAR T cells, or do you have an opinion about that?

The team is currently looking at this, and a paper is under revision with a journal. Early signs indicate that in certain contexts the cell phenotype shifts toward a memory phenotype, and the pulsed activation approach helps reduce exhaustion compared with having bispecific T-cell engagers constantly active. This enhanced phenotype may improve CAR T-cell persistence.

Q: How do you address concerns about the heat shock promoter being sensitive to various stress factors in the harsh tumor environment, which could lead to unwanted activation?

The promoter was a synthetic design derived from endogenous heat shock promoters but modified to be heat specific. They isolated the heat-responsive elements while removing components that respond to other stressors, such as hypoxia and pH changes. Testing confirmed that this synthetic promoter responds only to heat, not to hypoxia or pH changes.

Q: How does your technology address antigen heterogeneity in glioblastoma?

The technology uses NKG2D T-cell engagers that target stress ligands, which are broadly expressed across tumor cells, including glioblastoma. These ligands are also reported to increase in expression under cellular stress such as chemotherapy. This approach addresses tumor heterogeneity and provides treatment options even when other targetable antigens are unavailable.

Q: Is there a suicide mechanism built into the CAR T cells to prevent cytokine release syndrome and ensure safety?

Dr. Gamboa said that they have not incorporated a suicide mechanism yet. Safety switches are commonly considered as part of regulatory safety planning, but the CAR T-cells have been well-tolerated in brain treatments so far, so a suicide switch could be added if needed.

Q: Can you share insights from your FDA interactions, particularly regarding the combination of FUS with gene and cell therapy products?

Dr. Gamboa shared that the FDA was helpful and thorough in providing feedback through the INTERACT meetings. A key lesson is that the more detailed and specific you can be about your intended final product in the INTERACT submission, the more actionable the feedback the FDA can provide. INTERACT submissions lacking sufficient detail may not be granted a meeting until additional information is provided to support a productive discussion with FDA reviewers. Overall, the FDA has been receptive and open to innovative technologies combining FUS with cellular therapies.

AAV Capsid Engineering for Liver Detargeting and Redirecting Cellular and Tissue Tropism

Jared Smith, PhD, presented research on a promising AAV capsid modification that improves neuronal targeting. The work originated from efforts to develop AAV vectors for heart and muscle applications, particularly for conditions like Duchenne muscular dystrophy, with an emphasis on liver detargeting, since AAV serotypes like AAV8 and AAV9 naturally accumulate heavily in the liver after IV injection.[13] The team used the NAVIGATE platform to screen various capsid modifications in two rounds of directed evolution using peptide-insertion libraries on AAV9.AAA (the liver detargeted backbone), the team identified peptides NVG7 and NVG13 that enhance cardiac and muscle transduction.[13] Wild-type AAV9 typically transduces astrocytes in the brain primarily, with only about 25% of transduced cells being neurons.[14] However, AAV9.AAA.NVG7 shifted this tropism dramatically, with approximately 80% of transduced cells being neurons rather than astrocytes.[13] This shift was observed across multiple brain regions, including the motor cortex, thalamus, striatum, and hippocampus.[13]

Dr. Smith emphasized that even at high doses IV, AAV9 crossing the BBB naturally is highly inefficient, transducing less than 1% of brain cells. However, AAV.NVG7 capsids showed significant increases in neuronal transduction, with some regions, such as the thalamus, reaching approximately 20% transduction. He noted that AAV shows region-specific preferences, with the thalamus being particularly receptive to transduction. In their work with blood-brain barrier-penetrant capsids targeting the transferrin receptor, they achieved nearly complete transduction of thalamic neurons but approximately half in the cortex.

Dr. Smith emphasized that AAV transduction profiles are not determined by avoiding certain cells; instead, it is determined by the AAV's affinity to specific cell surface markers. Even when achieving better BBB penetration, work to modify capsids to target particular cell types of interest remains ongoing. Dr. Smith highlighted examples such as CA2 hippocampal neurons and Purkinje neurons, which AAV9-based capsids consistently transduce efficiently, whereas adjacent cell types, such as cerebellar granule neurons, show essentially no transduction. The findings suggest these liver-detargeted, cardiac-targeted modifications serendipitously created a more neuron-preferring AAV variant.

Questions

Q: Was the AAV vector self-complementary or single-stranded?

All the mouse experiments used single-stranded AAV, while the NHP experiments used self-complementary AAV.

Q: Which receptor do the mutated capsids bind to or avoid, and which liver cells are affected?

The mutations likely work by detargeting the AAV receptor (AAVR) rather than targeting a new receptor, and the capsids still transduce many hepatocytes but at reduced levels compared with wild type.

Q: Is the targeting difference due to receptor-mediated binding or an intracellular effect?

Since only the capsid was modified while the transgene cassette remained identical, the difference is capsid-mediated, though temporal dynamics at different timepoints could also play a role.

Q: Could the liver still act as a sink with post-entry differences causing the observed vector genome distribution?

Dr. Smith replied that this was possible, but they had not looked at this issue yet. Early timepoint measurements (hours or days post-transduction) weren't performed for liver tissue.

Q: Do these capsids have potential for enhanced central nervous system (CNS) delivery in humans?

Yes, these modifications could be valuable for CNS applications, particularly when combined with mechanical delivery methods like FUS to enhance BBB opening and improve neuronal targeting while reducing astrocyte transduction.

FUS-mediated Gene Replacement Therapy and Gene Editing for the Treatment of Hemophilia

Carol Miao, PhD, described the development of ultrasound-mediated gene delivery (UMGD) as an alternative gene therapy approach for hemophilia A. Hemophilia A results from a deficiency of Factor VIII, a clotting factor produced in the liver, leading to severe bleeding disorders. While AAV-mediated gene therapy has shown promising results and received clinical approval, it faces limitations, including declining therapeutic levels over time in many patients and inaccessibility to significant patient populations, including pediatric patients and those with inhibitory antibodies.

Dr. Miao's work has focused on developing UMGD to overcome these challenges. This approach uses ultrasound-mediated microbubble cavitation to create transient pores in cellular and nuclear membranes, facilitating plasmid DNA entry and nuclear delivery for transgene expression. UMGD offers several advantages, including inherent site-specific targeting, the possibility of re-dosing, and reduced immunogenicity and toxicity compared with viral vectors. To address the persistence problem typically associated with plasmid DNA delivery, the researchers developed plasmid constructs that incorporate transcriptionally active elements to maintain an open chromatin structure, thereby sustaining transgene expression.

The team has progressively improved gene transfer efficiency from an initial 2- to 3-fold increase to over 10,000-fold improvements through optimization of delivery routes, ultrasound systems, and transducers. They successfully scaled up from mice to rats, pigs, and dogs, ultimately achieving therapeutic Factor VIII expression in both hemophilia A mice and dogs.[15,16]

In hemophilia A dog studies conducted in collaboration with researchers at the University of North Carolina, the team used a FUS transducer with transcutaneous application. They employed a transhepatic venous delivery system to occlude hepatic blood flow, allowing microbubbles and plasmid to persist in the liver during ultrasound treatment. This approach increased Factor VIII activity levels of 10% to 35%, which is therapeutically significant, as increasing activity from below 1% to 5% can convert severe hemophilia into mild disease. The treatment demonstrated phenotypic correction, with improved blood clotting times, and a good safety profile, with only transient elevations of liver transaminases that normalized within days.

The team was able to target liver sinusoidal endothelial cells rather than hepatocytes by adjusting ultrasound parameters. This advancement enabled the team to perform gene editing for hemophilia A using UMGD to deliver Cas9 protein and guide RNA. In a mouse model with a 5-base pair deletion in Factor VIII, they achieved 5% Factor VIII production and 4% successful gene correction. This cell-specific targeting approach represents a significant step toward the clinical translation of UMGD-based gene therapy and gene-editing strategies for hemophilia A.

Questions

Q: Did you conjugate the plasmid onto the microbubbles to create a natural hepatic dwell, since the microbubbles get removed by the RES and stick to Kupffer cells?

Dr. Miao answered that they tried using cationic bubbles but found the plasmid loading was not particularly good, and they spent about a year trying to optimize the cationic agent for loading without success. However, with ultrasound exposure, the cavitation occurred quickly following infusion of MB/nucleic acids solution to allow cargo delivery into the cells.

Q: Does a hydrodynamic injection volume cause dilution-mediated natural transfection in mice?

While hydrodynamic injections with high volumes can achieve sound transduction in mice, it cannot be done in humans. In these experiments, they used only 4 mL in dogs and 300 to 400 microliters in mice, injected slowly over one minute, and background checks showed essentially no transfection from volume alone. In the UMGD experiments, the MB/nucleic acids cargoes were not delivered via hydrodynamic injection.

Dissecting Routes to The Brain: CSF Administration Versus Systemic AAV Delivery in NHPs

Michal Fortuna, PhD, discussed the biodistribution patterns of AAV vectors in NHPs across different administration routes, with emphasis on intracerebroventricular (ICV) delivery and intravenous (IV) administration using novel blood-brain barrier-penetrant capsids.[17]

The primary study compared AAV9, a benchmark capsid, with the engineered capsid AAVPHP.eB using unilateral ICV administration in NHPs.[18] Using a human synapsin promoter to drive neuronal expression, the researchers analyzed brain biodistribution using immunohistochemistry and molecular biology. Results showed that AAVPHP.eB achieved superior neuronal transduction compared with AAV9 across all examined brain regions. Expression was primarily cortical, with motor cortex, somatosensory cortex, and visual areas showing the highest transduction. In contrast, subcortical structures, including the putamen, cerebellum, and deep brain regions, exhibited minimal to absent transduction with both serotypes.

ICV delivery was uneven, with patchy expression patterns throughout the cortex, with clusters of highly transduced cells adjacent to areas devoid of expression. These patches often colocalize with blood vessels and are hypothesized to result from viral clearance mechanisms along lymphatic pathways. Importantly, this patchiness did not produce apparent neuronal toxicity, as evidenced by the absence of microglial or astrocyte upregulation and no neuronal loss at six weeks post-transduction. Quantification across five cortical regions revealed that AAVPHP.eB achieved transduction rates ranging from approximately 5% to over 20% of neurons, while AAV9 remained lower (3-5% on average). Interestingly, no significant difference was observed between the ipsilateral and contralateral hemispheres despite unilateral injection.

Spinal cord analysis showed similar trends, with slightly higher transduction in distal thoracic and lumbar segments, though AAVPHP.eB's advantage was less pronounced (1.5-2-fold improvement). Biodistribution analysis via qPCR confirmed histological findings and revealed a critical limitation: despite CSF delivery, substantial virus rapidly leaked into systemic circulation, with the liver sequestering the majority of the vector, suggesting that dose escalation would primarily increase peripheral exposure rather than brain transduction.

Testing of intracisternal magna (ICM) delivery yielded results comparable with ICV administration, with predominantly cortical labeling, limited penetration into deep structures, and characteristic patchy expression patterns. Dr. Fortuna concluded that better capsids and delivery methods are needed for effective brain-wide transduction in primates, as capsids selected through rodent screening do not translate well to other species.

The presentation concluded with promising preliminary data on distinct delivery types (ICV, ICM, and IT) using a novel vector (CN1839). ICM administration with this vector produced a similar expression pattern to ICV administration, but analysis was ongoing. A novel brain-penetrant capsid from Dyno Therapeutics (bCap1) delivered IV dramatically improved results compared with AAV9, achieving up to 40% neuronal transduction in some cortical areas. Deep brain structures, including the putamen, substantia nigra, thalamus, and hippocampus, showed robust transduction. Future directions include combining primate BBB penetrating capsids and optimizing cell-type enhancers for minimally invasive precision CNS gene therapy.

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Session 3: Overview Presentations

Cell Therapy for Brain Tumors

Catherine Bollard, MBChB, MD, described the development and application of T-cell therapies for solid tumors, with particular emphasis on brain tumors and combination strategies. T-cell therapy evolved from donor lymphocyte infusions and tumor-infiltrating lymphocytes (TILs). Despite early skepticism in the field, evidence suggests that both antigen-specific T cells and CAR T cells hold promise for treating solid malignancies.

She reviewed the success of virus-specific T-cell therapies, particularly EBV-specific T cells for post-transplant lymphoproliferative disease (PTLD).[19] These off-the-shelf, third-party T-cell products from partially HLA-matched healthy donors have demonstrated efficacy rates of 50% to 55% in solid organ transplant patients and 70% in bone marrow transplant patients, with dramatic viral clearance achieved without chemotherapy or preconditioning. Notably, CNS PTLD has shown remarkable responses exceeding 80% across multiple trials. While the European Medicines Agency approved an off-the-shelf EBV T-cell product, the FDA has not followed suit, leaving no commercially available virus-specific T-cell product in the United States.

CAR T cell therapy has developed rapidly for the treatment of B-cell malignancies starting with pediatric B-cell acute lymphoblastic leukemia, resulting in seven commercially available products but only one pediatric indication. For solid tumors, CAR T-cells have not achieved the dramatic successes seen in hematologic malignancies, with response rates around 10% across multiple trials. However, promising early results have emerged in pediatric neuroblastoma with GD2 CAR T-cells, particularly in patients with low-burden disease.[20,21] Patients with relapsed/refractory neuroblastoma who achieved long-term disease control after receiving GD2 CAR T cell therapy have survived for several years, with one patient surviving for over 18 years.[22] In diffuse intrinsic pontine glioma (DIPG), researchers demonstrated encouraging signals with patients surviving nearly twice the typical median overall survival, including one complete response with dramatic neurologic recovery.[23]

A major limitation of CAR T-cell therapy is antigen loss, which could be addressed with combination strategies. CAR T-cells only recognize extracellular antigens, limiting targetable antigens in solid tumors. In contrast, tumor-associated antigens (TAAs) are intracellular and recognized through MHC presentation to the native T-cell receptor.

Initial studies with TAA-T cell therapy in acute myeloid leukemia demonstrated safety with no cytokine release syndrome, neurotoxicity, or increased graft-versus-host disease in the post-transplant setting.[24] In solid tumors, these TAA-specific T cells showed prolonged progression-free survival correlating with antigen spreading.[25] In relapsed refractory brain tumors, the heavily pretreated patient cohort showed dose-dependent effects, with one complete remission sustained for over two years.

Dr. Bollard also discussed a novel combination strategy called CARTA (CAR-T combined with Tumor Antigen-specific T cells), specifically combining B7H3 CAR T-cells with PRAME-specific T cells. The hypothesis is that CAR T-cells will recognize tumors independent of MHC, release interferon-gamma to upregulate MHC expression, and thereby enable TAA-specific T cells to function effectively. In vitro data confirm that CAR T-cells upregulate MHC on tumor cells and demonstrate synergistic tumor killing in brain tumor and Ewing's sarcoma models, with promising preclinical results in mice.

The group has multiple ongoing clinical trials, including CARTA for sarcomas, parallel CAR-only trials for brain tumors, and, most importantly, protocols combining TAA-T cells with low-intensity FUS under the LIFT protocol. Future plans include dual CAR T-cell protocols and integration with intraoperative MRI-guided FUS for brain tumors, positioning the field to explore T-cell therapies combined with FUS in clinical settings.

Questions

Q: Do you think there is antigen escape in tumor-associated antigen T cells, with some cells not expressing those antigens persisting, and is there a need for lymphodepletion like with CAR T cells?

Dr. Bollard replied that lymphodepletion was tried for solid tumors but showed no benefit because it blunted the antigen spreading response. Antigen escape is not the primary mechanism of resistance when products recognize all three antigens at elevated levels, but MHC downregulation remains the biggest resistance mechanism, which is why combination strategies are being explored.

Q: Do you have any data on how these T cells traffic to the tumor sites?

For brain tumors, there is no trafficking data. In other settings using T-cell receptor (TCR) sequencing, T cells with unique TCRs from the product can be detected in bone marrow in acute myeloid leukemia cases and in lymph nodes in blood cancers.

Routes of Administration & Platform Approach for Gene Therapy

Bryan Pukenas, MD, described delivery routes for gene therapy to the CNS, focusing on methods to bypass biological barriers and the clinical implementation of ICM puncture for intrathecal delivery.

Multiple barriers restrict substrate entry into the brain, including the BBB, the blood-CSF barrier in the choroid plexus, the arachnoid barrier, the ependymal-CSF barrier, and the circumventricular organs. Delivery method selection is dose-dependent, with intraparenchymal injection requiring the lowest dose and intravenous administration requiring the highest. Intravenous delivery is exemplified by onasemnogene abeparvovec for SMA, which has demonstrated remarkable results: 91% of pre-symptomatic patients were free of permanent ventilation, and symptomatic patients showed significant motor improvements. However, high-dose IV administration carries risks including liver toxicity (resulting in a black box warning), immune responses, pre-existing neutralizing antibodies, dorsal root ganglia toxicity, thrombotic microangiopathy, and acute lung injury.

Intraparenchymal delivery encompasses two main approaches: direct parenchymal injection (straight diffusion) and convection-enhanced delivery. In Canavan disease trials, direct diffusion injections required up to 12 burr holes, with six placed in each hemisphere, to deliver gene therapy across multiple brain regions. Convection-enhanced delivery (CED) utilizes hydrostatic pressure to maximize interstitial spread through bulk flow rather than diffusion, using stepped cannulas to prevent backflow. CED can be monitored with co-injected gadolinium to visualize distribution and adjust trajectories. The technique achieves therapeutic effects through axonal transport, in which substrate is transferred to or from neurons via axons (manipulable by capsid selection), and cross-correction, in which transduced neurons secrete protein into the interstitial space for uptake by adjacent neurons.

CSF delivery bypasses the BBB but faces additional obstacles, including the CSF-brain barrier and tight junctions in ependymal cells that limit the efficacy of ventricular injections. CSF enters the brain parenchyma along perivascular spaces, particularly peri-arteriolar spaces, and

occurs on the brain surface, with possible circadian variation. Intracerebroventricular (ICV) injection, studied extensively in Canavan disease, shows limited uptake around ventricles due to the ependymal layer and risks including parenchymal hemorrhage from brain traversal and inflammatory responses along catheter tracts. Combination ICV plus IV therapy for Canavan disease demonstrated promising results, including remyelination, restoration of visual function, and sustained neurodevelopmental improvement.

Comparative studies demonstrate that substrate concentrations vary significantly based on injection route. ICM injection achieves approximately 100-fold higher GFP transduction efficiency in the brain than lumbar puncture, with IV delivery falling between the two.[26] All routes achieve spinal cord and some systemic transduction.

Implementation of the ICM procedure faced substantial challenges, as the technique carried historical associations with hemorrhages and brainstem punctures. Gaining institutional review board approval took over a year, and patient recruitment proved difficult until identifying a patient with fibrous dysplasia, preventing lumbar access. The procedure now involves comprehensive risk mitigation, including pre-procedural CT angiography to identify vessel positions, particularly posterior inferior cerebellar arteries (PICA), and intra-procedural CT angiography with the patient positioned to confirm safe needle trajectories. Following successful initial cases, systematic training programs were established, initially through laboratory sessions using cadavers. Training expanded into a formal program that certifies approximately 50 physicians across five continents, including neurosurgeons, radiologists, neurologists, and others.

The procedure has proven effective, with preclinical NHP studies showing widespread GFP uptake throughout the cerebral cortex and cerebellum after ICM delivery, compared with zero transduction after lumbar puncture. In patients, toxicities include dorsal root ganglia toxicity, which is clinically undetectable without invasive neurostimulation testing.[27] The most common procedural risks are inadvertent brainstem puncture and PICA laceration, which can be mitigated through surgical-level pre-procedural imaging and intra-procedural confirmation. FUS may have a future role in gene therapy delivery, potentially offering safer and more tolerable alternatives to current approaches.

Questions

Q: Do you have data on the depth of penetrance or the distribution of cellular therapy into deep-seated CNS structures like the brainstem?

There is no specific data, but the mechanism of penetration is assumed to be similar to viral vectors, with perforating arteries around the brain likely explaining good thalamic perforation. However, concerns exist about distribution when everything enters the intrathecal space.

Q: With intraparenchymal injections, do you get penetration and transduction in the basal ganglia?

Dr. Pukenas said that there is some penetration and transduction in the basal ganglia, though the exact amount is uncertain.

Q: How long does the cisternal injection procedure take?

The procedure takes about an hour with patients intubated, CT scanning, needle placement, slow injection by hand, and then removal. It also depends on the injection volume.

Q: What anesthetic is used for these procedures?

General anesthesia with paralysis is used, though adults could potentially undergo the procedure without sedation; however, for clinical trials, all patients are intubated and paralyzed for consistency.

Q: How do you determine needle depth and placement during CT procedures?

The depth is measured from skin to the intended needle tip location. CT appropriate length needles are selected from kits, with shorter being better, and some practitioners use bone wax markers on the needle as a stopping point.

Q: Is there a limit to how much can be injected into the intrathecal space?

There is no established limit so far, with trials using up to 20 mL while removing equivalent CSF volumes, and standard pressure hydrocephalus procedures routinely remove 50 to 60 mL safely.

Q: How can circadian rhythm and individual patient CSF dynamics be addressed as variables affecting vector distribution?

Individual patient CSF dynamics are not currently measured, and pressure is not assessed before procedures. CSF production peaks around 3:00 to 4:00 am, which is related to brain restoration during sleep. However, knowledge of circadian effects on gene therapy is limited.

Q: What is the maximum volume that can be transduced with a single needle trajectory?

The maximum volume depends on the study parameters, with current trials using around 20 mL in adults and 10 to 15 mL in children based on brain weight or CSF volume.

Q: Is cisternal injection better than ventricular injection?

Cisternal injections are believed to be better for most applications and are likely safer since they do not go through brain tissue. Ventricular injections may be appropriate, depending on the disease target, with the key consideration being the desired distribution of treatment in the CNS.

Session 4: Breakouts & Cross-pollinations

Flash Talks

Delivery of Antisense Oligonucleotide for Rare Pediatric Neurodegenerative Diseases

Laura Owlett, MD, PhD, explored antisense oligonucleotides (ASOs) in pediatric neurology and their potential combination with FUS. While AAV gene therapies like onasemnogene abeparvovec work remarkably well for conditions like SMA, they are high-stakes, one-time treatments, prompting interest in alternative approaches.

ASOs are synthetic nucleotide segments that bind to messenger RNA to disrupt the production of toxic proteins. Several have FDA approval in pediatric neurology, including nusinersin (Spinraza) for SMA, dosed intrathecally every four months. The most exciting applications involve ultra-rare neurogenetic disorders with fewer than 30 patients worldwide, where companies create personalized ASOs.

However, challenges remain. For example, repeated intrathecal dosing often requires anesthesia, and specific deep brain structures, such as the putamen and caudate, are not well reached by intrathecal delivery. FUS could enhance targeted ASO delivery to these difficult-to-reach areas, and since ASOs require repeated dosing, this creates valuable opportunities for trial design and therapeutic optimization.

Questions

Q: What type of delivery system is used for these ASOs; are they free ASOs or complexed with nanoparticles or other delivery vehicles?

These are free ASOs composed of modified nucleotides to improve stability. The specific formulations used are proprietary, so exact details are unknown. However, ongoing work involves modulating ASO structures to enhance delivery to particular cells, as RNA is inherently highly immunogenic and presents delivery challenges.

Noninvasive Focal Gene Transfer of Chemogenetic Proteins in the Primate Brain

David Schaeffer, PhD, described the development of transcranial FUS for focal gene delivery in marmoset monkeys. Marmosets are used because they are similar in size to rats but as primates, possess primate-specific cortical architecture, including a granular prefrontal cortex with connectivity patterns similar to those of macaques. Dr. Schaeffer's team first established parameters for safely opening the BBB in marmosets using FUS, determining optimal microbubble dosage and acoustic pressure while avoiding tissue damage.[28] They then tested AAV delivery, finding that AAV9 produced better transduction than AAV2, particularly in the frontal cortex.[29] while also showing subcortical viral delivery using AAVs with ubiquitous promoters.[29]

The primary goal of this research is focal delivery of chemogenetic proteins for functional neuromodulation. They successfully delivered designer receptors exclusively activated by designer drugs (DREADDs), which offer reversible, hours-long modulation of neural activity.[30] Using FDG-PET imaging after administering the DREADD activator deschloroclozapine, they observed profound increases in glucose metabolism at the injection site and in connected brain regions. Anatomical tracing and functional MRI confirmed that these metabolic changes occurred within functionally connected circuitry and spread across multiple synapses. Dr. Schaeffer's lab is also developing noninvasive PET methods to detect AAV expression over time.

Questions

Q: How long do the DREADD effects last in marmoset monkeys? Are there differences between acute and chronic behavioral assays?

Longitudinal data have not yet been collected from the FUS delivery experiments. However, based on direct injection studies from other groups, excitatory DREADDs persist for over a year. They have attempted both AAV2 and AAV9 delivery of inhibitory DREADDs via FUS but have been unsuccessful with different promoters, though excitatory DREADDs work well. Interestingly, direct injections of inhibitory interneuron-specific DREADDs are highly effective, and the team plans to test them with FUS-mediated delivery as well.

FUS-mediated Cell Therapy for Brain Tumors

Dalia Haydar, PharmD, PhD, presented on combining FUS with CAR T-cell therapy for pediatric brain tumors. The lab investigates why CAR T-cells, which can cure most xenograft models, have not yet succeeded in human patients. There are three major challenges: tumor heterogeneity and antigen downregulation, the immunosuppressive microenvironment that prevents CAR T-cell activation, and intrinsic product limitations affecting sustained anti-tumor efficacy.

The team uses FUS for two purposes: enhancing CAR T-cell trafficking across the BBB and modulating the tumor microenvironment. The microenvironment contains immune cells, cytokines, and physical barriers that can suppress CAR T-cell function. Rather than targeting individual pathways, they explored whether FUS could induce holistic microenvironmental changes.

In studies using suboptimal CAR T-cell doses across multiple brain tumor models (high-grade glioma, medulloblastoma, and DIPG), FUS showed minimal benefit when combined with functional CAR T-cells. However, it dramatically activated control CARs that could bind tumors but lacked the signaling domains required for T-cell activation. These control CARs, which generally show no efficacy, achieved tumor control and improved survival when combined with FUS. Importantly, this effect was absent in immunodeficient mice, confirming that FUS modulates the immune microenvironment.

The hypothesis is that FUS provides immunostimulation that compensates for the absence of co-stimulatory domains, while avoiding the exhaustion seen with overly activated functional CARs. Clinical trials in canines and patients with DIPG are planned.

Questions

Q: What is the proposed mechanism for why FUS works better with control CARs than functional CARs?

Control CARs lack activation and signaling domains, so they cannot trigger T-cell activation or cytokine release. The hypothesis is that functional CARs already release inflammatory cytokines that exhaust the cells, and FUS's immunostimulation doesn't help or may even hinder them. For control CARs that cannot independently release immunostimulatory cytokines, FUS appears to supplement this missing function. Since control CARs can still bind tumor targets, FUS may provide the immunostimulation that co-stimulation and activation domains typically supply.

Q: What happens with FUS alone? Have you measured T-cell exhaustion markers?

FUS alone shows very transient anti-tumor control in DIPG and no control in high-grade glioma models. Published studies document its immunomodulatory effects in the absence of T cells. Exhaustion measurements have not been completed yet, but are planned. The lab has observed that making T cells potent leads to rapid exhaustion, so the goal is to identify therapies that persist over the long term rather than becoming terminally exhausted.

Breakout Reporting & Open Discussion

Breakout 1

Session 1: From Small Animals to Primates to Humans

Elisa Konofagou, PhD, provided the group's report. Considerable progress has been achieved over the past two years, with more NHP data now available. Researchers demonstrated successful gene delivery using both MRI-guided and ultrasound-guided FUS systems, achieving both cortical and subcortical expression patterns. A critical need emerged for liver-detargeted capsids to reduce systemic doses and spare off-target organs, making FUS more relevant for pharmaceutical and biotechnology applications.

Translation from rodents to NHP models remains challenging due to species differences in AAV receptors expression between mice and humans. Huntington's disease was identified as a priority indication given its devastating prognosis and monogenic basis, though good NHP disease models are lacking. PET tracers with radiolabeled transgenes were highlighted as essential tools for tracking both vector delivery and expression kinetics. Infrastructure barriers persist, but costs remain prohibitively high for academic researchers, necessitating the use of FUS devices at national primate centers.

Session 2: FUS for Brain Cell Therapy

Dalia Haydar, PharmD, PhD, presented the report from this group. The group emphasized that delivery optimization may not be the primary question for CAR T and other cell therapies combined with FUS. Instead, researchers should leverage what is well-understood about the effects of FUS on the brain to enhance T-cell trafficking and tumor penetration. Multiple clinical trials are imminent, creating an urgent need for coordination among investigators.

Standardization of biospecimen collection (CSF and blood), processing, and storage protocols across institutions was prioritized to enable cross-trial learning. Cell tracking methodologies, particularly PET-based approaches that do not alter cell phenotype or biology, should be incorporated into trial designs. Patient eligibility criteria should eventually be standardized across trials to enable meaningful comparison of results, though this may not be feasible for initial studies.

Session 3: Routes of Administration

Isabelle Aubert, PhD, provided a summary of the group discussion, emphasizing a move away from a one-size-fits-all approach to CNS delivery. Participants agreed that optimal routes of administration must be tailored to the disease indication, therapeutic modality, gene carrier or vector type, and target organ. The brain was explicitly recognized as a multi-organ system with region-specific biology and delivery challenges that require differentiated strategies.

The limitations of intravenous delivery for brain applications were strongly highlighted. IV administration requires very high vector doses, even for low liver-tropic AAVs, leading to

systemic exposure, safety concerns, scalability challenges, and ineffective CNS targeting. As a result, the group reached a consensus to deprioritize IV delivery for CNS indications in the near term, while acknowledging that this assessment could evolve as liver-detargeted vectors and systemic delivery technologies improve.

Significant knowledge gaps were identified for intrathecal, particularly regarding vector clearance kinetics, CSF fluid dynamics, and their impact on biodistribution. ICM delivery was noted as a promising route for superficial brain regions and, when combined with FUS, for enhanced targeting of deeper structures such as the striatum.

Intra-arterial (IA) delivery was discussed as a technically feasible and efficient route, offering first-pass delivery to the brain while minimizing peripheral trapping. Preclinical studies, especially in combination with FUS-mediated BBB modulation, suggest superiority over IV administration for targeted delivery, though further validation in rodent and large-animal models is needed.

The discussion also highlighted that interactions between FUS, vector type, and tissue are highly context-dependent. Different gene carriers respond variably to FUS-BBB modulation, requiring route- and vector-specific optimization of FUS parameters. Regional vascular heterogeneity further influences delivery outcomes.

Finally, the group emphasized the importance of quantitative imaging and readouts to support translation and regulatory alignment. Caution was raised regarding overreliance on reporter genes, which may not accurately reflect true therapeutic biodistribution. PET imaging and tracer-based approaches were highlighted as valuable tools for real-time visualization, dose quantification, and longitudinal tracking of delivery, supporting optimization of FUS parameters and validation of regional targeting.

Overall, participants concluded that individualized delivery strategies, coupled with improved quantitative imaging and continued investigation of non-IV intrathecal routes, are critical priorities for advancing CNS gene therapy.

Breakout 2

Session 1: Ideal FUS System for Brain and Large Organ GCT

Eliza Konofagou, PhD, presented the discussion for the group. The field has progressed toward clinical translation compared with two years ago, though democratization of FUS access for large patient populations remains premature pending successful early-phase trials. There are approximately 100 Insightec systems clinically available, primarily for essential tremor ablation, suggesting infrastructure will follow successful therapeutic demonstrations.

A combination of microbubble formulation, concentration, and BBB opening parameters should be shared with the community as a safe and effective baseline. MRI gadolinium enhancement remains the standard for measuring barrier opening, despite the FDA's reluctance due to parenchymal penetration. For cardiac applications, further dose reduction would enhance safety profiles and accelerate adoption.

Early trials should employ spoke-and-hub models with centralized expertise rather than distributed sites, given variability in clinical assessments across centers. Diseases with clear unmet needs provide the optimal entry point, with Huntington's disease, SMA, and Duchenne muscular dystrophy offering precedents for using natural history controls rather than placebo arms in rare-disease trials.

The proposal to manufacture and distribute standardized AAV9-GFP reference material across laboratories remains viable, with identified capabilities that require dedicated funding for production, characterization, storage, and distribution. This would enable calibration of both FUS parameters across devices and quantification methods across laboratories.

Session 2: Large Organ GCT

Natasha Sheybani, PhD, summarized the discussion. Translating lessons from brain-focused research to peripheral organs presents both opportunities and challenges. Clinical toxicity criteria from established gene therapy paradigms should guide the definition of safety for large-organ targeting. A critical gap exists between brain-focused clinical systems (with image guidance and cavitation monitoring) and peripheral-targeting devices, raising questions about the necessary safety-monitoring infrastructure. The consensus suggested potentially higher risk tolerance and lower barriers to entry for peripheral applications compared with the brain.

Pancreatic applications emerged as a potential high-priority unmet needs for both pancreatic cancer and other pancreatic disorders, as FUS-mediated drug delivery to the pancreas is currently in clinical trials, with relatively little current activity in gene and cell therapy. mRNA-LNP formulations combined with immunotherapeutic approaches and potential synergies with ablative modalities warrant further exploration.

The percentage and type of cells requiring transduction vary dramatically by indication. Arrhythmogenic cardiomyopathy requires approximately 90% myocyte transduction for meaningful benefit, while hypertrophic cardiomyopathy achieves efficacy with 50% coverage. Placental insufficiency represents a successful extensive organ application requiring only 1 in 50 endothelial cells transduced, with cavitation agents remaining on the maternal side without crossing to the fetus.

Cavitation behavior differs across tissue types due to variations in capillary bed architecture and external mechanical constraints in the kidney versus the heart, skeletal muscle versus the brain, or matrix-dense pancreatic tumors, necessitating tissue-specific optimization.

Session 3: Overcoming Biological and Technical Barriers to FUS + GCT

Ramasamy Paulmurugan, PhD, led the discussion. Capsid engineering remains the primary challenge for AAV applications even beyond BBB opening, with optimization needed across different neuronal subtypes and brain regions. Companies are actively pursuing this, but standardization remains elusive.

Lipid nanoparticle size constraints for brain delivery limit particles to 50 to 60 nanometers, restricting payload to one or two plasmid DNA molecules, a few mRNA molecules, or 300 to 400 microRNAs per particle. Larger polymeric nanoparticles (150 nanometers) can carry approximately 1,000 microRNAs but are unsuitable for brain delivery.

Concerns exist about FUS effects on nanoparticle structural integrity. Immunogenicity from FUS treatment is inherent and cannot be eliminated, as tissue antigen release naturally triggers immune responses. This property benefits neuro-oncology applications but may complicate treatments for neurodegeneration or monogenic disorders, necessitating careful consideration of cumulative effects when using inherently immunogenic therapeutics.

Real-time monitoring should combine MRI and PET imaging with cavitation mapping. Emerging dual ultrasound transducer systems enable simultaneous treatment and monitoring through feedback mechanisms. For heterogeneous tumor geometries, CT-ultrasound image fusion with automated robotic arms can adjust focal length for homogeneous treatment across varying tissue depths, analogous to radiotherapy planning systems.

Acoustic lenses customized to individual patient anatomy could reduce procedure time by achieving target volumes in a single session rather than multiple openings. The field's advancement toward clinical translation requires establishing standardized workflows and uniform criteria across institutions to enable meaningful collaboration.

Session 5: Presentations

Novel Non-viral DNA Delivery Systems

Jeff Bartlett, PhD, began by discussing how durable transgene expression is mediated by circular AAV vector genomes. While traditional discussions separate delivery-dependent and payload-dependent barriers, emerging evidence suggests delivery methods can impact promoter utilization and genetic regulation in previously unexpected ways, including with AAV capsids.31 A fundamental challenge for DNA-based therapies is achieving nuclear entry and retention. Unlike mRNA, which functions in the cytoplasm, DNA must enter the nucleus for expression. Decades of AAV research suggest that most of the material in AAV preparations is ineffective, consisting of DNA fragments or single-stranded DNA that do not express unless they are self-complementary or given time to anneal into double-stranded vectors. Studies in patients and primates have demonstrated that long term, persisting genomes are circular, existing as monomers (in which AAV circularizes itself) or concatemers of multiple genes.

Cruciform DNA structures, also called Holliday junctions, form when AAV circularizes due to inverted terminal repeats. These structures occur naturally during DNA replication, repair, and homologous recombination. Cells possess highly conserved proteins that recognize these junctions and facilitate DNA binding and nuclear import. One Holliday junction resolvase resides in the cytoplasm specifically to bind these structures and transport them to the nucleus, preventing apoptosis from unresolved junctions during cell division.

The research team engineered optimized cruciform structures into circular DNA molecules. Initial work with AAV-based structures showed modest improvements in potency and nuclear uptake in post-mitotic cells. Further optimization, moving away from AAV structures to highly stable engineered cruciforms, achieved approximately a tenfold increase in potency in human iPSC-derived hepatocytes with clear nuclear DNA import.

In vivo mouse studies using hydrodynamic tail vein infusion revealed dramatic differences. AAV-based structures produced short-lived expression due to inflammatory responses, preventing re-administration. The optimized cruciform structure enabled both re-dosing and a tenfold increase in expression levels. Transcriptomic analysis showed that mini-circle DNA (considered an optimal non-viral payload) activated thousands of genes related to immune response, DNA repair, and cytokine pathways. Cruciform-containing DNA produced almost no immune activation.

LNP-formulated ccDNA is non-toxic, non-inflammatory, and mediates potent, durable transgene expression. These cruciform DNAs achieved stable physiological expression comparable to that of AAV following single-dose administration in wild-type mice. Critically, re-dosing three months later produced dose-responsive titration, enabling a biological-like approach rather than a one-time treatment. The optimized LNP formulation showed minimal induction of inflammatory cytokines, challenging the dogma that DNA toxicity precludes LNP use and suggesting that the LNP itself is the primary driver of toxicity. Compared with plasmid DNA, these engineered structures demonstrated increased potency and durability, with a tenfold potency advantage over mRNA due to continuous transcription from each DNA molecule.

These structural elements impact potency, delivery efficiency, nuclear entry and retention, and immune phenotypes while remaining largely agnostic to delivery method, making them compatible with both LNPs and FUS approaches.

Questions

Q: Does the construct contain ITRs (inverted terminal repeats)?

Dr. Bartlett clarified that while the construct has inverted repeats that form the hairpin cruciform structure, they are not AAV ITRs.

Q: Does DNA size affect nuclear import and expression?

This is a frequent question but has yet to be systematically studied. However, they have tested constructs up to approximately 40 kilobases for large genes, and the mechanism (comparing identical constructs with and without the cruciform structure) remains effective. Manufacturing challenges, rather than inherent biological constraints, are expected to be the main limitation for large constructs.

Q: Is there a need for matrix attachment regions (MARs) or scaffold/matrix attachment region (S/MAR) elements for DNA persistence?

Dr. Bartlett responded that they have incorporated standard S/MAR elements into constructs, but these additions did not significantly change outcomes. They hypothesize that the cruciform structure acts as a nucleation site for proteins. Holliday junction resolvases are just part of the story. These proteins then recruit DNA repair enzymes and deposit histones, thereby changing the epigenetic profile. Some of the proteins involved have been identified in human lethal genetic diseases, are essential for cell survival, and bind to chromatin structures such as telomeres.

FUS-mediated Delivery of Non-viral Gene Editing and Gene Therapy in the Brain

Rich Price, PhD, presented on FUS-targeted gene delivery to glioma endothelium and CRISPR Cas9 ribonucleoprotein delivery to gliomas. Both of these projects present unpublished work that still requires optimization.

The first project explores FUS as a tool to target gene delivery specifically to glioma endothelium. Building on previous work demonstrating that FUS combined with microbubbles enhances nanoparticle delivery across the blood-brain and blood-tumor barriers, the research addressed two key questions: whether molecular targeting improves responses and which cells exhibit transgene expression. Using nanoparticles modified with a chemical enhancement (termed TTNP) and loaded with the H2KK reporter gene, comparative studies between CED and FUS delivery revealed unexpected findings. While intravenous delivery had no effect due to the absence of enhanced permeability and retention, FUS-mediated delivery achieved approximately 25% to 30% transfection efficiency in tumor cells, comparable to that of CED. However, FUS dramatically outperformed CED in transfecting endothelial cells (75%), astrocytes, pericytes, and leukocytes, with endothelial targeting being particularly striking.

To achieve endothelial cell specificity, the researchers employed the CD144 (VE-cadherin) promoter, which reduced efficiency to 40% due to its lower activity relative to CMV, while maintaining endothelial specificity. As proof-of-concept, they delivered CXCL9, a chemokine that recruits T cells and natural killer cells when expressed by endothelium without promoting tumor growth. In vitro studies confirmed that transfected endothelial cells recruit T cells across transwells. In vivo delivery significantly increased CD4 helper T cells without increasing immunosuppressive regulatory T cells and also elevated natural killer cells and CD8 effector T cells. However, control plasmids showed some adjuvant effects. While CXCL9 delivery alone did not significantly improve survival, one complete responder showed tumor eradication with tissue-resident memory T cells present. Combining CXCL9 with PD-1 checkpoint inhibition increased survival, but only for a brief period (median 28 days).

The second project focused on using FUS to deliver CRISPR-Cas9 systems targeting P-glycoprotein, an efflux transporter that confers chemotherapy resistance in tumors and restricts drug penetration across the BBB. Initial CED delivery of ribonucleoprotein targeting specific exons successfully increased rhodamine retention in tumor and endothelial cells, indicating functional P-glycoprotein deletion, and enhanced paclitaxel efficacy at sub-therapeutic doses, improving survival and tumor control.

To avoid off-target effects in organs that naturally express P-glycoprotein (liver, kidney, intestines), they used lung-selective lipid nanoparticles (SORT-LNP). These LNPs incorporate a fifth lipid that tunes biodistribution, redirecting CRISPR cargo to the lungs where P-glycoprotein is absent. FUS successfully delivered these lung-sorted LNPs to brain tumors, achieving optimal delivery at mid-level acoustic pressures, as monitored by acoustic emission feedback. Subsequent paclitaxel treatment showed therapeutic benefit, although increasing the FUS pressure provided minimal additional benefit. Future improvements will focus on reducing LNP size to enhance penetration of the blood-brain and blood-tumor barriers. In conclusion, integrating FUS delivery into therapeutic engineering from the outset is essential, particularly for modular non-viral systems.

Questions

Q: There was a comment that small molecule inhibitors of MDA9 syntenin were created that enter the brain and shut down drug efflux transporters, causing chemotherapy to accumulate in tumors and kill cancer cells. This approach works in multiple tumor types, including paclitaxel-resistant triple-negative breast cancer, making them treatment-sensitive. There are nanocrystal versions for brain delivery, and this could test FUS systems since the molecules already cross the BBB. Published data show the inhibitors synergize with radiation and temozolomide in brain tumors.

Q: Can you monitor immune cell trafficking using cerebrospinal fluid or blood markers rather than requiring immunohistochemistry?

Dr. Price answered that there are currently no surrogate markers available for monitoring in real time. This could be tested through mechanistic studies, which would involve deleting CD4 and CD8 cells to see whether therapeutic responses disappear.

Q: What is the editing efficiency in this system, and why use paclitaxel instead of the standard glioblastoma chemotherapy temozolomide?

In vitro editing efficiency reaches up to 40% but is expected to be lower in vivo. Paclitaxel was selected because, although temozolomide can cross the BBB, paclitaxel is much more effective when it reaches the tumor and serves as a good exemplar of this system, which could be expanded to other drugs and therapies.

Q: Could multiple rounds of editing improve survival outcomes?

Dr. Price said that multiple deliveries and editing sessions are opportunities for the future and could improve the overall percentage of edited cells and survival. However, the preference would be to edit only once.

Q: Is this approach glioma-specific or applicable across different tumor types?

The approach works in both GL261 and CT26A models, and future work includes testing in genetically engineered mouse models to determine broader applicability, as gene editing itself is applicable across different contexts.

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Session 6: Regulatory

Regulatory Development in GCT

Michael Werner discussed the current regulatory climate for cell and gene therapies, noting a shift toward more centralized coordination across federal health agencies. Compared with prior regulatory practice, there is closer alignment among the Department of HHS, the FDA, the Centers for Medicare and Medicaid Services (CMS), and the White House in setting and advancing policy priorities relevant to biomedical innovation.

This increased coordination has been reflected in initiatives such as the Priority Review Voucher program, where senior FDA leadership has publicly highlighted the role of agency-wide and administration-level priorities in shaping regulatory incentives. While FDA leadership has expressed strong support for cell and gene therapies through multiple public announcements, a key challenge remains the limited availability of formal regulatory guidance accompanying these initiatives.

Several recently announced programs, including conditional approval pathways for ultra-rare diseases, the Priority Review Voucher system, and the Rare Disease Accelerated Pathway, have been introduced without traditional notice-and-comment rulemaking or detailed implementation frameworks. In some cases, companies reportedly received priority review vouchers without prior awareness that they had been nominated by FDA center leadership. This lack of procedural transparency and formalized guidance creates uncertainty for sponsors seeking to understand eligibility criteria, expectations, and the practical application of these emerging pathways.

Stakeholders were advised to proactively engage with agencies rather than waiting for formal guidance, to monitor administration officials' communications, including social media, and to align their priorities with stated administration goals such as onshoring manufacturing, addressing ultra-rare conditions, and ensuring product affordability.

ClearPoint's regulatory strategy for their SmartFlow device was discussed as an example of an innovative approach to FDA approval. The device was approved alongside gene therapy, with both products receiving FDA authorization on the same day through different review divisions. ClearPoint pursued de novo approval for the initial narrow indication since no predicate device existed on the market. Once approved, the company strategically used their own device as the predicate to file multiple 510(k) applications for broader indications, creating a versatile platform that could be used with various drug partners beyond the original gene therapy application.

Questions

Q: How do the proposed FDA processes fit with existing designations for rare diseases like orphan status, fast track, and priority review vouchers, and does one preclude the use of another?

There is no reason to think one designation precludes another as long as eligibility criteria are met, and a product could potentially receive orphan designation, fast track designation, and be eligible for a Priority Review Voucher simultaneously.

Q: When is the best time to leverage these designations with the FDA?

Each designation has different timing requirements, with some appropriate for the pre-IND phase and others requiring preliminary clinical evidence from human trials. The best approach is to review your technology against all available programs to determine which ones match and at what development stage to apply them.

Q: What are the potential pathways for translating a product approved for a rare disease into more prevalent indications, and do you have to start from nothing with a different pathway?

It is a legitimate regulatory strategy to gain orphan designation approval first and then pursue more prevalent diseases. This requires conducting all necessary trials for the new indication, demonstrating manufacturing capability and a supply chain at scale, and obtaining separate approval without orphan drug benefits such as market exclusivity. The advantage is that this method will already have the FDA's safety and efficacy determination, providing a foundation for support and real-world data about the product.

Panel Discussion: What Regulatory Pathways for FUS GCT?

Moderator: **Bob Smith**, MBA

Panelists: **Jon Lindner**, MD; **Bryan Pukenas**, MD; **Jared Smith**, PhD; and **Michael Werner**

This panel discussion focused on regulatory pathways for combining FUS with cell and gene therapies, with particular attention to navigating interactions with the FDA during a period of agency transition.

Current FDA Environment and Recent Experiences

The panel shared mixed experiences with recent interactions with the FDA. Several participants reported positive outcomes, including timely pre-IND meetings, helpful guidance on reducing animal study requirements, and responsive reviewers. One company received FDA encouragement to pursue more aggressive treatment approaches rather than overly cautious protocols. However, others experienced delays, with Biologic License Application review dates pushed back several months due to reduced staffing. Academic researchers face particular challenges, as they rely heavily on FDA guidance meetings but report difficulty securing timely pre-IND consultations. Communication delays create cascading backlogs that particularly affect physician-sponsored INDs and early-stage academic programs.

Regulatory Complexity and Strategic Considerations

Combining FUS with gene therapies creates multiple layers of regulatory complexity. For AAV-based therapies, companies must develop companion diagnostics to screen for pre-existing neutralizing antibodies. The panel emphasized the importance of early and frequent FDA engagement to understand evolving agency expectations, particularly for innovative approaches without clear precedents.

Several speakers noted that reviewers remain on the job at the FDA; these positions are funded by user fees from the previous fiscal year. However, the loss of senior leadership and policy expertise, particularly the 15% reduction in the Office of Tissues and Advanced Therapies staff, has created uncertainty about policy implementation despite supportive statements.

Data Quality and Trial Design

The panel stressed building robust data packages rather than relying on simple endpoints. One company invested significant effort in training clinical evaluators to ensure consistent patient assessments across sites, which improved data quality. For diseases lacking good animal models, innovative approaches, such as using human patient-derived cells to demonstrate enzyme-substrate relationships, have gained FDA acceptance.

Data Safety Monitoring Boards play a critical intermediary role, providing expert oversight that balances safety concerns against potential benefits. This becomes especially important when FDA expertise is stretched thin.

Agency Priorities Under Current Leadership

The FDA appears to prioritize transformational therapies that could represent cures over incremental improvements. Programs described as potentially transformational receive faster consideration, while extensive studies require years to complete and face more scrutiny. The Rare Disease Evidence Principles (RDEP) program exemplifies both promise and concern. While broadly positive, it includes an arbitrary threshold of 1,000 patients without a clear scientific justification or an opportunity for stakeholder input on how that number was determined. There was additional concern that high rates of staff turnover could result in a lack of appropriate expertise at the FDA, potentially causing problems with the regulatory process.

Industry Adoption Barriers

Pharmaceutical and biotech companies remain hesitant to incorporate FUS into their development programs. Safety concerns exist but represent only part of the reluctance. Many companies feel uncomfortable with unfamiliar technology that disrupts their established development pathways. Changing delivery methods also requires substantial regulatory work and funding that startup gene therapy companies may not have allocated.

The field needs visible clinical wins, particularly trials combining FUS with therapies that the industry already understands. Business-friendly terms for accessible device technology will help drive adoption, alongside demonstrated efficacy.

Role of Scientific Organizations

Organizations like the FUS Foundation and the Alliance for Regenerative Medicine can facilitate pre-competitive collaboration. Historical precedent exists for professional societies to conduct scientific workshops with the FDA to educate regulators on emerging technologies and help develop field standards. This becomes particularly valuable during transition periods when institutional knowledge is lost.

The FUS Foundation maintains active engagement with the FDA's device division, but faces more difficulty connecting with the biologics division relevant to gene therapy combinations. Patient advocacy groups increasingly fill expertise voids by championing promising therapies, though this raises questions about maintaining rigorous scientific standards.

Path Forward

The panel concluded that, despite regulatory volatility, strong science will continue to drive the field forward. Researchers should remain agile in adapting to short-term changes while maintaining focus on long-term goals. Increased public education about FUS, including media coverage and presence at major scientific conferences, will build broader awareness among potential collaborators and regulators. Clinical impact and patient stories resonate with the current administration's stated priorities and provide compelling justification for continued innovation.

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Session 7: Program Selection

Current Clinical Trials and Vector Usage in Gene Therapy

Michael Lehmicke provided an overview of the current state, challenges, and clinical progress in cell and gene therapy. The Alliance for Regenerative Medicine is a global advocacy organization made up of approximately 80% to 85% industry members (developers and supporting companies), 10% to 15% percent academic institutions, and the remainder patient organizations. The organization convenes the sector through major events, engages stakeholders, including the FDA and policymakers, on regulatory issues, and produces technical resources, such as their best-practice document for AAV vector process development using quality-by-design principles.

The field has moved away from describing gene therapies as curative, now characterizing them as durable and life-transforming, recognizing that not every treatment represents a cure. Safety issues, particularly in the past year, remain a concern that must be addressed openly. Manufacturing costs remain high despite progress in automated cell therapy production and improved gene therapy manufacturing methods, including producer cell lines and downstream purification. Commercialization has proven challenging, with disappointing uptake for approved hemophilia gene therapies as they compete with existing treatments.

However, there are some positive developments. Gene therapies entering phase 1 clinical trials have an 18.5% likelihood of approval, which is 2.5 times higher than the average for all other drugs. Accelerated approval pathways show promise, with potentially seven more gene therapies receiving accelerated approval between 2025 and 2027, compared with only three approved through this pathway from 1992 to 2024. Onasemnogene abeparvovec exemplifies commercial success, achieving blockbuster status in 2021 with over one billion dollars in sales, approved in over 60 countries with access established in 45 countries, and having treated over 5,000 children. Projected revenue is expected to double by 2028, underscoring the critical importance of establishing access capabilities early across regions.

Regarding the regulatory outlook, while 2024 saw a record ten cell and gene therapy approvals, 2025 is trending toward potentially five approvals. However, looking ahead, there could be as many as nine approvals, optimistically, in the coming year, based on pending submissions and decisions.

Data was presented contextualizing AAV gene therapy safety, noting that while every patient death is a tragedy, it's essential to consider disease context.32 With an estimated 7,000 patients treated and 14 known deaths, the resulting mortality rate compares favorably to or is better than checkpoint inhibitors and chemotherapy used in oncology for serious diseases, where patients have limited alternatives.32

Clinical trial data analysis showed that lentiviral and AAV vectors remain the most commonly used delivery systems, with lipid nanoparticles still representing a small but potentially significant emerging category, particularly for in vivo gene-editing applications. Cell-based immunotherapy trials predominantly use lentiviral vectors in ex vivo settings, while AAV is overwhelmingly used for in vivo gene therapy applications. There will likely be a major shift toward in vivo approaches over the next five to ten years, viewing ex vivo methods as a technological limitation. Despite investor questions about AAV's longevity, data show dramatic increases in AAV clinical trials over recent years, with no indication that the technology is declining.

Advances in AAV delivery were highlighted, noting the complexity of achieving effective BBB penetration, which varies by serotype and brain region. Improved delivery through lower, safer doses via alternative routes of administration could address AAV-related dosing safety concerns.

Another approach uses AI-curated databases alongside extensive NHP screening to engineer capsids that avoid the liver and better target the brain, demonstrating AI's potential in gene therapy development.

Questions

Q: Are investors really shying away from AAV given safety concerns, and what about next-generation vectors?

Investor reactions tend to show recency bias after adverse events occur, even though other delivery methods like lipid nanoparticles have their own safety issues. The plan is to provide a more holistic long-term view of AAV safety, with the American Society of Gene and Cell Therapy planning a workshop next year to convene experts on what is known about AAV safety.

Q: Does the Alliance for Regenerative Medicine work on access and affordability issues, given that gene therapies are often seen as prohibitively expensive with limited reimbursement, making them accessible only to the wealthiest patients?

Mr. Lehmicke answered that Alliance for Regenerative Medicine focuses on public payers and CMS in particular, working on issues like cross-border certification for providers when patients travel long distances, anti-kickback statutes that prevent travel stipends, and advocating for related legislation to improve access.

Q: What progress has been made on reimbursement models for gene therapies?

The CMS Administrator has been surprisingly supportive of the cell and gene therapy access model for sickle cell disease programs, with 35 or more states under federal and state Medicaid adopting value-based contract models that annuitize therapy costs over time. However, commercial payers prefer single lump-sum payments to avoid administrative burdens, since the healthcare system is designed for ongoing sick care rather than one-time curative payments. ARM is working to influence the evolution of thinking on these issues.

Panel & Discussion: How to Select a Program for FUS and GCT?

Panelists: **Estaban Engel**; **Lena Gamboa**, PhD; **Jennifer Johnston**, PhD; **Michael Lehmicke**; and **Bob Smith**, MBA

This panel discussion focused on the clinical translation potential of combining FUS with cell and gene therapies, addressing key challenges in indication selection, technology readiness, and industry adoption.

Indication Selection and Prioritization Framework

The FUSF developed a framework to evaluate which diseases are most likely to benefit from combining FUS with gene therapies. The analysis considered factors like scientific readiness, existing approved products, disease prevalence, and technical feasibility. Parkinson's disease emerged as a top candidate across multiple attribute dimensions, particularly because of its large patient population and multiple disease forms sharing similar pathology.

However, the framework received constructive criticism for not adequately accounting for life expectancy, disease severity across the lifespan, or the challenges of treating systemic conditions such as muscular dystrophy, which affect multiple organs. Pediatric diseases, which are nearly all orphan conditions, scored lower despite potentially compelling humanitarian arguments. The presenters acknowledged that this represented only the first round of analysis and requires substantial refinement based on community feedback.

Technology Readiness and Dose Reduction

Multiple speakers emphasized that FUS enables a significant dose reduction for AAV9, potentially 100-fold lower than that achieved with standard systemic administration. This decreases the doses needed, which is a meaningful improvement in safety. Other capsids that cannot cross the BBB, such as AAV2, can now access the brain using FUS. Liver-detargeted capsids also show promise for further dose reduction.

The balance between dose and invasiveness represents a critical trade-off. Systemic delivery requires extremely high doses, which carry associated safety risks. Intraparenchymal delivery works beautifully but requires highly invasive neurosurgery. FUS potentially occupies a middle ground, reducing systemic dose requirements while avoiding surgical procedures.

Capsid Selection and Tropism Challenges

No single capsid, payload, dose, or administration route will succeed across all indications. Separate groups use different capsids, animal models, promoters, and ultrasound systems, making direct comparisons difficult. One participant emphasized using AAV5 specifically because it targets dopamine neurons and astrocytes in the substantia nigra, demonstrating how tropism requirements vary by disease target. Achieving the right cell-type specificity while minimizing liver uptake remains challenging.

Species differences also matter significantly. AAAV5 becomes trapped in the mouse liver, but may distribute differently in rats, primates, and humans following FUS exposure. Adenovirus also shows species-specific trapping, accumulating in the liver of some species but in the lungs of Syrian hamsters. These variations complicate preclinical development.

Industry Adoption Barriers

Pharmaceutical companies face substantial uncertainty when considering the integration of FUS. They must evaluate which device to test, which animal species to use, which brain regions can be effectively opened, how many centers can perform the procedure, manufacturing cost implications, regulatory pathways, and the overall commercial viability. The heterogeneity of approaches across academic groups using different devices, capsids, and protocols makes informed decision-making difficult.

Risk-averse companies weigh whether adding FUS is justified by the additional trial complexity, cost, and regulatory burden. The calculation differs dramatically by indication. For devastating conditions like Huntington's disease, the approval bar sits lower than for early-onset Parkinson's patients who may have many quality life-years remaining. Companies also consider whether the benefits of FUS outweigh alternatives, including emerging non-viral vectors and improved capsid designs.

Approved Products as Starting Points

Multiple panelists advocated beginning with already-approved gene therapy products, such as AAV9-based onasemnogene abeparvovec (Zolgensma) or AAV2-based eladocagene exuparvovec (Kibilidi), rather than developing entirely new therapeutic constructs. This approach de-risks the FUS component by leveraging established safety profiles, known dosing parameters, and existing clinical experience. Regulatory pathways become clearer when one variable (the delivery enhancement) changes rather than introducing multiple novel elements simultaneously.

However, this strategy presents challenges. Patients and families may hesitate to use their one treatment opportunity with experimental delivery technology. Academic researchers developing therapies for young Parkinson's patients worry about translating findings to the typical population of 65 to 80-year-olds, questioning whether FUS performs differently in elderly patients with potential cerebrovascular disease.

Cost Considerations and Access

The economics remain complex. While FUS reduces vector requirements compared with IV administration alone, it adds device costs and requires specialized centers. Manufacturing savings from lower doses must offset implementation expenses. Small companies already struggling to fund phase 1 trials cannot easily add exploratory arms to test combinations of FUS. Patient enrollment also depends on the availability of procedures. The panel discussed whether FUS will lower costs and improve access, particularly as the technology matures. Some expressed hope that repeated successful trials would normalize the approach, reduce perceived risk, and expand center capabilities.

Beyond AAV: Alternative Therapeutic Modalities

Participants urged looking beyond AAV to antisense oligonucleotides, antibodies, and non-viral vectors. ASOs do not readily cross the BBB but could become game-changers with FUS enhancement. For antibody therapies like aducanumab targeting amyloid-β, FUS might reduce systemic exposure sufficiently to avoid adverse effects such as amyloid-related imaging abnormalities while maintaining efficacy.

Non-viral vectors also generated interest, despite limited discussion to date. Several speakers emphasized that classic biologics with established approvals and sales represent attractive candidates for demonstrating the utility of FUS in a lower-risk context before advancing to more complex gene therapy applications.

Immunomodulation Beyond Delivery

Some researchers prioritized FUS's immunomodulatory effects over delivery enhancement, particularly for cell therapies in brain tumors. Published work documents sterile inflammation following FUS, with various immunological tools available to investigate these effects. However, some questioned whether the field can adequately measure immunomodulation, citing challenges in obtaining appropriate patient tissue samples and the lack of biomarkers. An upcoming University of Virginia trial using radiolabeled bispecific CAR T cells with FUS will specifically measure cell recruitment after systemic delivery and FUS-mediated BBBo.

The discussion acknowledged that optimal biomarkers vary by tumor type. For some diseases, starting outside the brain, where biopsies are more accessible, might help establish the biology before moving to more challenging CNS indications. Disease dependence also affects whether immunomodulation or delivery represents the primary therapeutic opportunity for FUS.

Academic Trials and Industry Partnership Models

The current investment climate creates higher bars for industry funding, with investors wanting clinical data before committing. This leaves some companies in purgatory with proof-of-concept but insufficient resources for pivotal trials. Academic trials offer a path forward, generating human data that attract industry attention at lower cost and with greater investigator control. One example cited the preliminary treatment of three patients, generating substantial excitement and likely commercial interest.

Several speakers advocated for partnerships between academic centers with FUS capabilities, device companies, and therapeutic developers. Some pharmaceutical companies even consider acquiring FUS companies outright to control the technology and eliminate intellectual property negotiations. However, most attendees agreed the field needs more published data demonstrating reproducibility and efficacy across different therapeutic modalities, particularly in NHP, before major industry commitments materialize.

Ultra-Rare Disease Opportunity

One promising avenue involves ultra-rare or N-of-1 conditions, in which academic groups develop patient-specific gene-editing approaches. Recent success stories demonstrate regulatory

willingness to support transformational interventions. Combining these bespoke therapies with FUS for enhanced brain delivery might represent the fastest path to clinical application. The regulatory environment currently appears receptive to such approaches.

The BGTC, a public-private partnership supported by the NIH, the FDA, and industry, provides a model for addressing ultra-rare diseases where commercial incentives are insufficient. Discussions are underway about potential trials that could incorporate FUS alongside gene therapy for underserved patient populations.

Data Gaps and Research Priorities

The panel identified critical needs for additional NHP studies demonstrating the safety and efficacy of FUS with gene therapies. Only three to four groups have generated such data, which is insufficient to build industry confidence. More papers showing reproducibility across different capsids, devices, and therapeutic targets would substantially de-risk the approach.

The field also needs clarity on specific brain regions that can be effectively and safely opened, optimal ultrasound parameters for different applications, the durability of effects with repeated treatments, and comparative data on navigation-based versus MRI-guided systems. Safety data exists for FUS-BBB opening in patients with amyotrophic lateral sclerosis (ALS), Parkinson's and Alzheimer's disease, but without concurrent therapeutic administration.

Path Forward and The Role of the FUS Foundation

Participants agreed that science will ultimately prevail despite current regulatory and funding volatility. Good clinical data demonstrating clear patient benefit will drive field momentum. The FUS Foundation can facilitate progress by convening stakeholders, supporting comparative preclinical studies to establish standards, funding early clinical trials to de-risk the approach, and potentially partnering with initiatives such as the BGTC. The strategy should involve carefully selecting initial indications, generating convincing proof-of-concept data in humans, and leveraging that success to attract broader industry investment and enable expansion to more common conditions.

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Abbreviations

AAV	Adeno-associated virus
AAVR	AAV receptor
AML	Acute myeloid leukemia
ARIA	Amyloid-related imaging abnormalities
BBB	Blood-brain barrier
BGTC	Bespoke Gene Therapy Consortium
CAR	Chimeric antigen receptor
CED	Convection-enhanced delivery
CNS	Central nervous system
CRO	Contract research organization
CSF	Cerebral spinal fluid
CT	Computed tomography
DIPG	Diffuse intrinsic pontine glioma
DREAD	Designer receptors exclusively activated by designer drugs
FGTTC	FUS Gene Therapy Translation Consortium
FNIH	Foundation for the National Institutes of Health
FUSF	Focused Ultrasound Foundation
GDNF	Glial-derived neurotrophic factor
GMP	Good manufacturing practices
HHS	Health and Human Services
ICM	Intra-cisterna magna
IND	Investigational new drug
ITR	Inverted terminal repeats
LIFU	Low-intensity focused ultrasound
MAR	Matrix attachment regions
NHP	Non-human primates
NIH	National Institutes of Health
PICA	Posterior inferior cerebellar arteries
PTLD	Post-transplant lymphoproliferative disease
RDEP	Rare Disease Evidence Principles
RES	Reticuloendothelial system
SMA	Spinal muscular atrophy
TAA	Tumor-associated antigens
TCR	T-cell receptor
TIL	Tumor-infiltrating lymphocytes
UMGD	Ultrasound-mediated gene delivery

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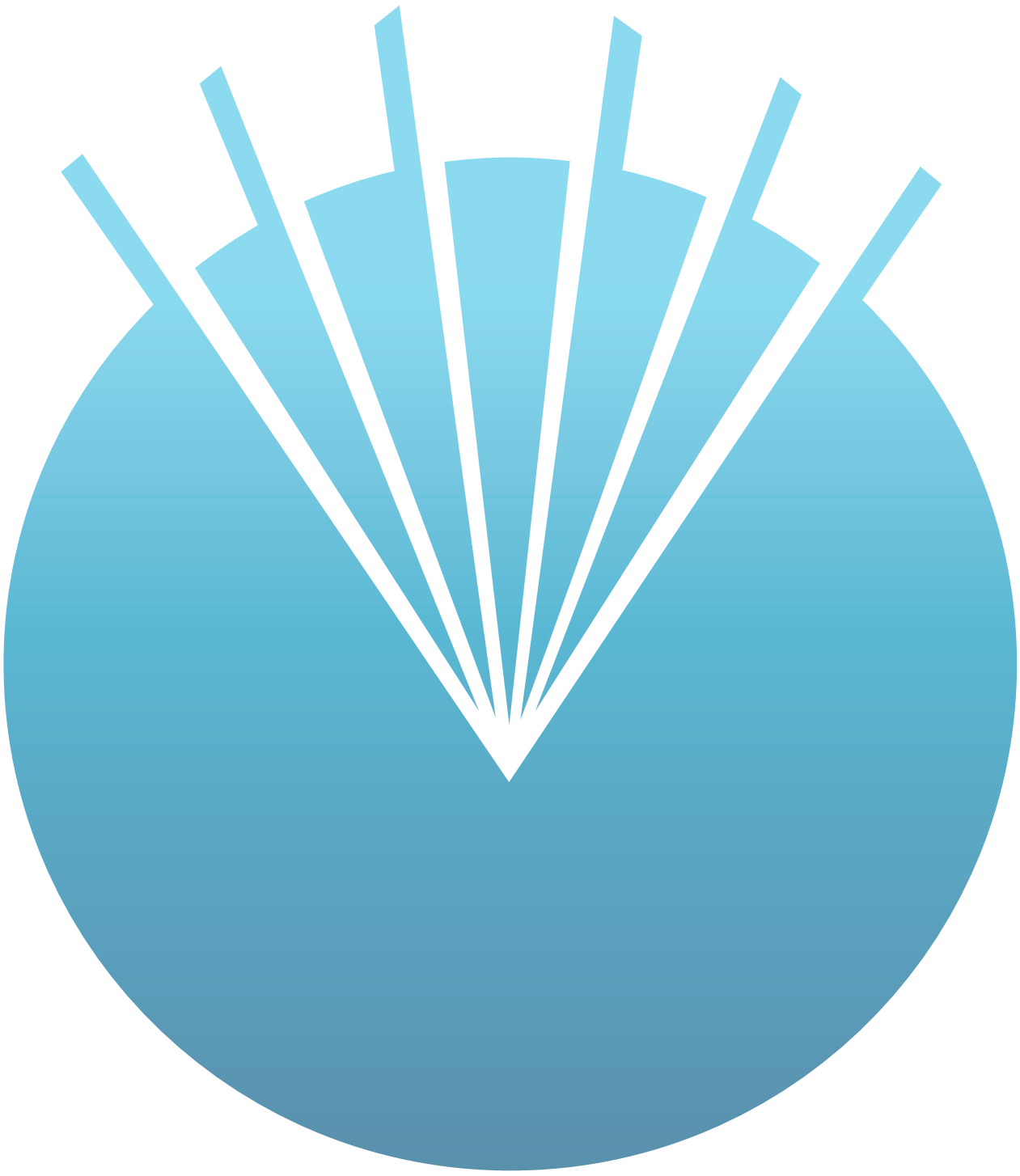

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