Translating Focused Ultrasound-Mediated Gene Therapy to the Clinic for Neurological Disorders

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Executive Summary

Focused ultrasound (FUS)–mediated blood–brain barrier opening (BBBO) offers a transformative opportunity to address one of the greatest bottlenecks in neurological gene therapy: safe, targeted, and efficacious delivery of adeno-associated virus (AAV) and other gene therapy vectors to the central nervous system (CNS). This landscape analysis, commissioned by the Focused Ultrasound Foundation, evaluates where FUS can provide the greatest translational and clinical impact, scoring candidate indications across four areas: **(1)** scientific **readiness, (2) operational feasibility, (3) commercial viability, (4) FUS delivery suitability**.

Purpose and Scope

The goal of this initiative is to identify priority CNS gene therapy programs and disease indications where FUS can unlock non-invasive or improved delivery advantages of AAVs, accelerate translation, and expand patient access. We propose a structured scoring framework to identify high-value opportunities. The analysis highlights most notably where FUS could overcome delivery barriers that have limited efficacy in past or ongoing trials.

Key Findings

- **Scientific & Clinical Opportunity:** Monogenic and genetically stratifiable disorders with well-defined biological or genetic targets, measurable biomarkers, and anatomically accessible and focal pathology are best suited for early clinical integration of FUS.
- **Regulatory Complexity:** FUS + gene therapy constitutes a combination product requiring joint oversight from CBER and CDRH divisions of the US FDA. Rare disease programs may benefit from Orphan Drug Designation, RMAT, and other expedited pathways. A platform approach, akin to the NIH-led Bespoke Gene Therapy Consortium (BGTC), could harmonize the complex regulatory approach and reduce redundancy between sites.
- **Operational Gaps:** The field lacks mid-stage translational infrastructure between academic proof-of-concept studies and full CRO-led IND-enabling toxicology packages. This gap slows progress and increases development costs.
- **Clinical Trial Design:** Rare disease trials may rely on surrogate biomarkers for accelerated approval, while AAV programs for non-rare diseases require more complex clinical trial design and long-term follow-up to capture delayed efficacy.
- **Commercial Considerations:** Strategic sequencing of indications, starting with rare diseases, can de-risk later expansion to larger, more complex populations. Potential cost savings from reduced surgical procedures and lower vector doses strengthen the case for payers' adoption.

Calls to Action

1. **Form a FUS–Gene Therapy Translational Consortium (FGTTC)** to coordinate regulatory strategy, share preclinical data, and engage early with FDA via INTERACT meetings.
2. **Designate expert clinical sites** matched to disease indication, vector capsid, route of administration, and FUS platform capabilities.

3. **Standardize preclinical platforms** for FUS-enabled gene therapy, including standardized cavitation monitoring, imaging protocols, and quality control assays for DNA, RNA, and protein endpoints.
4. **Bridge the mid-development gap** by funding intermediate-scale translational studies that are more robust than small animal studies but less resource-intensive than full IND-enabling toxicology programs—herein termed INTERACT-enabling studies
5. **Prioritize high-scoring rare diseases** for initial trials to leverage regulatory incentives, establish safety precedent, and build a path for expansion into non-rare indications.
6. **Develop and publish FUS parameter reporting guidelines**, with an open call for public and stakeholder feedback.
7. **Survey the broader community** including academic labs, biotech developers, CROs, device manufacturers, regulators, and clinicians to align on shared priorities and unmet needs.

Working Hypothesis

Without coordinated action, the momentum for FUS in CNS gene therapy delivery will stall, with programs advancing in isolation and duplicating regulatory, manufacturing, and safety work. With a consortium-led strategy, the Foundation can catalyze a scalable model for integrating FUS into gene therapy development thereby lowering barriers, accelerating timelines, and delivering transformative treatments to patients who currently have no viable options.

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Preface

Delivering therapeutic agents to the brain—whether small molecules, antibodies, cell therapies, or gene therapies—remains one of the most formidable challenges in medicine due to the blood-brain barrier (BBB), a tightly regulated endothelial interface that blocks ~98–99% of systemically administered drugs from reaching the central nervous system [1]. Gene therapies, in particular, represent a transformative class of biologics for treating neurodegenerative and genetic brain disorders, yet their clinical translation has been largely constrained by delivery challenges. The only FDA-approved gene therapy for a neurological disease, *Upstaza* (eladocagene exuparvovec), requires direct intracranial injection into the putamen. While this approach bypasses the BBB, it is invasive and has historically yielded limited tissue coverage, often falling short of achieving widespread and sufficient gene transduction in the brain as exemplified by early clinical trials in Parkinson's Disease [2].

Focused ultrasound (FUS) paired with intravenously administered microbubbles offers a powerful alternative. When applied at low intensities in a pulsed mode, FUS can transiently and non-invasively modulate the permeability of the BBB in targeted brain regions, enabling circulating gene therapy vectors to enter the brain parenchyma. This technique, known as FUS-mediated blood-brain barrier opening (FUS-BBBO), holds the potential to overcome the delivery bottleneck in neurotherapeutics, allowing for more effective, widespread, and repeatable administration of gene therapies without surgery.

As FUS-BBBO has only recently entered the clinical stage in neuro-oncology, its ultimate role in gene therapeutic delivery is still being defined. We hypothesize a spectrum of utility: at one end, FUS may serve as a wholly non-invasive delivery tool capable of replacing surgical approaches altogether; at the other, it may serve as an adjunct to improve the efficiency, distribution, or safety profile of existing or future administration methods. While we do not yet expect the efficacy of a fully non-invasive, intravenous FUS approach to always match that of direct intracranial injection, the clinical need for non-invasive alternatives is substantial. Patients may be hesitant to enroll in trials requiring neurosurgical procedures, and many may have contraindications or comorbidities that limit surgical eligibility. Therefore, complex neuro-surgical procedures may limit the widespread accessibility of new gene therapy treatments. FUS thus expands the therapeutic delivery toolkit, introducing new possibilities for non-invasive, targeted intervention in the brain.

This memo reviews the current landscape of in vivo gene therapy for the brain, approved indications and research programs, and outlines the critical factors that must be considered to design a project in this field. Its goal is to identify the major challenges that need to be addressed before advancing to first-in-human clinical trials and highlight the emerging role of FUS-BBBO as a delivery solution for gene therapies targeting the central nervous system.

1. Introduction

1.1 Gene Therapy Delivery: Scientific Opportunity and Clinical Unmet Need

While gene therapies hold extraordinary promise for treating neurological diseases, their success depends heavily on achieving adequate delivery to the central nervous system (CNS), a challenge that remains unresolved for many therapeutic platforms. The primary obstacle is the BBB, a highly selective endothelial interface that restricts the entry of nearly all large molecules, including viral vectors, antisense oligonucleotides (ASO), and gene editing tools. As a result, most gene therapy candidates for CNS disorders must rely on specialized routes of administration (ROA) to bypass or circumvent the BBB.

Current delivery strategies fall into three broad categories, each with distinct limitations:

- **Direct intraparenchymal injection (e.g., convection-enhanced delivery or CED)**
  This method enables precise targeting of deep brain structures but requires neurosurgical intervention, which carries procedural risks. Moreover, distribution is often highly localized and insufficient for diseases requiring widespread CNS coverage. For example, *Upstaza* (eladocagene exuparvovec), the only approved gene therapy for a CNS disorder, requires bilateral intraparenchymal infusion under general anesthesia. Extended to other diseases with broader patient population, the invasiveness of this approach may limit patient enrollment and broad adoption.

- **Intrathecal or intra-cisterna magna delivery (e.g., for ASOs or AAVs)**
  These cerebrospinal fluid (CSF)–based routes avoid brain surgery and can enable more diffuse distribution, especially to the spinal cord and brainstem. However, penetration into deeper parenchymal regions (e.g., striatum, hippocampus, or cortex) is limited, and distribution is influenced by CSF flow dynamics, age, and disease state. Intrathecal AAV administration has shown variable transduction patterns, with concerns about dorsal root ganglia toxicity at higher doses [3,4].

- **Systemic (intravenous or intra-arterial) delivery**

  Systemic administration is the least invasive option and is the route of administration for the approved gene therapy Zolgensma for the treatment of spinal muscular atrophy (SMA). IV administration of Zolgensma leverages more active transport mechanisms present in the developing BBB of infants under 2 years old, which allows for greater penetration of the AAV9 vector into the CNS. It should be noted, that although the BBB is considered fully matured at birth [5], upregulation in active transport systems and physiological differences in blood pharmacokinetics have been attributed to increased CNS drug penetration and risks of neurotoxicity in pediatric patients [6]. This is an important complexity to consider when designing gene therapies for children.

However, this route of administration is generally ineffective for CNS delivery in adults, due to reduced active transport, unless used with brain-penetrant engineered capsids (e.g., preclinical AAV-PHP.eB, or newer investigational and proprietary clinical analogues) or extremely high vector doses. The use of high doses raise the risk of peripheral organ toxicity and immune responses, as seen in AAV trials for SMA and Duchenne muscular dystrophy (DMD) [7]. Collectively, these limitations create a delivery bottleneck: therapies that are otherwise well-designed at the molecular level cannot reach enough target cells in the brain without unacceptable risk or invasiveness. Addressing this challenge requires approaches that can achieve broad, regionally selective, and non-invasive delivery.

1.2 Introduction to Gene Therapy: Techniques and Terminology

While the initial focus of gene therapy was on adding genes in recessive conditions, new modalities have now potential to enable the treatment of any kind of genetically defined disorder. Currently, "gene therapy" encompasses a range of approaches to alter gene expression in target cells. The following categories summarize the major classes currently under investigation for CNS disorders:

- **Gene Addition (also called gene replacement)**
  Introduction of a functional gene to compensate for a missing or mutated one. Typically uses AAV vectors due to their favorable safety profile and ability to mediate long-term expression. This approach is most advanced in the CNS (e.g., *Upstaza* AAV2-DDC for AADC deficiency; Zolengsma AAV9-SMN1 for SMA; current clinical trial with AAV2-GDNF for Parkinson's disease).

- **Gene Silencing**
  Reduction of toxic or aberrant gene products using antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), or CRISPR interference (CRISPRi). Targeted silencing is particularly relevant for dominantly inherited neurodegenerative diseases such as Huntington's (mutated-HTT silencing) or GRN-related frontotemporal dementia (FTD).

- **Gene Editing**
  Permanent correction or disruption of genomic sequences using nucleases such as CRISPR-Cas9, TALENs, or zinc-finger nucleases. Editing platforms are still largely preclinical for the brain and require careful consideration of off-target effects, vector size constraints, and delivery to dividing versus non-dividing cells.

- **Base and Prime Editing**
  Precision editing technologies that enable single-nucleotide changes or short sequence edits without creating double-strand breaks. These tools offer potential for highly targeted correction of pathogenic mutations but remain early in development for CNS use.

- **Repeat Expansion Targeting**
  Strategies to degrade, excise, or suppress repeat expansions associated with disorders such as Huntington's disease (CAG repeats) or C9orf72-linked ALS/FTD (GGGGCC repeats). Modalities include ASOs, RNA-targeted nucleases, and transcriptional silencing mechanisms.

- **Epigenetic Modulation**
  Use of engineered transcription factors, chromatin modifiers, or non-coding RNAs to upregulate or suppress gene expression without altering DNA sequence. These may be particularly suited for dosage-sensitive disorders or conditions requiring reversible regulation.

Each of these approaches presents unique challenges for delivery: some require high vector concentrations, others must reach specific cell types, and some involve tools (e.g., Cas9) that may exceed AAV packaging limits. Understanding how these therapeutic strategies interface with available delivery routes, especially those that are non-invasive or regionally selective, will be critical to advancing next-generation gene therapies for the brain, with or without FUS BBBO.

1.3 Opportunity for FUS BBBO Gene Therapy Delivery

Growing preclinical evidence indicates that focused ultrasound (FUS)-mediated blood-brain barrier opening (BBBO) addresses a key challenge in gene therapy for neurological disorders: achieving sufficient and targeted delivery of therapeutic vectors to deep brain structures with minimal invasiveness [8–12]. By enabling localized, noninvasive access to regions such as the striatum, FUS offers a delivery solution that could enhance the efficacy of both newly developed and previously tested gene therapies that were limited by suboptimal biodistribution.

2. Criteria for Selecting Gene Therapy Programs Suitable for FUS-Mediated Brain Delivery

2.1 Selection of Readiness Attributes for Gene Therapy Programs

Gene therapy programs typically progress through a series of development phases (after identification of a therapeutic target) beginning with vector design and in vitro validation, followed by manufacturing, preclinical efficacy and safety studies in relevant animal models, and culminating in clinical trials. Along this pathway, programs must demonstrate not only biological rationale and therapeutic potential, but also manufacturability, regulatory strategy, and eventual clinical feasibility. As more gene therapy candidates advance toward or through clinical testing, many encounter a plateau in development or scalability due to the aforementioned delivery limitations. It is at this juncture that focused ultrasound (FUS) presents a transformative opportunity, enabling noninvasive, targeted drug and gene delivery to the brain.

Because the goal of this initiative is to identify what delivery bottleneck can be solved with FUS-BBBO (and not to reinvent the gene therapy payload), the proposed framework prioritizes programs that already demonstrate a baseline level of translational "readiness." This includes scientific validity of the target and payload, feasibility of integration with FUS procedures, and the presence of commercial and clinical momentum. In this memo, programs utilizing adeno-associated viruses (AAVs) are prioritized. These vectors already have FDA approval for neurological and systemic indications, and their established safety profiles, manufacturing infrastructure, and regulatory precedent make them attractive candidates for early integration with FUS-mediated delivery. In contrast, emerging vectors such as non-viral vectors (e.g., lipid nanoparticles) currently lack the same translational maturity for CNS applications as of today.

The following section outlines these readiness attributes in more detail, highlighting the specific criteria that define an ideal candidate for a first-in-human trial of FUS-facilitated gene therapy to the brain.

2.1.1 Scientific Readiness

Given the motivation to translate FUS-mediated gene therapy delivery to clinical trials, a strong preclinical scientific rationale for selecting candidate indications is essential. This includes:

- evidence of a well-understood disease mechanism,
- identification of a validated genetic target,
- and proof that modulation of that target can meaningfully reverse or alter disease progression.

Gene therapy programs should demonstrate that the therapeutic approach is biologically and mechanistically grounded. In addition, the gene therapy vector must be designed for cell-type specificity, durable expression, and efficient CNS penetration. Vectors should employ strategies such as novel capsid engineering, whether through rational design or directed evolution, and promoter selection to enhance specific brain cells targeting while minimizing off-target effects. In particular, mitigating systemic exposure or hepatic transduction, which have posed challenges in earlier programs [13], are major technical priorities of many CNS gene therapy programs.

The route of administration (ROA) plays a pivotal role, not only in determining the invasiveness of the procedure but also in shaping manufacturing demands and patient accessibility. These considerations should be integrated early into preclinical study design to prevent downstream regulatory bottlenecks and support a clear path to first-in-human application.

Key questions for evaluating scientific readiness:

- Is the disease monogenic or multifactorial, and is the therapeutic target clearly defined?
- What cell types or tissues require gene delivery for clinical benefit?
- Is broad CNS distribution required, or is focal targeting sufficient?
- Are relevant in vivo models available to evaluate safety, distribution, and efficacy?
- Are there short-term biomarkers or clinical endpoints that are both measurable and predictive of long-term therapeutic benefit?
- Is a vector available with cell-type specificity, durable expression, and efficient CNS penetration?
- Is an optimal route of administration identified? (e.g. systemic vs. local)

2.1.2 Operational Feasibility

Translating a gene therapy program into a clinical trial requires more than scientific merit alone; it demands operational readiness across manufacturing, regulatory, clinical, and patient-facing domains.

First, the program must be supported by manufacturing capabilities that meet the necessary technological demands and can scale under current Good Manufacturing Practices (GMP). This includes plasmid production, viral vectors (e.g., AAV), and quality control systems that can support both early clinical studies and eventual commercialization. The vector production must be scalable for clinically feasible dosing.

Regulatory expertise is equally critical. Programs need access to teams experienced in Chemistry, Manufacturing, and Controls (CMC), Investigational New Drug (IND) applications, and regulatory engagement specific to gene therapy. This includes navigating the requirements for combination products, should the program integrate a delivery modality such as FUS, and preparing for interactions with FDA divisions including the Center for Biologics Evaluation and Research (CBER), the Center for Devices and Radiological Health (CDRH), and the Office of Combination Products (OCP).

Clinical trial execution requires access to specialists who understand the complexities of conducting gene therapy trials, particularly in neurological populations. These experts bring knowledge of dosing protocols, safety monitoring, vector biodistribution analysis, and trial design considerations for rare or progressive diseases. Importantly, the program must identify mechanisms for recruiting eligible patients, whether through national registries, institutional networks, or natural history studies. Early partnerships with patient advocacy groups and disease foundations can strengthen trial enrollment, improve protocol design, and ensure alignment with patient needs and expectations.

Once these aspects of operational feasibility are established, additional planning is required to incorporate focused ultrasound (FUS) as a delivery method. This includes consideration of device regulatory classification, site training, procedural standardization, and integration of FUS into the overall clinical development plan. FUS offers a noninvasive, targeted delivery strategy, but its implementation must be synchronized with the logistical and regulatory demands of gene therapy to achieve clinical success.

Key questions for evaluating operational feasibility:

- What manufacturing capabilities are required (technology, scale, GMP)?
- Is regulatory expertise available for CMC, IND trial design and FDA interactions?
- Is there access to experts experienced in running gene therapy neurological clinical trial (for this indication)?
- Is there an access secured to eligible patients (via registries, patients advocacy groups, disease foundations, etc.)?
- Is the logistics optimized for FUS implementation, training and procedure standardization?

2.1.3 Commercial Viability

Bringing a gene therapy for the brain to market can require over $2 billion in development costs before reaching a single patient, with much of that investment driven by GMP-grade AAV manufacturing, non-human primate (NHP) toxicology studies, and early-phase clinical trials [14].

While these figures reflect typical industry-led programs, alternative academic and public-private translational routes—such as the NIH-led Bespoke Gene Therapy Consortium (BGTC)—are actively working to reduce redundant preclinical and manufacturing efforts. BGTC is funded with around $76 million over five years and has been establishing standardized AAV-vector manufacturing, analytical, and regulatory "playbooks" to de-risk and streamline preclinical-to-clinical translation for rare diseases [15].These efforts aim to provide reusable protocols and common platforms that could significantly lower costs across multiple programs. Nonetheless, even after approval, the financial burden remains substantial: prices for gene therapies can exceed $3 million per treatment, and projected U.S. spending is expected to reach $35-40 billion over the next decade [16].

A scientific breakthrough is only the first step; without a viable commercial path, even the most promising gene therapies risk failing to reach patients. For a FUS-mediated gene therapy program to advance toward clinical adoption, commercial readiness must be assessed alongside preclinical and regulatory milestones. Key questions include: who holds the intellectual property rights for the therapeutic vector, and are there freedom-to-operate concerns, particularly for proprietary capsids, promoters, or delivery enhancers that may be encumbered by blocking patents? If FUS is to be integrated, device-related IP may also require licensing or joint development agreements, especially in the case of novel sonication sequences or combination product claims.

The funding model is another critical determinant. AAV vector manufacturing remains capital-intensive, and early-stage programs must secure partnerships or financing that can support GMP-grade vector production, IND-enabling studies, and multi-phase clinical trials. In parallel, device development and validation, if a new FUS system is required, may demand investment from either medtech or biopharma stakeholders.

Importantly, commercial viability relies on a clear understanding of the market. Are there enough patients with the target condition to justify investment? While rare diseases may involve small populations, regulatory incentives — such as the FDA's Orphan Drug Designation, Rare Pediatric Disease Priority Review Voucher, and expedited pathways like RMAT and Fast Track— they can significantly reduce development risk and enhance return on investment. These programs offer benefits including tax credits, user fee waivers, market exclusivity, and accelerated timelines, which have helped catalyze gene therapy development even in ultra-rare indications. However, early engagement with payers and providers remains essential to anticipate reimbursement challenges and articulate a compelling value proposition. A strong product profile must demonstrate not only therapeutic benefit but also potential cost savings, particularly if FUS can reduce vector dose requirements, eliminate surgical procedures, or shorten hospitalization time.

When FUS is introduced as a delivery method, additional considerations come into play. The regulatory classification of the combined biologic-device product must be clearly defined, and manufacturing, training, and reimbursement pathways for the FUS system must be feasible and scalable. Therefore, strategic partnerships across gene therapy developers, device manufacturers, academic centers, and patient groups will be essential to navigate these intersecting domains and bring FUS-enabled therapies to market.

Key questions for evaluating commercial viability:

- Who holds the intellectual property rights for the therapeutic vector?
- Are there freedom-to-operate concerns, particularly for proprietary capsids, promoters, or delivery enhancers that may be encumbered by blocking patents?
- Is there a need to license FUS device-related IP or to develop joint development agreements, for a combination product FUS + GT?
- Are fundings secured to support the development, manufacturing, clinical trials of the gene therapy?
- Are fundings secured to support FUS system development and validation, if needed?
- Can the therapy benefit from rare diseases regulatory incentives to reduce development risks?
- Is there data or market research to support a product commercial potential
- Are strategic partnerships across gene therapy developers, device manufacturers, and academic centers possible?

2.2 FUS delivery relevance

A final dimension to consider when identifying programs and indications for FUS-mediated brain gene therapy, is the suitability of the gene therapy programs for FUS-BBBO. From the preclinical literature, it is now established that not all capsids and/or routes of delivery will

benefit from FUS-BBBO for enhanced delivery [17]. For example, systemic administration of AAV9 has demonstrated feasibility of targeted CNS transduction with FUS in primate studies [9,18], whereas intrathecal delivery or AAV2 has shown limited preclinical efficacy [12].

Additionally, novel engineered capsids such as AAV.FUS [11] and CCP16 [19] have been developed specifically to leverage FUS-BBBO for efficient and localized brain transduction. Other capsids, such as AAV2-HBKO have shown enhanced delivery and transduction when combined with FUS-BBBO and intra-CSF delivery [20,21].

This FUS delivery relevance dimension also incorporates additional considerations, and important parameters to assess include:

- the localization of the pathology — favoring anatomically discrete targets,
- the compatibility of capsid and route of administration with FUS-BBBO paradigms,
- with engineered BBB-penetrant capsids, the potential to either reduce systemic dosing, or to locally boost the delivery if needed.

Key questions for evaluating FUS delivery relevance:

- Is there a need for spatially discrete FUS-mediated delivery (e.g. striatum)?
- Is there a validated FUS-BBBO paradigm for this capsid and route of administration?
- Is there an expected systemic dose-reduction when combined with FUS-BBBO?
- For engineered brain-penetrant capsids, is there a need of local delivery boost?

3. Framework for Identifying Indications and Gene Therapy Programs suitable for FUS-mediated BBBO as a Delivery Approach

3.1 Proposed framework for indications selection

To support indication prioritization for first-in-human trials of FUS-mediated AAV gene therapy, a composite scoring framework was developed, integrating gene therapy development readiness and FUS delivery relevance.

In this framework, an indication is first evaluated across three categories:

- scientific readiness (e.g. targetability, disease model reliability),
- operational feasibility (e.g. clarity of regulatory path, patient identification, trial coordination),
- commercial viability (e.g. market size, differentiation),

resulting in a readiness score reflecting the maturity and translational potential of the gene therapy program.

Additionally, the indication is evaluated in terms of its FUS delivery relevance to assess:

- Compatibility of the proposed approach with current FUS-BBBO paradigms.

We propose to then sum the two scores to prioritize indications where both the gene therapy strategy is clinically actionable, and utility of FUS is clearly defined.

This framework is not designed to encourage replacement of well-functioning surgical strategies, such as intraputaminal *Upstaza* delivery in pediatric AADC deficiency, but rather to broaden treatment options by offering less invasive, patient-friendly alternatives that could improve recruitment, reduce surgical burden, and unlock new clinical use cases for gene therapies previously constrained by delivery limitations. Specifically, it should identify indications where FUS offers a plausible route to solve a delivery bottleneck by:

- avoiding neurosurgical infusion,
- reducing vector dose,
- or enhancing regional expression in sub-optimally transduced brain regions

3.2 Applying the Framework: Identification of Indications

The framework was applied to score several neurological gene therapy indications, with results summarized in

Table 1. The associated data are accessible in our companion database (http://www.fusfgenetherapy.com/). These results reflect our current assessment of the landscape of gene therapy development, focusing specifically on existing vector designs and routes of administration already evaluated in preclinical or clinical settings. As such, the analysis is intended to identify indications and associated gene therapy programs that are presently amenable to integration with validated FUS-BBBO delivery methods—most notably intravenous administration of compatible serotypes such as AAV9.

However, a distinct and complementary line of inquiry concerns those indications where gene therapy development may benefit from strategic redesign to enhance compatibility with FUS. This includes re-evaluating vector capsids, promoters, and routes of administration to better align with the unique advantages of FUS-facilitated delivery. These capsids represent compelling candidates for future preclinical validation, especially in cases where existing programs, particularly those relying on intrathecal or intracranial delivery of non-FUS-compatible serotypes, encounter translational bottlenecks, whether due to subtherapeutic efficacy, safety concerns, or logistical challenges such as limited patient willingness to undergo surgery.

Table 1: Framework for Indication Selection. Indications were sorted based on score summing the overall gene therapy development readiness (itself the average of scientific readiness, operational feasibility and commercial viability scores) with the FUS suitability score. The most up-to-date set of data can be found in our publicly accessible database http://www.fusfgenetherapy.com/

Disease/Indication	Scientific Readiness Score	Organizational Readiness Score	Commercial Readiness Score	Avg. Gene Therapy Development Readiness	Avg. FUS Suitability Score	Total Score
Parkinson's Disease (GBA1)	2.8	2.8	3.0	2.9	3.0	**5.9**
Spinal Muscular Atrophy (SMA)	3.0	3.0	3.0	3.0	2.0	**5.0**
Parkinson's Disease (idiopathic)	2.4	2.7	2.5	2.5	2.5	**5.0**
Huntington's Disease	2.8	2.5	3.0	2.8	2.0	**4.8**
Duchenne Muscular Dystrophy (DMD)	2.8	3.0	3.0	2.9	1.5	**4.4**
Canavan Disease	2.8	2.3	2.3	2.5	2.0	**4.5**
GM1 Gangliosidosis	2.8	2.3	2.3	2.5	2.0	**4.5**
AADC deficiency	2.8	2.2	2.0	2.3	2.0	**4.3**
Gaucher Disease (neuronopathic)	2.8	2.2	2.0	2.3	2.0	**4.3**
Frontotemporal Dementia (FTD, GRN)	2.6	2.2	2.0	2.3	2.0	**4.3**
Alzheimer's Disease	1.8	2.2	2.5	2.2	2.0	**4.2**
Giant Axonal Neuropathy (GAN)	2.8	1.7	2.0	2.2	2.0	**4.2**
Adrenomyeloneuropathy (AMN)	2.8	2.2	2.0	2.3	1.5	**3.8**
Dravet Syndrome	2.8	2.2	2.0	2.3	1.5	**3.8**
MPS I (Hurler syndrome)	2.8	2.2	2.0	2.3	1.5	**3.8**
MPS II (Hunter syndrome)	2.8	2.2	2.0	2.3	1.5	**3.8**
MPS III (Sanfilippo A-D)	2.8	2.2	2.0	2.3	1.5	**3.8**
Mucolipidosis Type IV (ML4)	2.0	1.8	1.3	1.7	2.0	**3.7**
Krabbe Disease (Globoid cell LD)	2.6	1.5	2.0	2.0	1.5	**3.5**
Batten Disease CLN2	2.8	2.3	2.3	2.5	1.0	**3.5**
Metachromatic Leukodystrophy (MLD)	2.8	2.2	2.0	2.3	1.0	**3.3**
Tay-Sachs & Sandhoff (GM2)	2.8	2.2	2.0	2.3	1.0	**3.3**
MPS VII (Sly syndrome)	2.8	2.2	2.0	2.3	1.0	**3.3**
ALS (SOD1)	2.6	2.3	2.3	2.4	1.0	**3.4**
CLN3 (Juvenile Batten)	2.6	2.2	2.0	2.3	1.0	**3.3**
CLN6 (Late-infantile Batten)	2.6	2.2	2.0	2.3	1.0	**3.3**
Rett Syndrome	2.6	2.2	2.0	2.3	1.0	**3.3**
Multiple System Atrophy (MSA)	2.0	1.2	1.3	1.5	1.5	**3.0**
CLN7 (Late-infantile Batten)	2.6	1.3	1.5	1.8	1.0	**2.8**

4. Disease Context and Therapeutic Targets Case Studies

4.1 Parkinson's Disease

4.1.1 Parkinson's Disease Overview

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease (AD), affecting over 10 million people globally and approximately 1 million individuals in the United States alone. Its prevalence increases with age, and incidence is expected to rise substantially with global aging demographics. Clinically, PD is defined by progressive motor dysfunction with symptoms including tremor, rigidity, bradykinesia, as well as a wide array of non-motor symptoms including cognitive impairment, depression, and autonomic dysfunction [22]. The pathological hallmark is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to dopamine depletion in downstream basal ganglia structures, primarily the putamen and caudate.

From a therapeutic perspective, this neuroanatomical circuitry, i.e. SNpc input and striatal output, forms a rational and spatially defined target for gene therapy. Unlike Alzheimer's disease, where pathological substrates are diffuse, PD allows for focused intervention within discrete subcortical regions. Key delivery targets for FUS-mediated gene therapy include the putamen, caudate, and substantia nigra, each of which can be reached non-invasively via FUS-BBB opening, as evidenced by important large animal pre-clinical studies [9,18].

4.1.2 Monogenic forms of PD

A small but crucial subset of Parkinson's disease (PD) cases, approximately 10-15%, arise from inherited mutations in single genes—so-called monogenic forms of PD. These cases are particularly well-suited for gene-targeted approaches, as they provide a direct link between genotype and disease mechanism. Specifically, genes with clearly measurable enzymatic or protein targets are especially amenable to gene therapy development, as they allow for pharmacodynamic tracking of treatment effects. Three major types of genetic contribution have been described: autosomal dominant mutations, autosomal recessive mutations, and incomplete-penetrance risk variants that modify disease susceptibility. Understanding these distinctions is essential when selecting candidates for early-phase gene therapy trials, especially those employing focused ultrasound (FUS) for targeted, noninvasive delivery to the brain.

In autosomal dominant inheritance, a mutation in just one copy of a gene is sufficient to cause disease. This is often due to a toxic gain-of-function, in which the mutated protein acquires abnormal activity or interferes with normal cellular processes. One example is the *LRRK2* gene, where the G2019S mutation leads to increased kinase activity and neuronal toxicity. This mutation is the most commonly known cause of familial PD and also occurs in sporadic cases. While not every carrier of a *LRRK2* mutation will develop PD, the risk increases with age. Estimates suggest penetrance (the probability that a mutation carrier will manifest disease) ranges from 30% to over 80% by age 80, depending on genetic background and population [23].

Another autosomal dominant gene is *SNCA*, which encodes α-synuclein, the primary component of Lewy bodies. In this case, mutations or multiplications of the gene increase the expression or aggregation of α-synuclein, directly contributing to PD pathogenesis. Though rare, *SNCA* mutations have high penetrance and cause aggressive, early-onset PD often accompanied by

dementia [24]. Because α-synuclein aggregation is a hallmark of most PD forms, *SNCA*-related PD offers a particularly disease-relevant target for gene silencing strategies.

Taken together, *LRRK2* and *SNCA* represent autosomal dominant, monogenic causes of PD with well-defined molecular mechanisms. Despite differences in their biological functions—*LRRK2* being a kinase with measurable enzymatic activity, and *SNCA* encoding a structural protein without enzymatic function—both genes offer clear, quantifiable biomarkers of target engagement. In the case of *LRRK2*, kinase activity can be tracked via phosphorylation of Rab substrates such as Rab10 [25], while *SNCA* burden can be monitored through α-synuclein levels in cerebrospinal fluid or emerging PET tracers [26]. Measurable biomarkers like these are essential for translational success, especially in early-phase trials where confirming molecular engagement is critical for dose selection and regulatory approval. As discussed earlier, the presence of a measurable enzymatic or protein target enables not only rational therapeutic design but also real-time pharmacodynamic assessment of treatment effects.

Given that both LRRK2 and SNCA mutations result in toxic gain-of-function effects, they are not amenable to AAV-based gene replacement strategies. Instead, gene-silencing approaches such as antisense oligonucleotides (ASOs) have emerged as the leading modality. For LRRK2, a Phase 1 ASO trial (BIIB094; NCT03976349), employing intrathecal injection of the therapeutic, was initiated but later paused, while small-molecule kinase inhibitors such as BIIB122 remain under active investigation. For SNCA, an ASO targeting α-synuclein mRNA (ION464; NCT04165486), delivered by recurring IT injections, is currently being evaluated in a Phase 1/2a trial in multiple system atrophy with relevance to Parkinson's disease. These trials leverage the availability of protein-level biomarkers to assess target engagement and guide therapeutic development in the absence of a gene replacement strategy.

The third major gene, *GBA1*, differs in that it does not follow classic Mendelian inheritance (i.e. dominant or recessive). Instead, it acts as a risk gene with a modifier effect. Individuals with a single (heterozygous) mutation in *GBA1* have a 5- to 10-fold increased risk of developing PD, but not all carriers will be affected. Penetrance is incomplete, estimated between 10% and 30% [27]. *GBA1* encodes the lysosomal enzyme glucocerebrosidase (GCase), and loss-of-function mutations reduce its activity, promoting α-synuclein accumulation and impairing cellular waste clearance. Despite its complex genetic role in PD, *GBA1* is a strong therapeutic target because GCase activity is directly measurable in CSF or blood. This provides a clear readout of drug effect and makes *GBA1* both biologically well-understood and clinically actionable. Building on this strong biological rationale and biomarker accessibility, AAV9-mediated gene therapy targeting *GBA1* (e.g., Prevail's PR001), administered ICM, is already in clinical trials (NCT04127578).

In contrast, mutations in the autosomal recessive gene *PRKN* (also called *PARK2*) must be inherited on both alleles (one from each parent) to cause disease. These mutations result in loss-of-function of the Parkin protein, an E3 ubiquitin ligase involved in mitochondrial quality control. *PRKN*-associated PD typically manifests in adolescence or early adulthood and is characterized by slow progression and a good response to levodopa. Notably, many cases lack Lewy body pathology, suggesting a different disease mechanism compared to synucleinopathy-driven PD [28]. Although clearly monogenic, *PRKN*-PD poses translational challenges: there is no validated pharmacodynamic biomarker for Parkin activity, as its function is context-dependent and lacks a measurable enzymatic output in CSF or blood. This makes it difficult to confirm target engagement in vivo, limiting its suitability for gene therapy approaches that rely on

biological biomarker-guided development and may therefore necessitate alternative endpoints, such as wearable-derived digital biomarkers and other functional/behavioral measures. However, because heterozygous carriers are generally unaffected and the genetic cause is well-defined, *PRKN*-PD may still hold relevance for cell-based or neuroprotective interventions.

In addition to possible early motor response outcomes, imaging biomarkers are also in development. FDG-PET-derived network biomarkers, such as the Parkinson's disease–related pattern (PDRP) and cognition-related pattern (PDCP), have demonstrated sensitivity to treatment effects, with measurable changes in PDRP expression observed within months of intervention, including evidence of treatment-induced network remodeling following AAV2-GAD gene therapy in PD, making them practical endpoints for short-term (3–6 month) response assessment in gene therapy trials for PD [29].

Table 2: Monogenic forms of Parkinson's Disease

Inheritance Pattern	Gene	Protein	Mechanism
Autosomal Dominant (typically toxic gain of function or dominant-negative mechanisms)	SNCA	α-synuclein	Protein aggregation
	LRRK2	Leucine-rich repeat kinase 2	Kinase hyperactivity
	VPS35	Vacuolar protein sorting 35	Retromer dysfunction
Autosomal Recessive (typically result from loss-of-function mutations leading to impaired mitochondrial or lysosomal function)	PRKN (PARK2)	Parkin (E3 ubiquitin ligase)	Impaired mitophagy
	PINK1	PTEN-induced kinase 1	Mitochondrial dysfunction
	DJ-1 (PARK7)	Antioxidant protein DJ-1	Oxidative stress response failure
	FBXO7	F-box protein 7	Ubiquitin-proteasome dysfunction
	PLA2G6	iPLA2β	Lipid metabolism
	ATP13A2	Lysosomal ATPase	Lysosomal dysfunction
Risk Genes	GBA1	Glucocerebrosidase (GCase)	Heterozygous mutations increase PD risk ~5–10×; associated with faster progression, α-synuclein aggregation
	SMPD1	Acid sphingomyelinase	Also a lysosomal risk gene for PD, especially with dementia
	GCH1	GTP cyclohydrolase 1	Causes dopa-responsive dystonia (DRD); can mimic PD
	MAPT	Tau protein	H1 haplotype is a risk factor for PD and PSP, but not a monogenic PD cause

From a translational standpoint, these genetic subtypes (*GBA1*, *LRRK2*, and *SNCA*) align strongly with scientific readiness criteria for first-in-human (FIH) gene therapy trials using focused ultrasound. Each has a well-characterized molecular mechanism, available animal models, emerging or established biomarkers, and either current or near-clinical vector-based therapies. Importantly, the regions most affected in these forms of PD (e.g., the putamen and substantia nigra) are anatomically accessible to FUS-mediated blood-brain barrier opening,

enabling localized, noninvasive delivery. Among them, *GBA1* is especially attractive due to the availability of pharmacodynamic biomarkers, known lysosomal mechanisms, and existing regulatory incentives such as Orphan Drug Designation and RMAT eligibility – incentives which could also apply to other monogenic forms (e.g., LRRK2, SNCA and PRKN) with further validation of disease-modifying therapies targeting these genes.

4.1.3 Non-monogenic forms of PD

While monogenic forms of PD provide clear molecular drivers that can be directly targeted with gene therapy, the vast majority of PD cases are non-monogenic and clinically heterogeneous, with no single causal mutation. These idiopathic forms likely arise from a complex interplay of genetic risk variants, aging-related cellular dysfunction, and environmental exposures. As a result, identifying a single, universal molecular target for gene therapy is challenging, and target engagement cannot be readily confirmed through genotype-driven stratification. This biological complexity makes non-monogenic PD less suited for first-in-human trials of FUS-mediated gene delivery. Nevertheless, therapeutic strategies have focused on modulating crucial pathways implicated in PD pathophysiology, such as dopaminergic enzyme replacement, neurotrophic support, and synuclein reduction, regardless of the genetic cause. The next section outlines the rationale behind current clinical trials that apply gene therapy to idiopathic PD populations by targeting these convergent mechanisms.

Several gene therapy approaches targeting idiopathic Parkinson's disease have explored both symptomatic (non–disease-modifying) and disease-modifying strategies. The symptomatic approach with AAV2-hAADC (Voyager's VY-AADC01) aimed to restore aromatic L-amino acid decarboxylase (AADC) in the putamen to enhance dopamine synthesis. In a phase 1 clinical trial, while it yielded motor improvements and increased 18F-DOPA PET signal, intraputaminal MRI-guided convection-enhanced infusions achieved only limited coverage. Approximately 21%, 34%, and 42% of the putamen in escalating-dose cohorts exhibited infusion coverage, despite intraoperative infusion monitoring techniques used to map injectate volume [30]. Even in the highest-dose cohort, variable enzyme activity and PET responses reflected inconsistent tissue transduction. Moreover, invasive neurosurgical delivery carried procedural risks, including headaches, dyskinesia, and rare deep vein thrombosis—events classified as treatment emergent but manageable [31].

Disease-modifying trials with AAV2-GDNF and AAV2-NTN, which aimed to provide neurotrophic support, similarly suffered from constrained anatomical distribution. Phase 1 studies reported putamen coverage averages of just ~26%, with clinical measures remaining largely unchanged. Such outcomes were again attributed to insufficient vector spread despite MRI-coordinated cannula placement [32]. Reviews have highlighted that inadequate transduction due to inadequate coverage contributed to trial failures and spurred design modifications in later studies [33,34].

These delivery limitations are particularly consequential in non-monogenic PD, where diffuse degeneration across the basal ganglia, brainstem, and cortex requires broader therapeutic coverage compared to focal, monogenic forms. The instability to achieve consistent vector distribution has constrained both symptomatic and disease-modifying efficacy. These challenges, i.e. limited coverage, variable outcomes, and procedural complexity**,** underscore the potential advantage of focused ultrasound (FUS)-mediated blood-brain barrier opening as a noninvasive

method to achieve targeted, widespread transduction when coupled with innovations in clinical development as demonstrated in ongoing trials.

Table 3: Summary of past and ongoing clinical trials for Parkinson's Disease

Trial Number	Phase	Start (End) Date	Sponsor	ROA	ROA details	Transgene	Capsid
NCT00195143	Phase 1	8/2003 (8/2005)	Neurologix, Inc.	IP	Unilateral subthalamic nucleus [28]	GAD	AAV2
NCT01621581	Phase 1	3/2013 (2/2022)	NINDS	IP	Bilateral putamen	hGDNF	AAV2
NCT00400634, ext NCT05894343	Phase 2	11/2006 (11/2008)	Sangamo Therapeutics	IP	Bilateral putamen CED	Neurturin	AAV2
NCT00985517	Phase 1/2	10/2009 (11/2017)	Sangamo Therapeutics	IP	Bilateral putamen and substantia nigra	Neurturin	AAV2
NCT04167540	Phase 1b	4/2020	Brain Neurotherapy Bio/UCSF	IP	Bilateral putamen	GDNF	AAV2
NCT06285643	Phase 2	6/2024	AskBio/Bayer AG	IP	Bilateral putamen	GDNF	AAV2
NCT05603312	Phase 1/2	10/2022 (9/2024)	Meira GTx	IP	Bilateral STN	GAD	AAV(?)
NCT00643890	Phase 2	8/2008 (12/2010)	Neurologix, Inc.	IP	Bilateral STN	GAD	AAV(?)
NCT01973543	Phase 1	10/2013 (1/2020)	Neurocrine Biosciences	IP	Bilateral striatum	hAADC	AAV2
NCT03562494	Phase 1	10/2018 (10/2024)	Neurocrine Biosciences	IP	Bilateral	hAADC	AAV2
NCT03065192	Phase 1	5/2017 (8/2021)	Neurocrine Biosciences	IP	Bilateral putamen	hAADC	AAV2
NCT00229736	Phase 1	11/2004 (3/2013)	Genzyme (Sanofi)	IP	4 striatal infusions	hAADC	AAV(?)
NCT04127578	Phase 1/2	1/2020	Prevail Therapeutics	ICM	Cisterna Magna	GBA1	AAV9
NCT07011771	Phase 1/2	8/2025	Capsida Biotherapeutics, Inc.	IV	IV	GBA1	AAV(?)

One of the most advanced ongoing trials in idiopathic PD is sponsored by Asklepios BioPharmaceutical (AskBio), evaluating AB-1005, an AAV2 vector encoding glial cell line–derived neurotrophic factor (GDNF), delivered by intraputaminal infusion (NCT04167540). AB-1005 is designed as a disease-modifying therapy aimed at restoring trophic support to degenerating dopaminergic neurons. Unlike earlier GDNF trials, this program employs a single bilateral cannula, navigated with robot-assisted convection-enhanced delivery, and leverages updated clinical imaging protocols to improve vector coverage and safety. As of 2024, interim

data from the open-label Phase 1b trial suggest favorable tolerability and stable GDNF expression, with no vector-related serious adverse events reported and sustained clinical follow-up beyond one year [35]. The most recent safety and efficacy data emerging from this trial indicates stability or improvement in MDS-UPDRS scores in mild or moderate dose cohorts, respectively [36].

Another promising effort includes PR001 program of Prevail Therapeutics (a wholly owned subsidiary of Eli Lilly), an AAV9 gene therapy encoding GBA1, originally developed for GBA1-PD, but relevant due to its targeting of lysosomal dysfunction, a pathway also implicated in idiopathic PD pathophysiology. Although the current Phase 1/2 PROPEL trial (NCT04127578) is limited to genetically stratified participants, it showcases the emerging direction of mechanism-based stratification that could inform future approaches for idiopathic disease.

Together, these insights position PD, especially monogenic forms, as a lead candidate for first-in-human trials of FUS-mediated gene therapy. The disease offers well-defined neuroanatomical targets, strong translational precedent from surgical gene therapy trials, measurable biomarker and imaging endpoints, and the possibility of genetically stratified enrollment to de-risk early clinical testing.

4.2 FTD (GRN) Overview

Frontotemporal dementia (FTD) is a clinically and pathologically heterogeneous group of neurodegenerative disorders, accounting for up to 20% of dementia cases in individuals under 65 years of age. It is characterized by progressive atrophy of the frontal and temporal lobes, leading to changes in behavior, language, and executive function. Unlike Alzheimer's disease, memory is relatively preserved in early stages, but patients typically exhibit disinhibition, apathy, and impaired social cognition. The average age of onset is between 45 and 65, and disease progression is rapid, with a median survival of 6-8 years post-diagnosis.

Approximately 30-40% of FTD cases are familial, and among these, mutations in the *GRN* gene (encoding progranulin) are a major cause of autosomal dominant FTD. *GRN* haploinsufficiency leads to reduced levels of progranulin, a secreted glycoprotein involved in lysosomal function, neuroinflammation, and neuronal survival. Importantly, *GRN*-associated FTD represents a compelling target for gene therapy because the disease mechanism is a loss-of-function, and thus amenable to rescue via gene replacement strategies. Biomarkers such as plasma progranulin levels and T1-weighted MRI of frontotemporal atrophy provide reliable diagnostic and pharmacodynamic endpoints.

Target regions for therapeutic delivery in *GRN*-FTD include the anterior frontal cortex, temporal cortex, and cingulate gyrus, as well as thalamic and hippocampal regions in later stages. These cortical and subcortical regions are amenable to transcranial focused ultrasound (FUS)-mediated blood-brain barrier opening, especially emerging techniques leveraging multi-spot beam steering or hemispheric grid sonication strategies to facilitate delivery to broad brain areas.

Multiple therapeutic programs are advancing for *GRN*-FTD. Prevail Therapeutics is conducting a Phase 1/2 trial (NCT04408625) of AAV9-GRN administered via intra-cisterna magna injection, which has demonstrated encouraging progranulin restoration and cortical uptake in preclinical

models, and promising interim clinical data [37]. Passage Bio and Denali Therapeutics have pursued similar AAV9-based programs targeting *GRN*. Though elevated CSF progranulin levels in patients were observed [38], these intra-CSF administration approaches still result in non-uniform cortical distribution and are highly dependent on cerebrospinal fluid dynamics, often failing to reach deep cortical layers or exhibiting variable transduction across patients.

FTD caused by *GRN* mutations is therefore an ideal candidate for early FUS gene therapy trials. It presents with a genetically defined, fully penetrant mutation, a clear therapeutic mechanism (restoration of progranulin levels), validated biomarkers (plasma NfL), and anatomically accessible targets. The rapid clinical decline in *GRN*-FTD and lack of existing disease-modifying therapies further underscore the need for a platform capable of safe, repeated, and region-specific gene delivery. FUS enables this precision, offering a non-invasive route to cortical regions traditionally inaccessible by intrathecal or surgical means, and holds promise for extending therapeutic window and efficacy in this aggressive neurodegenerative condition.

4.3 ALS (SOD1) Overview

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease characterized by selective loss of upper and lower motor neurons, leading to muscle weakness, atrophy, spasticity, and ultimately respiratory failure. Median survival is 3-5 years from symptom onset. While the majority of ALS cases are sporadic, approximately 10% are familial, and mutations in *SOD1* (superoxide dismutase 1) account for roughly 20% of these hereditary forms. The *SOD1*mutation is autosomal dominant and toxic gain-of-function in nature, resulting in misfolded protein aggregation, mitochondrial dysfunction, and oxidative stress in motor neurons.

SOD1-ALS is among the best-characterized monogenic forms of the disease and was the first to be modeled in transgenic animals. These models recapitulate important features of motor neuron degeneration and have enabled the preclinical development of targeted therapies. Notably, the advent of antisense oligonucleotide (ASO) therapeutics has yielded promising translational progress. Tofersen, a *SOD1*-targeted ASO developed by Biogen and Ionis, received accelerated FDA approval in 2023 for patients with confirmed *SOD1* mutations, based on reduction in neurofilament light chain (NfL) levels and stabilization of respiratory function in early-treated individuals.

Due to the ASO platform, Tofersen requires repeated intrathecal administration, raising challenges around patient burden, CSF pharmacokinetics, and variable spinal cord biodistribution. Moreover, while lumbar administration is optimal for targeting the lower spinal cord, effective delivery to upper cervical and motor cortical regions remains difficult, particularly in early phases of the disease when upper motor neurons may still be salvageable [39]. These limitations motivate the use of FUS to enhance delivery of not only AAVs, but also ASOs to anatomically precise CNS targets.

Key targets in *SOD1*-ALS include the motor cortex, cervical and thoracolumbar spinal cord, and brainstem nuclei such as the hypoglossal and facial motor neurons. FUS-mediated blood-spinal cord barrier opening has been demonstrated in preclinical studies [40] and feasibility of blood-brain opening in the motor cortex of ALS patients has also been reported [41]. Transcranial and interlaminar sonication of these areas provides a route to non-invasively enhance therapeutic uptake in afflicted regions of the motor system.

AAV-based strategies are also in development for *SOD1*-ALS. AAVrh10 and AAV9 vectors encoding microRNAs or shRNAs targeting *SOD1* transcripts have shown efficacy in reducing protein levels and extending survival in preclinical models. However, like ASOs, these gene therapy vectors currently rely on intrathecal or cisterna magna administration, which often leads to non-uniform CNS penetration.

Given its monogenic cause, measurable biomarkers (NfL, SOD1 protein levels), validated ASO efficacy, and anatomically mappable pathology, *SOD1*-ALS also represents a strong candidate for FUS-enabled gene therapy intervention. The ability to reach both cortical and spinal targets non-invasively addresses a core limitation of current delivery strategies and could enable earlier, more comprehensive neuroprotection in this otherwise fatal disease.

4.4 Huntington' Disease Overview

Huntington's disease (HD) is a fatal, autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the *HTT* gene, which encodes the huntingtin protein. Pathogenic expansions (≥36 CAG repeats) lead to production of a toxic, aggregation-prone mutant huntingtin (mHTT) that disrupts numerous cellular pathways, including transcription, mitochondrial function, proteostasis, and synaptic signaling. Disease onset typically occurs between ages 30 and 50, with progressive motor dysfunction (chorea, dystonia, rigidity), psychiatric symptoms (depression, irritability), and cognitive decline culminating in death within 15-20 years. HD affects an estimated 3-10 per 100,000 individuals worldwide.

Neuropathologically, HD is marked by early and selective atrophy of the caudate nucleus and putamen, followed by progressive degeneration of the cortex, globus pallidus, and thalamus. These striatal and cortical structures are well-defined and anatomically accessible, making them attractive targets for localized therapeutic delivery. Notably, disease onset and progression in HD are relatively well-classified, with longitudinal imaging and biomarker studies (e.g., in the TRACK-HD and Enroll-HD cohorts) providing robust anatomical and biochemical readouts for trial design and monitoring.

As a monogenic gain-of-function disorder with a fully penetrant mutation, HD is particularly amenable to gene-targeted interventions. Several therapeutic modalities are under investigation to suppress mHTT production or promote its degradation, including:

- Antisense oligonucleotides (ASOs): Ionis and Roche developed tominersen, a non-allele-specific ASO targeting *HTT* mRNA. While the Phase 3 GENERATION HD1 trial (NCT03761849) was halted due to lack of clinical benefit and potential toxicity at higher doses [42], subgroup analyses and biomarker improvements in early-stage patients have motivated renewed interest in optimizing delivery and dosing. Wave Life Sciences is pursuing allele-selective ASOs targeting SNP-linked mHTT transcripts, aiming to reduce off-target suppression of wild-type *HTT* [43]
- RNA interference and gene editing: AAV-based delivery of microRNAs or shRNAs targeting *HTT* is under preclinical and early clinical investigation. UniQure's AMT-130, an AAV5-delivered microRNA targeting *HTT*, is currently in Phase 1/2 trials via intra-striatal injection (NCT04120493), and preliminary data have demonstrated mHTT lowering in cerebrospinal fluid and early signs of safety.

FUS-mediated blood-brain barrier opening, again, presents a transformative alternative: enabling repeatable, region-specific delivery of ASOs, AAVs, or other modalities directly to the caudate

and putamen without surgical intervention. Moreover, the presence of reliable imaging biomarkers (e.g., volumetric MRI for caudate atrophy detection, diffusion tensor imaging) and fluid markers (e.g., CSF mHTT, neurofilament light chain) enables pharmacodynamic assessment of gene therapy efficacy.

HD is therefore another well-suited candidate for FUS-enabled gene therapy. It combines a single known genetic driver, clear anatomical targeting, robust biomarkers, and pressing clinical need. FUS can overcome current limitations in biodistribution and invasiveness, enabling broader delivery to the striatum and cortex, potentially with fewer systemic side effects.

4.5 Platform approach for rare diseases

It should be noted, that while Parkinson's disease, FTD, ALS, and Huntington's disease represent relatively more prevalent neurodegenerative conditions, there exists a broader landscape of rare monogenic disorders that are also well-suited for gene therapy (see Table 4). Each affects only a small number of patients, but their cumulative impact is large. These ultra-rare conditions often have clear genetic etiologies and well-defined anatomical targets, making them compelling indications for focused ultrasound–mediated delivery. In such cases, the therapeutic strategy may trend toward a more bespoke capsid engineering approach, in which AAV vectors are customized for specific cell types, brain regions, or transport properties to meet the unique demands of each disorder. FUS offers the flexibility to pair with these tailored vectors for safe, targeted delivery, potentially enabling scalable precision medicine for diseases that would otherwise remain untreatable.

Table 4: Subset of rare CNS disorders for which gene therapy is being developed.

Disease Name	Estimated Current # of Patients (USA)	Incidence (USA)	Prevalence (USA)
Metachromatic Leukodystrophy (MLD)	<8000	~1 in 40,000–160,000 live births	~1 in 40,000–160,000
Krabbe Disease	<4000	~1 in 100,000 live births	~1 in 100,000
GM1 Gangliosidosis	<3500	~1 in 100,000–200,000 live births	~1 in 100,000–200,000
Friedreich's Ataxia	<1200	~1 in 50,000 live births	~1 in 50,000
Adrenoleukodystrophy (ALD)	<1700	~1 in 20,000 male births	~1 in 20,000 males
Batten Disease (CLN1–CLN8)	<3500	~1 per 100,000 live births	~2–4 per 100,000
Lafora Disease	<1500	~1 in 1,000,000 live births	~1 in 1,000,000
Tay–Sachs Disease	<1000	~1 in 320,000 live births	~1 in 320,000
Rett Syndrome	<12000	~1 in 10,000–15,000 live births	~1 in 10,000–15,000 females
Mucolipidosis Type IV (MLIV)	<10000	~1 in 40,000 live births	~1 in 40,000
Canavan Disease	<3500	~1 in 100,000 live births	~1 in 100,000
MPS III (Sanfilippo Syndrome)	<3000	~0.26 per 100,000 live births	0.26 per 100,000 live births
SOD1-associated ALS	<17000	~0.04 per 100,000	~2% of ALS cases

A translational roadmap for synergizing focused ultrasound (FUS) with rare monogenic central nervous system (CNS) disorders can draw from the model of the Bespoke Gene Therapy Consortium (BGTC) by implementing a platform-based, modular pipeline for capsid discovery, vector optimization, and delivery customization, tailored to the anatomical and molecular features of each rare condition. This FUS-integrated strategy centers on scalable yet disease-

803 specific development, especially for disorders with localized CNS pathology and known genetic
804 etiology.
805

5. Evidence Supporting FUS-Mediated Delivery

Focused ultrasound (FUS)-mediated blood-brain barrier opening (BBBO) has crossed a major translational threshold. In just the past 5-6 years, clinical FUS-mediated BBB opening has evolved from an experimental concept to a versatile technology being tested in multiple patient populations with neurological diseases.

5.1 Safety and feasibility of FUS-BBBO for neurological indications

Safety and feasibility have been consistently demonstrated across small trials: on the order of 100+ patients (AD, PD, ALS, brain tumor patients combined) have now undergone at least one FUS BBB opening procedure without significant adverse effects directly attributable to the procedure [44]. The reversibility of the BBB opening (typically restoring within 24 hours) has been confirmed with serial MRI and by lack of sustained toxicity [41].

Given the accumulating clinical evidence supporting the safety and breadth of utility of FUS BBBO, the focus must now turn to optimizing the use of FUS as an enabling technology for the delivery of complex therapeutics, such as gene therapy vectors. Gene therapies that use adeno-associated virus (AAV) vectors or similar large payloads (~25 nm, ~4 MDa) pose unique delivery challenges due to their size, poor passive permeability across the BBB, and their susceptibility to immune clearance.

5.2 Current FUS technologies for brain gene therapy deliveries

FUS enables localized delivery of these vectors across the BBB, but doing so safely requires careful consideration of sonication parameters. Notably, there is a trade-off between achieving sufficient BBBO to allow therapeutic levels of transduction and avoiding tissue damage. Larger BBBO volumes produced in a single sonication increase the risk of edema, microhemorrhage, and inflammatory responses [8].

Recent preclinical studies have begun to address these trade-offs by using real-time feedback control based on acoustic emissions from microbubbles. Cavitation imaging and passive cavitation detection enable monitoring of microbubble activity during FUS sonication, allowing the operator (or algorithm) to modulate exposure and minimize off-target effects. A key translational advance is the emergence of cavitation dose as a predictive biomarker for therapeutic delivery. For instance, Batts et al. demonstrated that the stable cavitation dose correlates linearly with transduction efficiency in non-human primates (NHPs), establishing a quantitative relationship between acoustic exposure and biological outcome [18]. This correlation is particularly robust for systemic delivery of AAV9, where up to a 200-fold enhancement in brain uptake was achieved with FUS in targeted brain areas for gene therapy delivery including the caudate, putamen, and substantia nigra.

The value of large-volume targeting with FUS was further highlighted by Blesa et al., who used MRgFUS to safely open wide regions of the basal ganglia in Parkinson's disease patients, including the putamen and caudate [45]. There are trade-offs between MRgFUS and ultrasound-guided FUS (USgFUS) systems. MRgFUS systems offer advantages of real-time thermal and anatomical imaging, pinpoint targeting, and established regulatory pathways, at the expense of a potential limited procedural throughput, cost, and compatibility with flexible treatment settings. USgFUS systems may provide a more agile alternative for widespread clinical translation of

gene therapies, but do not yet offer intrinsically definitive confirmation of the success of the BBB opening procedure.

5.3 Looking forward: the next generation of FUS BBBO systems

In this context, emerging technical strategies are critical to overcome limitations seen in previous gene therapy trials. For example, Batts et al. introduced a novel rapid beam steering pulse sequence that enables significantly larger BBBO volumes without increasing total sonication duration or changing transducer placement [46]. While initially demonstrated in mice, the technique shows promising translatability to non-human primates, as suggested by dual-sonication data previously presented [18]. These methods directly address challenges such as those encountered in the initial AAV2-hNTN (CERE-120) gene therapy trial for Parkinson's disease, where insufficient coverage of the putamen limited clinical efficacy. Moreover, these advancements offer a path to target the substantia nigra (SN), a deeper and less accessible structure that is difficult to reach via intraparenchymal injection. FUS enables targeted delivery to the SN cell bodies which are more amenable to AAV transduction than the degenerating axon terminals in the striatum, offering a biologically sound strategy to improve therapeutic outcomes in PD.

The distinction between MRgFUS and USgFUS systems becomes particularly important in the context of gene therapy delivery, where the timing between vector administration and BBBO is critical. Studies have shown that gene vector administration should occur as close as possible to the time of BBBO to maximize transvascular delivery during peak vascular exposure. USgFUS systems allow for this temporal coordination with more flexibility than MR-based systems. For instance, in unpublished work by Batts et al., NHPs received intrathecal AAV injections and FUS treatment in the same room, streamlining the workflow in a way that would be infeasible with MRgFUS. Such logistical advantages will be critical in future clinical gene therapy trials.

Nonetheless, a remaining gap in USgFUS development is the lack of direct BBBO confirmation. MRI remains the gold standard for assessing BBB permeability post-sonication, and must currently be used in conjunction with USgFUS systems to validate their effects. There is an urgent need for reliable ultrasound-based BBBO imaging and quantification methods to independently verify treatment safety and efficacy in USgFUS-only procedures.

Despite these logistical and technological considerations regarding the development of next generation FUS devices and protocols, the principal therapeutic advantage of FUS remains: it enables dose reduction. By transiently increasing BBB permeability, FUS allows lower systemic or intra-CSF doses of AAV to achieve therapeutic brain concentrations. This is especially important for mitigating systemic toxicity, reducing costs, and expanding the therapeutic window in vulnerable populations.

Taken together, the preclinical literature supports the following conclusions:

- FUS BBBO is clinically validated and well-tolerated.
- AAV and other large gene therapy vectors can be safely and effectively delivered with FUS in NHP
- Cavitation monitoring and dose quantification offer tools for safe, predictable, and reproducible delivery.
- USgFUS systems enable logistical flexibility and procedural efficiency.

- MRI-based confirmation remains essential, though ultrasound-based techniques are in development and validation stages of research
- FUS enhances the biodistribution and transduction of viral vectors in preclinical models, especially in NHPs which comprise the final translational stage before human trials.

This body of work strongly argues that the field is entering a critical window: the infrastructure and tools for FUS-mediated gene delivery are maturing, and the translational path for indication-specific trials is now both scientifically and operationally viable.

6. Routes of Administration (ROAs) and Tradeoffs

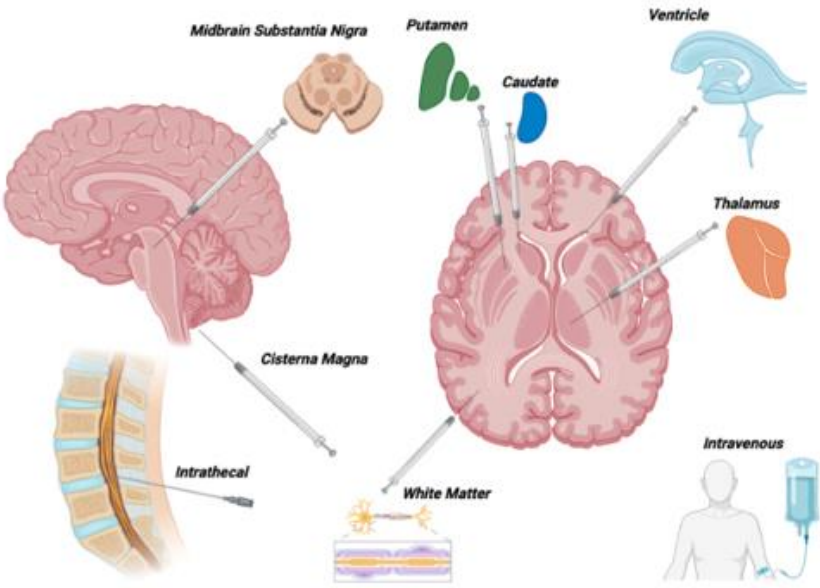

Figure 1: Gene therapy routes of administration (ROA). Figure from Daci & Flotte, 2024

6.1 ROAs for approved gene therapy and biologics for neurological diseases

Gene therapy for central nervous system (CNS) disorders began over two decades ago with the use of AAV vectors delivered via intraparenchymal stereotactic injection, initially targeting indications such as Parkinson's disease (PD), Canavan disease (CD), and Alzheimer's disease (AD) [47]. Since then, the field has advanced to include several routes of administration and biologic platforms.

To date, four therapies targeting the brain or other CNS structures have received regulatory approval (see Table 5). These include both gene therapies and biologics with CNS-directed effects. Additionally, at least three more programs are seeking accelerated approval in the United States: an AAV9-based intra-CSF therapy for Hunter syndrome, a systemic AAV9 therapy for MPS IIIA, and an AAV5-based intra-striatal therapy for Huntington's disease [48]. Collectively, these therapies span intra-parenchymal, intravenous (systemic), and intra-CSF routes of administration, reflecting a growing toolkit for CNS delivery. Around 30 clinical trials are currently ongoing for AAV-based gene therapy programs for CNS diseases (see Appendix Table 1 and our online database https://www.fusfgenetherapy.com).

Focused ultrasound (FUS) has demonstrated the ability to enhance AAV delivery and transduction in preclinical models when combined with a range of administration routes, including intravenous, intra-CSF (e.g., intrathecal and intracisternal), and even intranasal delivery. The optimal route to pair with FUS will depend on multiple factors, including the anatomical spread of disease and the capsid serotype used. The following sections discuss each route in detail and how FUS-BBBO may enhance their clinical utility.

Table 5: Approved therapies for brain neurological disorders, including gene therapy and biologics, as of Q2 2025

Name	Upstaza (Kebilidi in USA)	Zolgensma	Qalsody	Spinraza
Generic Name	eladocagene exuparvovec	onasemnogene abeparvovec	tofersen	nusinersen
Indication	Aromatic L-amino acid decarboxylase (AADC) deficiency	Spinal muscular atrophy (SMA)	Amyotrophic lateral sclerosis (ALS)	Spinal muscular atrophy (SMA)
Therapy	Gene Therapy	Gene Therapy	RNA therapy	RNA therapy
ROA	Intra-Parenchymal	Systemic	Intrathecal	Intrathecal
Vector / Biologics	AAV2	AAV9	ASO	ASO
Locations Approved	US, EU, UK, Israel	US, EU, UK, Japan, Australia, Canada, Brazil, Israel, Taiwan, South Korea	US, EU, Japan, China	US, EU, UK, Canada, Japan, Brazil, Switzerland, Australia, South Korea, China, Argentina, Colombia, Taiwan, Turkey, Hong Kong, Israel
First Year Approved	2022	2019	2023	2016
FDA approval	2024	2019	2023	2016
Originator Company	PTC Therapeutics	Novartis	Ionis Pharmaceuticals	Ionis Pharmaceuticals
Primary target	Brain, CNS	CNS, Spinal Cord	CNS, Spinal Cord	CNS, Spinal Cord

6.2 Systemic delivery

6.2.1 Intravenous Delivery

Since the discovery that certain wild-type AAV serotypes—particularly AAV9—can cross the blood-brain barrier (BBB) (Foust et al., 2009), intravenous (IV) delivery has emerged as a less invasive alternative to intraparenchymal injection for CNS gene therapy. The most prominent clinical example is Zolgensma (AAV9-SMN1), approved for the treatment of spinal muscular atrophy (SMA). In this context, systemic AAV9 delivery enables transduction of motor neurons and peripheral tissues in infants, whose immature BBB allows for greater CNS penetration. A similar strategy is under regulatory review for MPS IIIA, which also involves both CNS and systemic pathology.

Despite these advances, the efficiency of CNS transduction following systemic administration remains low. AAV9 accumulates in the liver and peripheral organs at levels exceeding those in the brain by more than 1,000-fold [49]. Achieving disease-modifying effects in the CNS therefore requires extremely high systemic doses.

6.2.1.1 Limitations of Systemic AAV9 for CNS Delivery

- High-dose toxicity has been documented at doses exceeding 1×10^{14} vg/kg, including hepatotoxicity, thrombotic microangiopathy, sensory neuronopathy, and cases of fatal liver failure [3,13,50,51].

- Manufacturing and cost constraints become prohibitive at these dose levels due to the sheer quantity of vector required for broad biodistribution [52].
- Wild-type AAV9 exhibits limited neuronal specificity after systemic or FUS-enhanced delivery, with transgene expression often restricted to astrocytes and vascular-associated cells [18,53,54].

To address these barriers, preclinical studies have investigated combining IV AAV delivery with focused ultrasound–mediated blood-brain barrier opening. This approach aims to achieve therapeutic CNS expression at significantly lower vector doses by enabling localized BBB disruption and facilitating transvascular vector entry into targeted brain regions.

6.2.1.2 Potential Advantages and Remaining Challenges of FUS + IV AAV9

- FUS-enhanced delivery can increase local AAV9 transduction by 10- to 100-fold, offering a potential path to reduce total systemic dose below 5×10^{13} vg/kg while maintaining therapeutic CNS expression.
- Spatially targeted delivery to deep brain structures (e.g., striatum, hippocampus) can be achieved noninvasively, avoiding neurosurgical infusion.
- However, cell-type specificity remains a challenge; AAV9 continues to preferentially transduce astrocytes in many FUS-treated regions, and systemic exposure, though reduced, still carries risk for off-target toxicity.

While systemic AAV9 delivery has demonstrated clinical utility in select pediatric and multisystemic diseases, its application to CNS disorders is constrained by dose-limiting toxicities, manufacturing demands, and limited brain transduction. FUS-BBBO offers a compelling strategy to overcome these limitations by focusing delivery to specific brain regions and reducing required vector doses. The success of this approach will depend on continued capsid innovation and translational validation in large-animal models.

6.2.1.3 Beyond wild-type serotype and towards engineered capsids

These challenges have motivated the development of engineered AAV capsids tailored for FUS-enabled delivery. Promising candidates include:

- AAV.FUS and AAV.FUS3, developed through in vivo selection for FUS-enhanced CNS delivery [11].
- CCP16, an engineered capsid designed for improved neuronal transduction after FUS [19]
- AV2-HBKO after ICM delivery

Failures of early engineered capsids which achieved high CNS transduction in rodents but failed to translate to non-human primates, highlight the challenge of species specificity in capsid design. This has driven the development of a new generation of engineered capsids with cross-species activity and refined tropism. Voyager Therapeutics (TRACER platform) has produced capsid variants like VCAP-101/102, which demonstrate 20–400× enhanced brain transduction in rodents and NHPs and rely on conserved endothelial receptors (e.g., ALPL) for BBB passage. Capsida Biotherapeutics has developed CAP-003, an engineered capsid tailored for broad CNS delivery with reduced off-target transduction. Sangamo Therapeutics, Dyno Therapeutics and other academic groups are also advancing additional capsids using multiplexed in vivo screening.

These discoveries reflect a clear shift from wild-type AAV to rationally engineered, BBB-crossing capsids with built-in tropism. Moreover, capsid engineering also offers the opportunity to design capsids optimized to work with FUS BBBO. By combining these capsids with FUS-mediated BBB opening, there lies opportunities to further reduce systemic doses, enhance regional targeting, and move more efficiently toward safe, scalable CNS gene therapies.

Table 6: Representative Engineered AAV Capsids for CNS Gene Therapy (last updated Q2, 2025)

Capsid Name	Developer	Key Features	Delivery Route	Species Validated	FUS Compatibility Notes
PHP.eB	Deverman / Caltech	High CNS transduction in mice via LY6A; not active in NHPs	IV	Rodents only	Not suitable for FUS in humans/NHPs
VCAP-101/102	Voyager Therapeutics	High CNS uptake via ALPL; 20–400× ↑ vs AAV9	IV	Rodent + NHP	Good candidate for IV + FUS dose de-escalation
CAP-003	Capsida Biotherapeutics	Broad CNS + low liver/DRG; robust expression	IV	NHP	Strong synergy with FUS-BBBO targeting regions
CCP16	Brigham and Women	Designed for FUS-BBBO and neuronal specificity	IV + FUS	Rodent + NHP	Specifically designed for FUS enhancement
AAV.FUS3	Szablowski / Shapiro Labs	In vivo-selected for FUS-targeted CNS delivery	IV + FUS	Rodent	Useful tool for optimizing FUS regimens
ST-AAVs	Sangamo Therapeutics	NHP-based screens for BBB-penetrant tropism	IV	NHP	Multiple leads for neuron/glia specificity
BI-hTFR1	Deverman / Apertura	Enhanced active transport across brain endothelium	IV	Rodent	Neurons and glia specificity

6.2.2 Intra-arterial (IA) delivery

Intra-arterial (IA) delivery of AAV vectors offers an attractive middle ground between systemic and stereotactic administration by leveraging the cerebral vasculature to increase transgene exposure to the brain while reducing peripheral organ exposure. Compared to intravenous delivery, IA injection can achieve up to 10-fold higher vector concentrations in the brain and approximately 10-fold lower systemic biodistribution, thereby mitigating off-target toxicity [55,56]. However, IA delivery alone remains constrained by the integrity of the blood-brain barrier (BBB), which limits transduction to perivascular cells without additional permeabilization techniques.

6.2.2.1 Limitations of Standalone IA Delivery

- BBB remains a barrier**:** Without BBB modulation, IA AAV delivery primarily transduces endothelial and perivascular cells, with minimal neuronal uptake [44]
- Procedure complexity: IA delivery requires endovascular catheterization which is minimally invasive relative to open neurosurgery, but more invasive than IV or intrathecal approaches.
- Limited targeting precision**:** Without additional modulation (e.g., FUS), IA injections broadly perfuse vascular territories, limiting regional specificity.

6.2.2.2 Advantages of Combining IA with Focused Ultrasound (FUS)

- Enhances CNS entry: FUS-mediated BBB opening can be synchronized with IA infusion to dramatically increase brain parenchymal uptake in targeted regions. This has been demonstrated with small molecules and nanoparticles, and is now being extended to AAV vectors in large-animal studies.
- Reduces systemic toxicity**:** Because IA delivery already lowers peripheral exposure relative to IV, combining it with FUS may further reduce the systemic AAV dose required for CNS transduction, potentially falling below known toxicity thresholds for AAV9.
- Leverages regional vascular targeting: IA delivery can selectively perfuse vascular territories (e.g., internal carotid vs. vertebral), and FUS adds an additional spatial filter, enabling double-gated targeting to maximize brain region specificity.
- Compatible with clinical practice**:** IA catheterization is routinely used in stroke intervention, embolization, and chemotherapy for CNS tumors, making the technique clinically familiar and feasible, especially in high-risk or surgically ineligible populations.

6.2.2.3 Challenges and Developmental Status

- Limited preclinical data: While proof-of-concept of IA delivery has been shown in rodent and porcine models [44], FUS+IA AAV delivery remains in early stages of validation, especially in non-human primates.
- Timing and control: Synchronizing FUS with bolus IA infusion requires precision in timing and understanding of flow dynamics
- Vector-specific factors**:** Capsid tropism and promoter activity may vary based on transit time and tissue exposure dynamics during IA infusion, requiring optimization.

FUS-enhanced intra-arterial delivery represents a promising strategy for maximizing local CNS transduction while minimizing systemic vector exposure. Its applicability will depend on continued translational validation, capsid engineering suited to short vascular transit times, and integration into clinical neurointerventional procedures. As the field progresses, IA + FUS could emerge as a minimally invasive yet highly targeted alternative to both IV and surgical routes for AAV-mediated gene therapy to the brain.

6.3 Intra-parenchymal delivery

Direct intraparenchymal delivery remains the most established method for achieving high-efficiency, region-specific gene transfer to the brain. By bypassing the blood-brain barrier entirely, this route ensures that therapeutic vectors reach their intended targets within deep brain nuclei, white matter tracts, or cortical regions. It is particularly advantageous when the disease pathology is localized and when high, localized transduction efficiency is required.

6.3.1 Clinical Precedents and Advantages

Intraparenchymal delivery has been clinically validated through multiple gene therapy trials. The approved therapy Upstaza® (eladocagene exuparvovec, also known as Kebilidi in the U.S.) exemplifies this approach, delivering AAV2-hAADC via bilateral intraputaminal infusion using a stereotactically guided cannula. This therapy, designed for patients with aromatic L-amino acid

decarboxylase (AADC) deficiency, demonstrated durable improvements in motor function and developmental milestones in pediatric patients.

Similarly, uniQure's Huntington's disease program employs AAV5 delivered by convection-enhanced delivery (CED) directly into the striatum. This program has received FDA Breakthrough Therapy Designation, highlighting the clinical and regulatory feasibility of intraparenchymal routes for neurodegenerative diseases with focal pathology [57].

One important advantage of this method is the ability to co-infuse a contrast agent with the vector solution, enabling real-time MRI-guided monitoring of vector distribution during the infusion [58]. This allows for intraoperative assessment of target coverage and safety margins—a feature not available with systemic or CSF-based delivery routes.

Additionally, intraparenchymal gene delivery benefits from validated surgical platforms, such as the SmartFlow Neuro Cannula, which has received De Novo FDA clearance for the intraputaminal administration of gene therapy for AADC deficiency [59]. These devices are now incorporated into procedures in several gene therapy trials.

6.3.2 Key Advantages of Intra-Parenchymal Delivery:

- Maximal transduction efficiency in targeted deep brain regions, especially for diseases with localized pathology.
- Bypasses the BBB, eliminating the need for dose escalation or capsid engineering to overcome vascular barriers.
- MRI-visible delivery through co-infusion of contrast agent, allowing real-time visualization of vector spread.
- Regulatory precedent and FDA-cleared devices support its clinical use.

6.3.3 Limitations and Barriers to Broad Use:

- Invasive neurosurgery is required, often under general anesthesia, increasing procedural risk and complexity [60].
- Procedures are long and labor-intensive, typically requiring hours in the OR [61].
- Infusion rates and volumes are physiologically limited, constraining total brain coverage [60].
- Scalability is a concern for high-prevalence diseases, where surgical capacity, cost, and patient burden may limit broader adoption [61].

6.4 Intra–Cerebrospinal Fluid (Intra-CSF) Delivery

Intra-CSF administration enables broader CNS distribution than intraparenchymal delivery while limiting systemic exposure common with intravenous or intra-arterial injections. The intra-CSF space is accessed via three primary routes—intrathecal lumbar (IT), intracerebroventricular (ICV), and intracisterna magna (ICM)—each with distinct pharmacokinetics and anatomical reach.

6.4.1 Intrathecal (IT) Delivery

Intrathecal injections are typically performed via lumbar puncture and are widely used in clinical practice, especially for antisense oligonucleotide (ASO) therapies. Notable examples include nusinersen (Spinraza®, for spinal muscular atrophy) and emerging ASOs for Huntington's disease and SOD1-mediated amyotrophic lateral sclerosis (tofersen, brand name Qalsody). While

these therapeutics are not viral gene therapies, they provide valuable insights into the distribution profiles and logistical feasibility of CSF-based administrations. ASOs diffuse slowly through CSF and are absorbed primarily via spinal cord and dorsal root ganglia. Although intrathecal AAV delivery is less common, it mainly targets spinal cord and cortical surfaces with limited deep brain penetration, and leakage into systemic circulation is frequently observed.

6.4.2 Intracerebroventricular (ICV) Delivery

ICV administration involves injecting directly into the lateral ventricles, offering improved access to deep brain structures, including periventricular white matter and hippocampus. Used in preclinical models of lysosomal and metabolic disorders, ICV delivery can achieve more uniform periventricular transduction compared to IT. However, leakage into systemic circulation and uneven spread across cortical and subcortical regions remain challenges, similar to IT.

6.4.3 Intracisterna Magna (ICM) Delivery

Approximately a decade ago, the ICM route—administered via suboccipital puncture—was introduced as an effective and clinically translatable alternative for widespread CNS gene transfer [62]. In non-human primates, ICM injection of AAV9-GFP achieved nearly 100-fold greater brain transduction compared to lumbar puncture, substantially outperforming equivalent intravenous doses and avoiding peripheral organ exposure and associated toxicity. This method was well tolerated, with no histological evidence of inflammation, reinforcing its safety for potential clinical application.

One notable clinical translation of ICM delivery is a gene therapy program for MPS II (Hunter syndrome), currently seeking accelerated approval in the USA [63].

An important limitation of ICM administration is the non-uniform biodistribution of the vector:

- AAV spreads effectively along CSF trajectories including the spinal cord, hippocampus, medulla, pons, midbrain, and cortical layers.
- However, deep brain nuclei such as the dentate cerebellar nuclei, putamen, caudate, and thalamus receive approximately tenfold lower viral load compared to regions adjacent to CSF pathways [64].

This uneven distribution provides an opportunity for FUS-BBBO to amplify delivery in deep structures after ICM administration. Mechanistically, AAV clearance from the CSF into the systemic circulation has been documented in several non-human primate studies—liver and spleen contain significant vector DNA after ICM or IT injections [62,65]. AAV in the bloodstream thus becomes available for FUS-targeted delivery, provided the timing of FUS is optimized with respect to blood pharmacokinetics of the vector. This combined strategy has shown promise in rodent and primate models for enhancing both vector penetration and regional tropism.

ICM delivery offers a compromise between CNS scope and invasiveness among intra-CSF methods, and already demonstrates superior brain distribution compared to IT or IV. Yet, deep-brain regions remain underrepresented. When combined with appropriately timed FUS-BBBO, intra-CSF routes (especially ICM) offer a strategic two-pronged delivery: broad surface and central transduction via CSF, plus enhanced focal delivery to deep nuclei via vascular crossing. This represents a compelling next-generation approach for disorders requiring widespread but regionally intensive CNS gene transfer.

6.5 Intranasal Delivery and Focused Ultrasound (FUSIN)

Intranasal delivery offers a noninvasive route to bypass the blood-brain barrier, but its use has been historically limited to small, highly potent molecules due to poor absorption across the nasal mucosa. Recent advances combining this method with focused ultrasound—termed FUS-mediated intranasal delivery (FUSIN)—have significantly enhanced targeting efficiency in preclinical models.

6.5.1 Highlights from Preclinical Studies

- FUSIN enables spatial targeting: in mice, intranasal delivery of fluorescent dextran, when combined with transcranial FUS and microbubbles, increased localized brain uptake ~8-fold compared to intranasal delivery alone and approached levels seen with intravenous + FUS delivery [66]
- AAV vector delivery achieved noninvasively: In a key study, FUSIN of AAV5-EGFP achieved 414× higher expression in targeted brain regions—both cortex and brainstem—than intranasal delivery alone, with minimal peripheral biodistribution [67]
- Enhanced cellular targeting and tropism: FUSIN delivery shifted transgene expression closer to neurons and microglia compared to systemic or intraparenchymal approaches [68]

6.5.2 Limitations of Intranasal Delivery

- Low bioavailability: Only a small portion of intranasal dose reaches the CNS due to mucociliary clearance and enzymatic degradation [66].
- Dose constraints: Intranasal administration limits total volume (~20–30 µL per nostril in rodents), restricting the achievable vector dose.
- Anatomical spread variability: Distribution within the brain can be influenced by nasal anatomy, airflow dynamics, and CSF flow, introducing heterogeneity between subjects.

While intranasal gene delivery combined with FUS represents a promising noninvasive alternative to bypass both surgical procedures and systemic exposure, it remains in early-stage development with significant dosing and distribution constraints. Nonetheless, preclinical studies provide compelling rationale for further optimization and translation of FUSIN for CNS gene therapies.

6.6 Summary – ROA and opportunities for FUS

Table 6: Route of administration summary.

ROA	Overview	Opportunities for FUS	Limitations for FUS
Intravenous (IV)	Clinically feasible but requires high dose, high systemic exposure, requires capsid engineering	Reduce systemic dose for focal brain delivery	Variable performance depending on brain region targeted with FUS
Intra-arterial (IA)	Clinically feasible but requires high dose, high systemic exposure, may require capsid engineering	Improved first-pass delivery	Risk of embolic complications with microbubble and AAV administration
Intra-cisterna magna (ICM)	minimally invasive, high CSF exposure, potentially risky procedure	Enhance cortical and deep structure penetration	Brain region-dependent benefit (minimal added benefit in cortical, CSF-facing regions)

Intracerebroventricular (ICV)	Direct CSF access ideal for periventricular targets	Enhance parenchymal penetration from ventricular interface	Potentially minimal added benefit
Intrathecal (IT Lumbar)	Spinal CSF access, good for spinal cord targets; less invasive than ICV	Enhanced but still minimal brain distribution	Regional mismatch (primary structure transduced is spinal cord)

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7. Technical Requirements for FUS

As the therapeutic landscape for central nervous system (CNS) gene delivery evolves, focused ultrasound (FUS) is emerging as a critical technology for enabling targeted, non-invasive access across the blood-brain barrier (BBB). Yet to transition from preclinical success to routine clinical use, FUS systems must meet a distinct set of engineering and operational requirements tailored to the demands of gene therapy. These include not only safe and reproducible BBB opening but also compatibility with surgical procedures, scalability across patient populations, and real-time control features that optimize delivery precision and reduce the risk of off-target effects.

At the core of a FUS system is the transducer: a device that converts electrical energy into acoustic pressure waves. Transducers may be single-element, geometrically focused units or, more commonly in therapeutic systems, multi-element arrays that permit electronic beam steering. This flexibility allows acoustic energy to be directed with high spatial precision to specific brain regions. A function generator defines the pulse sequence, including the frequency, pressure, burst duration, and duty cycle required to achieve transient BBB opening. An amplifier drives the transducer at the prescribed power level. These components are typically controlled through a software interface that integrates patient imaging and sonication planning parameters.

A distinguishing feature of therapeutic FUS systems for gene delivery is the inclusion of cavitation monitoring. By detecting acoustic emissions from circulating microbubbles during treatment, these systems can provide indirect yet spatially resolved feedback on BBB opening. Importantly, recent work in non-human primates has shown that the strength and distribution of cavitation emissions correlate with transgene delivery efficiency, specifically with the amount of AAV vector detected in brain tissue. This positions cavitation monitoring not just as a safety measure but also as a predictive biomarker for therapeutic efficacy. Systems now exist that modulate acoustic exposure in real time to maintain a consistent cavitation dose, minimizing risk while maximizing delivery performance.

Two classes of FUS systems are leading candidates for clinical translation in gene therapy: MR-guided FUS (MRgFUS) and ultrasound-guided or neuronavigation-guided FUS (USgFUS or NgFUS). MRgFUS systems feature large, hemispherical phased arrays designed to deliver sonications under real-time MRI guidance. This configuration enables precise spatial targeting and confirmation of BBB opening through post-contrast imaging. These systems, exemplified by devices such as INSIGHTEC's Exablate Neuro, are already deployed in major medical centers for approved thermal ablation procedures. However, their use for gene therapy introduces logistical challenges. The need for co-administration of gene vectors, potentially via routes incompatible with the MRI suite as described previously, and the high cost and fixed-site design of MRgFUS systems pose significant barriers to scalability.

In contrast, USgFUS systems, especially those integrated with neuronavigation, offer greater procedural flexibility. These systems use external tracking (e.g., optical or electromagnetic) to co-register patient anatomy with the FUS device, allowing for accurate targeting without MRI. Though traditionally limited in focal precision due to acoustic aberration through the skull, they are lightweight, portable, and more compatible with intraoperative gene therapy administration. Modular by design, these systems can accommodate various transducers, cavitation detectors, and imaging probes, making them highly adaptable to different clinical settings. Clinical-stage systems such as NaviFUS and Delsona's UltraNav are at the forefront of this approach.

A physical limitation that also influences clinical utility is the geometry of the ultrasound focus itself. In most USgFUS systems, the focal zone is elongated in the axial direction (perpendicular to the transducer face) while being narrower in the lateral plane. This means that BBB opening, and consequently AAV transduction, is not confined to a single compact region, but rather distributed along the beam axes. For many gene therapy targets in the brain such as the hippocampus, striatum, or substantia nigra, this may result in partial or off-target exposure of adjacent tissue. MRgFUS systems, by contrast, can more precisely localize the focal spot and offer sub-millimeter control over targeting, but at the cost of substantially longer treatment times if large or complex structures must be covered. These tradeoffs between spatial precision and treatment efficiency underscore the need for thoughtful matching of system design to anatomical and therapeutic goals in FUS-facilitated gene therapy.

Regardless of system type, a fundamental technical challenge lies in the heterogeneous structure of the human skull. Acoustic energy must pass through two dense cortical bone layers and an intermediate porous region (diploë), which variably scatter, absorb, and distort the beam. Accurate targeting therefore requires individualized acoustic simulations, often based on CT scans that capture patient-specific skull morphology. To reduce reliance on ionizing radiation, emerging research focuses on MRI-based alternatives such as UTE and ZTE sequences that can provide similar resolution. Some investigational techniques also explore intraoperative ultrasound imaging to assist with skull mapping and targeting, though these methods are not yet compatible with MR-based systems.

The technical strategy does not end with the FUS system alone. Adjunct approaches are gaining attention for their ability to improve vector transduction and therapeutic consistency. Corticosteroids are increasingly used to mitigate immune responses to AAV vectors, especially in repeat dosing scenarios. Meanwhile, a recent finding that nitrous oxide anesthesia can transiently enhance BBB permeability, and thereby improve AAV uptake, offers an intriguing, clinically accessible adjunct for FUS gene delivery.

As the field progresses toward human trials, the ideal FUS system will be one that combines precision targeting, real-time safety control, clinical workflow integration, and scalability. Bridging the engineering challenges with the clinical realities of gene therapy delivery will be essential to realizing the full potential of FUS as a platform technology. A wave of next-generation FUS devices is beginning to address these demands directly. Cordance Medical is developing a wearable, portable FUS headset designed for outpatient use and repeatable BBB opening, commencing early clinical investigation for brain tumors [69] and neurodegeneration. Simultaneously, Kullervo Hynynen's lab I Sunnybrook Research Institute (Toronto) has introduced a novel hemispherical "dome" transducer array with full head coverage and electronic beam steering, allowing for rapid, noninvasive sonications with sub-millimeter spatial accuracy [70], currently in clinical trial for BBBO. These advances, along with modular, lower-cost systems in development across academia and industry, represent critical steps toward making FUS a scalable, routine modality for gene delivery to the brain.

Table 7: Comparison of MRgFUS vs. USgFUS systems for facilitating targeted brain gene therapy

Feature	MRgFUS (MRI-Guided Focused Ultrasound)	USgFUS / NgFUS (Ultrasound- or Neuronavigation-Guided Focused Ultrasound)
Guidance Modality	Real-time MRI targeting with gadolinium contrast enhancement	Neuronavigation (optical or magnetic tracking), often co-registered with pre-op MRI/CT
Transducer Design	Hemispherical phased array	Modular linear or circular arrays; can use single-element or small arrays
Focal Spot Geometry	Compact, spherical focal region with high spatial precision	Elongated focal region along axial beam axis; broader exposure in beam direction
Spatial Precision	High sub-millimeter resolution; tightly confined focal zone	Lower spatial confinement; requires careful alignment and targeting compensation
Treatment Volume Coverage	Requires point-by-point rastering; long treatment time for large targets	Larger coverage per sonication; faster but less confined dosing
Cavitation Monitoring	Integrated passive detectors within phased array; good for binary confirmation of activity	Greater flexibility for mapping cavitation in space and time using ultrasound imaging arrays; allows for real-time localization and dose estimation
Therapy Confirmation	Immediate MRI-based verification of BBB opening	No direct imaging of BBB opening; confirmation relies on cavitation signals and procedural alignment
System Footprint	Large, stationary system in MR suite	Portable; adaptable to OR, ICU, or bedside workflows
Compatibility with Surgical Workflows	Limited; difficult to coordinate with gene therapy requiring non-systemic delivery	Highly compatible with surgical or interventional workflows (e.g., intrathecal, intracisternal)
Clinical Availability	Established for ablation (e.g., INSIGHTEC Exablate Neuro); BBBO clinical trials ongoing	Clinical-stage development (e.g., NaviFUS, UltraNav) with expanding interest in gene therapy applications
Treatment Scalability	Limited throughput due to MR time and cost; potential clinical bottleneck	Higher throughput potential; decentralized use and reduced infrastructure demands
Targeting Adaptability	Rigid but highly accurate; MR-based alignment	Flexible and modular; requires accurate co-registration but supports diverse anatomical targets
Patient-Specific Planning	Requires CT (or UTE/ZTE MRI) for skull correction and acoustic simulation	Also benefits from CT or MRI-based planning; easier to adapt to alternative imaging workflows
Cost and Accessibility	High capital and procedural costs; limited to select centers	Lower cost, more accessible; potentially deployable in lower-resource settings

7.1 Summary of Technical Requirements

- Spatially focused acoustic energy delivery through electronically steered or geometrically focused arrays
- Cavitation monitoring with real-time control to ensure safe, effective BBB opening and predict transgene delivery
- MR-guided systems offer high targeting accuracy but limited workflow flexibility and scalability
- Neuronavigation-guided systems provide portability and procedural adaptability but require improvements in targeting precision
- Skull heterogeneity necessitates patient-specific treatment planning, traditionally via CT, with ongoing work on MRI-based alternatives
- Adjunct strategies (e.g., corticosteroids, nitrous oxide) may enhance vector delivery and reduce immunogenicity.

8. Regulatory and Clinical Trial Design Framework: Implications for FUS-Mediated Gene Therapy

The preceding sections have reviewed the groundwork for advancing FUS-facilitated AAV gene therapy toward clinical translation. We began with a framework for identifying and prioritizing candidate indications for first-in-human trials, examined the landscape of gene therapy modalities and their distinct characteristics, evaluated routes of administration and their implications for therapeutic delivery, and reviewed FUS technology in detail—particularly the mechanisms by which it enables precise, targeted gene delivery while reducing systemic exposure. We also introduced the Bespoke Gene Therapy Consortium as an instructive model: their regulatory playbook represents years of sustained coordination among academic researchers, industry partners, regulatory authorities, and clinical trial leaders, demonstrating what coordinated effort can achieve. Here, we turn to the regulatory pathway, clinical trial design considerations, anticipated challenges, and strategic recommendations required to move this combined approach into human trials.

This section provides a preliminary overview, acknowledging both the baseline complexities of gene therapy approval and the additional regulatory burden introduced by combination product designation. The intrinsic complexity and regulatory history of gene therapies demand strategic, not incremental, advancement. We identify critical junctures requiring particular attention, most notably the need for intermediate-scale studies that enable early FDA engagement to align on data requirements and endpoints for this novel combination modality—what we term INTERACT-enabling studies. Our recommendations focus on three priorities: first, the coordinated development of a FUS-gene therapy regulatory playbook modeled after the BGTC effort; second, prioritization of intermediate-scale INTERACT-enabling studies as the immediate next step; and third, selection of specific indications identified through our indication prioritization framework developed earlier in this report. This approach is intentionally preliminary, designed to invite community input and establish a foundation for consortium-driven learning, where each clinical trial advances the pathway for those that follow. The goal is clear: to strategically position FUS as a synergistic enabler that expands gene therapy's reach to deliver transformative treatments to patients who need them most.

8.1 Gene Therapy Regulatory Pathway

Before addressing the additional complexities introduced by FUS delivery, it is essential to understand the baseline regulatory pathway for gene therapy clinical trial approval. The following regulatory terminology will be used throughout this section:

- **IND (Investigational New Drug)** refers to the application to FDA requesting authorization to administer an investigational drug or biologic to humans, required before clinical trials can begin.
- **BLA (Biologics License Application)** is the application for permission to introduce or deliver biologics (including gene therapies) into interstate commerce, equivalent to a New Drug Application (NDA) for biologics.

- **IDE (Investigational Device Exemption)** allows investigational devices to be used in clinical studies to collect safety and effectiveness data.
- **PMA (Premarket Approval)** is the most stringent type of device marketing application required by FDA for Class III devices (highest risk category), requiring scientific evidence demonstrating device safety and effectiveness.
- **510(k)** is a premarket submission to FDA demonstrating that a device is substantially equivalent to a legally marketed predicate device, and is less burdensome than PMA.

Based on the BGTC playbook [71], the pathway to gene therapy clinical trial approval typically spans in the best cases 2-3 years from early development to first patient dosing and involves several distinct phases (Table 8). Early development (12-18 months) focuses on proof-of-concept studies in vitro and in vivo, establishment of preliminary manufacturing processes, initial dose range studies, and disease model validation. At this stage, sponsors may request an optional INTERACT meeting with FDA to obtain guidance on manufacturing approaches, preclinical models, and study design for novel regulatory situations. Pre-IND preparation (6-9 months) requires completion of dose-range finding studies, production of GMP manufacturing lots, establishment of chemistry, manufacturing, and controls (CMC) specifications, drafting of the clinical protocol, and engagement with patient advocacy groups. A Pre-IND meeting is highly recommended at this stage to secure FDA agreement on the adequacy of the IND package, clinical design, and CMC approach before submission. IND submission itself typically requires 1-2 months to compile the complete eCTD package (five modules), finalize toxicology studies, identify clinical sites, and document investigator qualifications. FDA review takes 30 days, during which sponsors must respond to FDA questions and address any clinical hold issues. If no clinical hold is issued or once hold issues are resolved, clinical readiness activities (2-4 months) include site activation and training, patient recruitment initiation, and long-term follow-up planning before first patient dosing.

Phase	Duration	Key Activities & Prerequisites for FDA Meetings	FDA Meetings	Expected Outcomes
Early Development	12-18 months	• Proof-of-concept studies (in vitro/in vivo) • Preliminary manufacturing process • Initial dose range studies • Disease model validation	INTERACT Meeting (Optional)	Novel regulatory guidance on manufacturing, pre-clinical models, and study design
Pre-IND Preparation	6-9 months	• Complete dose-range finding studies • GMP manufacturing lots produced • CMC specifications established • Draft clinical protocol • Patient advocacy engagement	Pre-IND Meeting (Highly Recommended)	FDA agreement on IND package adequacy, clinical design, and CMC approach

IND Submission	1-2 months	• Complete eCTD package (5 modules) • Toxicology studies finalized • Clinical sites identified • Investigator qualifications	IND Submission (No meeting)	Study May Proceed letter or Clinical Hold with required modifications
FDA Review	30 days	• Respond to FDA questions • Address clinical hold issues • Finalize site preparations	Clinical Hold Resolution (If needed)	Authorization to initiate clinical studies
Clinical Readiness	2-4 months	• Site activation and training • Patient recruitment initiation • Long-term follow-up planning	Study Initiation	First patient dosing and ongoing trial execution

Table 8: Required Pathway for Clinical Trial Approval.

In parallel with this required pathway, sponsors may pursue optional strategic designations that can facilitate development without delaying clinical trial initiation **(**Table 9). **Orphan Drug Designation**, which can be sought at any time but is preferably obtained early, applies to diseases affecting fewer than 200,000 people in the US and provides tax credits for clinical testing, waiver of approximately $3 million in FDA fees, and seven years of market exclusivity [72]. **Fast Track Designation**, available after preliminary data are available, applies to serious conditions addressing unmet medical needs and enables more frequent FDA meetings, rolling review capability, and enhanced clinical guidance. **Breakthrough Therapy designation**, requiring preliminary clinical evidence of substantial improvement over existing therapy for serious conditions, provides intensive FDA guidance with senior manager involvement and rolling review. It is important to note that these special designations are strategic business and development tools rather than regulatory requirements, and are not gatekeepers to starting clinical trials. However, the BGTC playbook recommends applying for these designations early because they can make clinical development more efficient and cost-effective, and some designations like Fast Track and Breakthrough Therapy provide more intensive guidance that can influence clinical trial design.

Table 9: Optional Strategic Designation (Parallel Track)

Designation Type	Timing	Key Requirements	Benefits
Orphan Drug Designation	Any time, preferably early	Disease affects <200,000 people in US Scientific rationale for treatment	Tax credits for clinical testing Waiver of ~$3M FDA fees 7-year market exclusivity
Fast Track Designation	After preliminary data available	Serious condition Addresses unmet medical need	More frequent FDA meetings Rolling review capability Enhanced clinical guidance
Breakthrough Therapy	After preliminary clinical evidence	Serious condition Substantial improvement over existing therapy	Intensive FDA guidance Senior manager involvement Rolling review
Rare Pediatric Disease	Before September 2024*	Primarily affects patients birth-18 years	Priority Review Voucher upon approval

Serious/life-threatening condition	Voucher can be sold or transferred

8.2 Combination Product Complexity and Dual Regulatory Pathways

FUS-mediated gene therapy represents a complex combination product that adds substantial regulatory complexity to the baseline gene therapy pathway described above. These combinations are anticipated to require dual regulatory oversight from FDA's Center for Biologics Evaluation and Research (CBER) for the gene therapy component and from the Center for Devices and Radiological Health (CDRH) for the FUS device, although the regulatory assessment of the FUS device combined with microbubbles remains unclear and will likely be clarified through early regulatory interactions. The primary mode of action (PMOA) determination will dictate lead center assignment, with most FUS-gene therapy combinations likely falling under CBER leadership given that the therapeutic effect derives primarily from the genetic payload rather than the FUS delivery device. However, this determination must be made on a case-by-case basis through early FDA consultation, ideally during INTERACT meetings.

This regulatory complexity is compounded by fundamental differences between combining FUS with an already-approved gene therapy product versus developing a novel gene therapy in conjunction with FUS delivery. For approved products, such as Zolgensma (onasemnogene abeparvovec) or Upstaza (eladocagene exuparvovec), the regulatory pathway may involve supplemental applications including a PMA or 510(k) for the device component with bridging clinical data demonstrating that FUS enhancement does not compromise safety or efficacy of the approved gene therapy. This pathway is potentially less burdensome as the gene therapy component has already undergone comprehensive review, allowing the focus to remain on demonstrating that FUS delivery does not introduce new safety concerns or alter the established risk-benefit profile. However, for novel gene therapy constructs developed specifically for FUS-enhanced delivery, the combination requires comprehensive evaluation of both components simultaneously, necessitating a full IND and eventual BLA submission for the biologic alongside device PMA or IDE clearance. This dual pathway substantially increases regulatory complexity, development timelines, and resource requirements, as both the gene therapy and the delivery technology must independently meet regulatory standards while their interaction must be thoroughly characterized.

The regulatory landscape heavily favors rare disease applications, offering multiple expedited pathways that could strategically accelerate FUS-gene therapy translation [73]. These advantages suggest a potential approach for the device side of the combination: establish regulatory precedent through rare disease applications that can serve as predicate for future indications before expanding to more prevalent conditions. The ClearPoint Neuro SmartFlow Cannula provides an instructive example of this strategy in practice. The cannula device received FDA De Novo authorization specifically for intraputaminal administration of the gene therapy KEBILIDI™ from PTC Therapeutics for the treatment of AADC deficiency, a rare pediatric neurometabolic disorder [74]**.** Future gene therapies can reference this clearance as a predicate device in 510(k) submissions, simplifying and accelerating regulatory approval for new CNS-targeted gene therapies using the same delivery method. Importantly, this precedent assures

pharmaceutical partners that the regulatory path is achievable and that ClearPoint can serve as a device development and delivery partner for multiple gene therapy programs. The device is currently used in other clinical trials, including the AskBio Parkinson's disease trial [75], demonstrating how initial rare disease approval can support expansion to additional indications.

Whether FUS BBB opening can achieve similar regulatory clearance as a delivery technology for gene therapy remains to be explored and represents a key strategic question for the field. There is an added layer of complexity for FUS-mediated gene therapy delivery, as the combination may be linked to a specific route of administration (systemic, intrathecal, or intraparenchymal), and the relationship between FUS parameters, microbubble formulations, and different gene therapy vectors may require indication-specific optimization. Nevertheless, if a path similar to the ClearPoint Neuro Cannula could be followed, this would present a clear advantage. De Novo clearance for delivering gene therapy to treat a rare disease could serve as predicate for clinical trials or regulatory approval of gene therapies targeting more prevalent indications under the FDA 510(k) process. The key factors for 510(k) clearance are substantial equivalence in intended use and technological characteristics, not the rarity or prevalence of the disease itself. This regulatory strategy would significantly benefit from early coordination through a consortium model, as discussed below.

8.3 Consortium Model and Standardized Development Pathway

BGTC published a standardized operational playbook for developing AAV-based gene therapies for rare diseases that provides a roadmap for streamlining product development and navigating the regulatory pathway, addressing critical steps before IND submission for first-in-human studies [71]. The playbook includes regulatory best practices, case studies of existing AAV gene therapies, and standardized approaches to manufacturing, analytical methods, and chemistry, manufacturing, and controls (CMC). This coordinated effort aims to significantly reduce per-program costs and development timelines by eliminating redundant work across individual programs, allowing gene therapy developers to build on established precedent rather than independently solving the same problems.

A similar consortium model for FUS-mediated gene therapy could establish standardized protocols, regulatory templates, and shared learning to accelerate clinical translation. Key elements would include:

- common device specifications and quality control standards,
- cavitation monitoring parameters and safety thresholds with validated methodologies,
- safety assessment protocols including imaging and neurological evaluation,
- adverse event reporting guidelines specific to FUS-BBB opening procedures,
- and regulatory submission templates that address the unique aspects of combination product applications.

This consortium-driven approach would prevent each investigator from independently navigating the complex combination product pathway, reducing barriers to entry and accelerating progress across the field. Just as BGTC's playbook allows gene therapy developers to build on established precedent rather than starting from scratch, a FUS-gene therapy consortium would create shared infrastructure that benefits all subsequent trials. Critically, this

consortium model would facilitate coordination on the device predicate strategy discussed above, ensuring that early regulatory successes in rare disease applications are structured to maximally benefit subsequent trials in other indications.

8.4 The case for Clinical FUS-GT Center of Excellence

Gene therapy clinical trials carry fundamentally different risk profiles compared to conventional drug development, and these considerations become even more critical when combined with FUS delivery. The irreversible nature of genetic modification, inability to withdraw treatment, potential for immune-mediated adverse events, and limited reversibility of overdosing create unique safety considerations that distinguish gene therapy from traditional pharmaceuticals [76,77]. When combined with FUS-mediated BBB opening, additional variables including cavitation dose quantification, timing of vector administration relative to FUS application, potential for enhanced immune responses due to increased CNS exposure, and the durability of BBB opening must be carefully managed. These specific risks mandate specialized expertise spanning viral vector manufacturing and quality control, neurosurgical or interventional techniques for catheter-based delivery, FUS device operation and cavitation monitoring, advanced neuroimaging interpretation, and gene therapy-specific adverse event management including immune response monitoring. Not every clinical site possesses this multidisciplinary capability, arguing for concentration of early trials at designated centers of excellence with proven track records in both FUS and gene therapy applications. International expansion should follow successful safety and feasibility demonstration at these initial centers of excellence, with the consortium model facilitating knowledge transfer and capability building at additional sites.

8.5 Clinical Trial Design: Biomarkers, Endpoints, and Temporal Considerations

Successful translation of FUS-gene therapy combinations requires careful attention to trial design elements that address both the constraints of rare disease populations and the unique temporal characteristics of gene therapy efficacy. Rare disease gene therapy trials can operate under fundamentally different design principles than traditional drug development. Small patient populations typically preclude large randomized controlled trials, leading to innovative designs including single-arm studies, historical controls, and adaptive protocols [78]. RMAT designation specifically acknowledges these constraints, allowing accelerated approval based on surrogate endpoints that are "reasonably likely to predict clinical benefit," provided they are validated in natural history studies and supported by mechanistic rationale [79]. The FDA's 2019 guidance on rare disease endpoints emphasizes flexibility in accepting biomarker-based primary endpoints when direct clinical measures are impractical due to disease rarity or long natural history timelines [80]**.** This regulatory accommodation is particularly relevant for FUS-gene therapy applications, where traditional clinical efficacy measurements may require years to manifest while molecular and imaging biomarkers can demonstrate target engagement and delivery success within weeks to months.

Gene therapy trials require multilayered biomarker approaches that span multiple levels of assessment: vector delivery (biodistribution via qPCR for vector DNA), transgene expression (RT-qPCR for mRNA), and functional outcomes (enzyme activity assays, protein quantification, or clinical measures). The selection and timing of these biomarkers directly impact indication prioritization and trial feasibility. The temporal characteristics of available biomarkers should inform indication selection, as diseases with validated early biomarkers that respond within weeks to months of intervention are better suited for Phase 1/2a trials than those requiring years of follow-up to demonstrate clinical benefit. Several approved gene therapies demonstrate this principle in practice. AADC deficiency treatment with Upstaza relies on CSF measurements of dopamine metabolites (HVA, 5-HIAA) that provide biochemical confirmation of enzyme activity within weeks, preceding motor improvements by months [81]. Spinal muscular atrophy treatment with Zolgensma used motor milestone achievement and survival as accelerated approval endpoints, but SMN protein levels and motor neuron counts provided earlier proof of target engagement [82]. Parkinson's disease gene therapy trials have employed dopamine transporter PET imaging and AADC activity measures as intermediate biomarkers before assessing motor clinical outcomes at later timepoints [30]. These examples illustrate how early surrogate biomarkers enable faster proof-of-concept and dose-finding studies while long-term clinical endpoints confirm therapeutic benefit.

For FUS-enhanced delivery specifically, additional pharmacodynamic markers become critical components of the biomarker strategy. Cavitation dose quantification during treatment provides real-time feedback on FUS delivery parameters, MRI-based BBB opening confirmation immediately post-procedure demonstrates successful execution of the delivery protocol, and enhanced vector distribution assessment via PET imaging [83] for example, or CSF analysis in the acute post-treatment period quantifies the delivery advantage conferred by FUS. These FUS-specific biomarkers can demonstrate successful delivery within days, providing early go/no-go signals for dose escalation independent of therapeutic effect. This capability is particularly valuable in early-phase trials where distinguishing inadequate FUS-mediated delivery from insufficient therapeutic construct activity is essential for appropriate protocol modifications. Demonstrating enhanced delivery efficiency through FUS could support dose reduction strategies, potentially improve safety profiles while maintaining efficacy which is a particularly important consideration given the dose-limiting toxicities observed in systemic AAV administration.

The availability and temporal characteristics of biomarkers were explicitly weighted in the indication prioritization framework presented earlier in this report. Indications with validated surrogate biomarkers that have established correlation to clinical benefit (such as enzyme levels in lysosomal storage disorders or CSF protein biomarkers in proteinopathies), early-responding biomarkers that change within weeks to months rather than years (enabling faster dose-finding and proof-of-concept), quantifiable target engagement measures that confirm both vector delivery and transgene expression (distinguishing delivery failure from therapeutic failure), and accepted regulatory precedent where surrogate endpoints have supported approval in related indications are advantageous for early FUS-gene therapy trials. Conversely, indications lacking reliable early biomarkers or requiring multi-year follow-up for clinical assessment pose higher risk for Phase 1 trials, as the extended timeline to assess efficacy increases development costs

and delays subsequent trials while the inability to distinguish delivery from therapeutic failure complicates interpretation of negative results.

AAV-based gene therapies exhibit delayed therapeutic effects, with peak transgene expression often occurring 4-12 weeks post-administration and clinical benefits potentially delayed by months to years [84]**.** This temporal disconnect between treatment administration and efficacy assessment requires carefully planned interim analyses using predictive biomarkers to guide dose escalation and safety monitoring without waiting for distal clinical endpoints. The irreversible nature of gene modification necessitates extended follow-up periods, typically 15 years for AAV studies, to monitor for delayed adverse events including insertional mutagenesis, immune-mediated toxicity, and potential oncogenic effects [85]. FUS-enhanced trials might additionally monitor for delayed BBB integrity effects and potential chronic inflammatory responses at sonication sites. This extended follow-up requirement has implications for trial design, site selection, and patient consent processes, as participants commit to long-term surveillance that extends far beyond the active treatment period.

Early-phase FUS-gene therapy trials should prioritize endpoints structured temporally to provide incremental decision points that inform dose escalation and protocol modifications. In the immediate period (days 0-7), trials should assess FUS delivery success through cavitation dose quantification and MRI-confirmed BBB opening, alongside acute safety monitoring including neurological examination and imaging for edema or hemorrhage. The early period (weeks 2-12) should focus on vector transduction through biodistribution assessment (CSF vector genome copies, imaging if available using PET ligands for transgene detection), target engagement through transgene mRNA expression and early protein or enzyme activity measures, and continued safety and tolerability monitoring including immune response assessment and neurological function. The intermediate period (months 3-12) should evaluate functional biomarkers including disease-specific surrogate endpoints (enzyme activity, protein levels, imaging biomarkers), preliminary efficacy signals through clinical scales and quality of life measures, and optional tissue confirmation through dermal biopsy for peripheral transduction (if systemically administered) or surgical biopsy if clinically indicated. Long-term follow-up (years 1-15) must assess clinical outcomes using disease-specific functional endpoints, maintain safety surveillance for long-term adverse events, immune responses, and BBB integrity, and evaluate durability of both transgene expression and clinical benefit. This temporal structure allows for early identification of delivery failures or safety signals while providing sufficient time to assess the durability and clinical meaningfulness of therapeutic effects.

8.6 Strategic Recommendations and Path Forward

The regulatory and clinical trial design considerations outlined above converge on several strategic recommendations for advancing FUS-mediated gene therapy toward clinical translation.

First, the field would benefit substantially from coordinated development of a FUS-gene therapy regulatory playbook modeled after the BGTC effort, establishing standardized approaches to device specifications, monitoring parameters, safety assessments, and regulatory submission strategies. This consortium-driven approach would prevent redundant efforts and accelerate progress across the field by allowing subsequent trials to build on established precedent.

Second, prioritization of intermediate-scale INTERACT-enabling studies represents the critical next step, as early FDA engagement to align on data requirements and endpoints for this novel combination modality will determine the feasibility and efficiency of subsequent development. These studies should focus on demonstrating the delivery advantage conferred by FUS through robust biomarker assessment while establishing safety profiles that support advancement to efficacy trials.

Third, indication selection should prioritize diseases identified through the evaluation framework developed earlier in this report, with particular emphasis on rare diseases that offer regulatory advantages, possess validated early biomarkers for proof-of-concept, and demonstrate clear unmet medical need that FUS-enhanced delivery could address.

9. Roadblocks and Mitigation Strategy

The clinical translation of focused ultrasound (FUS)-facilitated gene therapy for the brain presents a complex landscape of technical, regulatory, financial, and strategic risks. These challenges are compounded by the novelty of combining a device-based blood-brain barrier opening modality with biologics such as AAVs or ASOs. While the therapeutic potential is substantial, successful clinical advancement requires early, proactive mitigation strategies across all dimensions of development. The following section outlines the key categories of anticipated roadblocks and their corresponding risk mitigation approaches.

9.1 Scientific and Technical Risks

A central technical concern is the consistency and reliability of BBB opening. Inter-patient variability in skull composition, targeting accuracy, and transducer coupling can lead to unpredictable vector penetration and variable transgene expression. To address this, FUS protocols must incorporate real-time acoustic feedback (e.g., cavitation monitoring) and, where feasible, MRI guidance to confirm BBB disruption during treatment. Standard operating procedures (SOPs) should be established for device calibration, bubble dosing, and patient positioning. Moreover, studies in non-human primates (NHPs) using clinical-grade systems are essential to characterize the expected variability and define acceptable margins for first-in-human applications.

Subtherapeutic or toxic levels of transgene expression may result from nonlinear relationships between vector dose and tissue exposure, driven by heterogeneous BBB permeability or host immune clearance. These effects can be mitigated through rigorous dose-escalation studies in NHPs, ideally accompanied by gene expression and biodistribution analyses. In parallel, engineering gene therapy vectors with tunable or cell-type-specific promoters can improve safety and minimize off-target effects.

Immunological responses remain a challenge, particularly in the context of AAV delivery. Pre-existing neutralizing antibodies, T-cell activation, or complement cascade engagement could compromise efficacy or safety. To minimize these risks, clinical protocols should include pre-screening for AAV seropositivity and consider transient immunosuppression regimens during the vector dosing window.

9.2 Funding and Commercialization Constraints

The cost of integrating FUS systems with GMP-grade vector production is substantial. Clinical systems, acoustic monitoring hardware, and supporting imaging infrastructure can exceed ~$1 million per site. Simultaneously, GMP AAV manufacturing remains cost-prohibitive for many academic or early-phase programs. Mitigation strategies include pursuing modular funding via public-private partnerships, such as NIH U01 grants or disease foundation support. Collaborations with academic vector cores or commercial contract manufacturing organizations (CMOs) can also reduce cost and logistics barriers.

Commercial hesitancy from gene therapy sponsors is another foreseeable hurdle. Biotech companies may be reluctant to adopt FUS if it introduces delivery complexity, requires unfamiliar regulatory oversight, or dilutes control over therapeutic administration. To overcome this, it is crucial to position FUS not as a competing platform but as a delivery enabler that expands the range of CNS indications and improves localization. Joint intellectual property

structures, co-publication agreements, and collaborative preclinical studies may also help align interests.

9.3 Legal and IP Complexity

The intellectual property landscape for FUS gene therapy is highly fragmented. Patent rights may be held separately for the therapeutic vector (capsid, promoter, payload), the delivery protocol, the FUS system, and its software. Early freedom-to-operate (FTO) analysis is essential to define potential obstacles. Multi-party licensing agreements should be pursued proactively, ideally with clearly delineated fields of use and sublicensing rights to reduce downstream conflicts.

9.4 Strategic and Operational Coordination

Even with technical feasibility demonstrated, the translation of FUS gene therapy depends on alignment between academic investigators, clinical sites, industry stakeholders, and philanthropic funders. Divergent priorities, ranging from speed of development to risk tolerance and publication preferences, can impede progress. Establishing a transparent and mission-driven consortium can help unify goals. Shared charters, milestone-based collaboration, and pre-agreed policies on authorship, IP, and data sharing are essential governance tools.

Another operational challenge lies in the limited number of clinical sites with dual capability in both FUS infrastructure and gene therapy administration. Rather than diffusing capacity too broadly, early-phase trials should concentrate activity at 1–2 expert centers and invest in rigorous training. Collaboration with experienced contract research organizations (CROs) specializing in neurosurgical biologics can further streamline execution.

9.5 Patient-Centered Challenges

Finally, successful trial execution hinges on thoughtful engagement with the patient community. Many target indications such as monogenic neurodevelopmental or neurodegenerative disorders are rare and require genotypic confirmation for eligibility. Early partnerships with patient advocacy organizations and natural history registries can support patient identification and pre-trial genetic screening.

Moreover, patient communities are key stakeholders for articulating unmet needs and assessing the acceptability of a new treatment paradigm that integrates focused ultrasound (FUS) with gene therapy.

Finally, the dual novelty of FUS and gene therapy may present ethical or communication hurdles for patients, families, and institutional review boards. Proactive development of accessible educational materials, inclusion of patient advocates on advisory boards, and early engagement with IRBs are key to building trust and ensuring informed participation.

Table 10: Risk Assessment and Mitigation Strategy Summary

Roadblock	Likelihood (1–5)	Impact (1–5)	Score	Mitigation Strategy
Unreliable BBB opening or inconsistent vector delivery	4	5	20	Use real-time acoustic feedback; validate in NHPs with clinical FUS device; establish SOPs across sites
Subtherapeutic or toxic transgene expression	3	5	15	Preclinical dose-finding in NHPs; incorporate tissue-specific promoters or self-regulatory systems
Immune responses to vector or transgene	4	4	16	Screen for neutralizing antibodies; include immunosuppression protocols; monitor cytokines and T-cell activation
Regulatory uncertainty around device-biologic combination	5	5	25	Engage FDA early via INTERACT and Pre-IND; retain experienced regulatory consultant; clarify lead review center (CBER vs. CDRH)
High FDA bar for dual-novelty therapeutic	3	5	15	Strengthen GLP safety package; consider starting with ASO delivery or severe indication with high unmet need
High cost of GMP vector and device integration	4	4	16	Pursue co-funding: NIH U01, FUS Foundation, MJFF; explore use of academic GMP cores; apply for RMAT designation
Biotech partner hesitancy	3	4	12	Show FUS expands delivery potential; offer co-IP opportunities; secure pilot data in NHPs with existing vector
Fragmented IP landscape	3	4	12	Conduct early FTO analysis; establish co-licensing agreements; involve tech transfer offices from day one
Misaligned incentives among partners	4	4	16	Create consortium charter; define common milestones and publication policies; convene quarterly PI meetings
Clinical site inexperience with both FUS + gene therapy	4	4	16	Limit trial to 1–2 expert centers; develop training protocols; partner with CRO experienced in complex neurosurgical trials
Recruitment challenges in rare CNS populations	3	3	9	Partner with patient advocacy groups; build registries and genetic screening programs in advance
Ethical and risk communication issues	3	3	9	Develop risk communication plans; engage IRBs early; include patient reps on steering committees

10. Recommendations for FUS Foundation Action

10.1 Consortium Formation and Governance Structure

We recommend the FUS Foundation lead the establishment of a formal FUS-Gene Therapy Translation Consortium, modeled after successful initiatives like the Bespoke Gene Therapy Consortium. This consortium should include academic centers with dual FUS-gene therapy expertise, industry partners spanning device manufacturers and gene therapy developers, regulatory consultants, and patient advocacy organizations.

The consortium's governance structure should include: (1) A Scientific Steering Committee to prioritize indications and standardize protocols; (2) A Regulatory Working Group to engage FDA and establish combination product pathways; (3) A Clinical Operations Committee to coordinate multi-site trials and data sharing; (4) An Industry Liaison Board to facilitate academic-industry partnerships and technology transfer.

10.2 Designated Centers of Excellence

Not every clinical site possesses the multidisciplinary expertise required for FUS-gene therapy trials. We recommend identifying and formally designating 3-5 centers of excellence based on specific capabilities:

Tier 1 Centers (comprehensive capability): Sites with established FUS clinical programs, GMP vector manufacturing access, neurosurgical gene therapy experience, and regulatory track records.

Tier 2 Centers (specialized capability): Sites with focused expertise in specific disease areas, routes of administration, or FUS technologies. These centers would collaborate with Tier 1 sites for specialized patient populations or novel delivery approaches.

10.3 Preclinical Platform Standardization

The consortium should establish standardized preclinical assessment platforms to reduce redundancy and improve data quality across programs. Key initiatives include:

Standardized FUS Protocols: Develop consensus protocols for cavitation monitoring, safety parameters, and efficacy assessment across different FUS systems and animal models. Charles River Laboratories' acquisition of Insightec MRgFUS instrumentation provides a potential centralized facility for standardized large animal studies.

Biomarker Harmonization: Establish common analytical methods for vector biodistribution (qPCR), transgene expression (RT-qPCR), and protein activity assessment. This includes standardized sample collection, processing, and analysis protocols to enable cross-program comparisons.

Quality Control: Implement standardized quality metrics for AAV vector characterization, FUS device calibration, and outcome assessment to ensure regulatory-grade data generation from early-stage studies.

10.4 Addressing the "Translational Valley of Death"

A critical gap exists between initial proof-of-concept studies and expensive IND-enabling toxicology studies. We propose establishing an intermediate tier of **"INTERACT-enabling studies"** that provide more robust efficacy and safety data than initial academic studies while remaining more cost-effective than full GLP studies.

This intermediate tier would include: (1) Multi-dose efficacy studies in disease-relevant animal models; (2) Preliminary toxicology assessment in low sample size of non-human primates; (3) Vector biodistribution and expression durability studies; (4) Optimized FUS parameter development with treatment monitoring.

10.5 Strategic Disease-Specific Matching

As we advance in the characterization of each indication and apply this framework to prioritize potential programs, we expect these recommendations to evolve.

Applying the framework today suggests that several indications warrant near-term attention, given their high gene therapy readiness and strong suitability for integration with focused ultrasound (FUS). An illustrative implementation timeline is as follows:

- **Immediate (0–18 months): GBA1-associated Parkinson's disease**, leveraging existing AAV9-GBA1 programs and established putamen targeting protocols. This indication offers a strong scientific rationale, relevant regulatory precedent, and measurable biomarkers.
- **Mid-term (18–36 months): Frontotemporal dementia (GRN mutations)** and select ultra-rare diseases (e.g., **type II neuronopathic Gaucher disease**), where unmet need is high and development can benefit from rare-disease regulatory advantages.
- **Long-term (3–5 years):** Expansion to more prevalent conditions such as **idiopathic Parkinson's disease** and **Alzheimer's disease**, building on clinical experience and precedent established through earlier rare-disease programs.

This strategic sequencing leverages the relative development and regulatory advantages of rare diseases while building the clinical experience and safety database needed to support broader indications. It also aligns with common industry incentives, as rare-disease programs often offer clearer development pathways and more predictable regulatory interactions.

By operationalizing these recommendations, the Focused Ultrasound Foundation can organize the infrastructure and expertise needed to realize the transformative potential of FUS-enhanced gene therapy for neurological disorders.

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APPENDIX

Appendix Table 1: Previous and ongoing AAV clinical trials for CNS disorders. See most up to date in the associated database: https://www.fusfgenetherapy.com

Indication	Trial Number	Link	Phase	Recruitment Status	Start Date	End Date	Sponsor	ROA	ROA Details	Transgene	Capsid
AADC deficiency	NCT02852213	https://clinicaltrials.gov/ct2/show/NCT02852213	Phase 1	Recruiting	01-Jul-16		Krystof Bankiewicz	IP	Bilateral Substantia nigra compacta and Ventral tegmental area (VTA) [19]	hAADC	AAV2
Adrenomyeloneuropathy (AMN)	NCT05394064	https://clinicaltrials.gov/ct2/show/NCT05394064	Phase 1/2	Active, not recruiting	17-Nov-22		SwanBio Therapeutics, Inc	IT	Lumbar (IT)	ABCD1	AAV9
Alhzeimer's Disease	NCT00876863	https://clinicaltrials.gov/ct2/show/NCT00876863	Phase 2	Completed	01-Nov-08	13-Aug-15	Sangamo Therapeutics	IP	Basal forebrain (which contains the Nucleus basalis of Meynert)	NGF	AAV2
Alhzeimer's Disease	NCT05040217	https://clinicaltrials.gov/ct2/show/NCT05040217	Phase 1	Recruiting	07-Feb-22		Mark Tuszynski	IP	unspecified	BDNF	AAV2
Alhzeimer's Disease	NCT04133454	https://clinicaltrials.gov/ct2/show/NCT04133454	Phase 1	Unknown status	10-Oct-19	01-Jan-21	Libella Gene Therapeutics	IV, IT	IV and IT	hTert	AAV2
ALS	NCT06100276	https://clinicaltrials.gov/ct2/show/NCT06100276	Phase 1/2	Recruiting	01-Aug-24		UniQure Biopharma B.V.	IT	Lumbar (IT)	miR-SOD1	AAVrh10
Batten Disease CLN2	NCT00151216	https://clinicaltrials.gov/ct2/show/NCT00151216	Phase 1	Completed	01-Jun-04	01-Jun-19	Weill Medical College of Cornell University	IP	12 intraparenchymal locations (six on each side)	hCLN2	AAV2
Batten Disease CLN2	NCT01161576	https://clinicaltrials.gov/ct2/show/NCT01161576	Phase 1/2	Completed	19-Aug-10	31-Dec-20	Weill Medical College of Cornell University	IP	12 intraparenchymal locations (six on each side)	hCLN2	AAVrh10
Canavan Disease	NCT04833907	https://clinicaltrials.gov/ct2/show/NCT04833907	Phase 1/2	Recruiting	01-Apr-21		Myrtelle Inc.	ICV	Intracerebroventricular	ASPA	AAV-oligo001
Canavan Disease	NCT04998396	https://clinicaltrials.gov/ct2/show/NCT04998396	Phase 1/2	Recruiting	08-Sep-21		Aspa Therapeutics	IV	IV	BBP-812	AAV9
CLN3	NCT03770572	https://clinicaltrials.gov/ct2/show/NCT03770572	Phase 1/2	Active, not recruiting	13-Nov-18		Amicus Therapeutics	IT	Lumbar (IT)	CLN3	AAV9
CLN6	NCT02725580	https://clinicaltrials.gov/ct2/show/NCT02725580	Phase 1/2	Completed	09-Mar-16	27-Oct-21	Amicus Therapeutics	IT	Lumbar (IT)	CLN6	AAV9
CLN7	NCT04737460	https://clinicaltrials.gov/ct2/show/NCT04737460	Phase 1	Active, not recruiting	04-May-21		Benjamin Greenberg	IT	Lumbar (IT)	CLN7	AAV9
Dravet Syndrome	NCT05419492	https://clinicaltrials.gov/ct2/show/NCT05419492	Phase 1/2	Recruiting	14-May-24		Encoded Therapeutics	ICV	ICV one time	eTFSCN1A	rAAV9
Duchenne Muscular Dystrophy (DMD)	NCT05096221	https://clinicaltrials.gov/ct2/show/NCT05096221	Phase 3	Completed	27-Oct-21	25-Oct-24	Sarepta Therapeutics, Inc.	IV	IV	micro-dystrophin	rAAVrh74
Duchenne Muscular Dystrophy (DMD)	NCT05881408	https://clinicaltrials.gov/ct2/show/NCT05881408	Phase 3	Active, not recruiting	31-May-23		Sarepta Therapeutics, Inc.	IV	IV	micro-dystrophin	rAAVrh74
Frontotemporal Dementia	NCT04408625	https://clinicaltrials.gov/ct2/show/NCT04408625	Phase 1/2	Recruiting	09-Nov-20		Prevail Therapeutics	ICM	Cisterna Magna	GRN	AAV9
Gaucher Disease	NCT04411654	https://clinicaltrials.gov/ct2/show/NCT04411654	Phase 1/2	Active, not recruiting	29-Jun-21		Prevail Therapeutics	ICM	Cisterna Magna	GBA1	AAV9
Gaucher Disease	NCT05324943	https://clinicaltrials.gov/ct2/show/NCT05324943	Phase 1	Active, not recruiting	15-Apr-22		Spur Therapeutics	IV	IV	GBA1	AAVS3
Giant Axonal Neuropathy	NCT02362438	https://clinicaltrials.gov/ct2/show/NCT02362438	Phase 1	Active, not recruiting	24-Apr-15		NINDS	IT	Lumbar (IT)	Gigaxonin	scAAV9
GM1	NCT04273269	https://clinicaltrials.gov/ct2/show/NCT04273269	Phase 1/2	Terminated	11-May-21	22-May-23	LYSOGENE	ICM	Cisterna Magna	GLB1	AAVrh10
GM1	NCT03952637	https://clinicaltrials.gov/ct2/show/NCT03952637	Phase 1/2	Recruiting	19-Aug-19		NHGRI	IV	IV	GLB1	AAV9
Huntington's disease	NCT04120493	https://clinicaltrials.gov/ct2/show/NCT04120493	Phase 1/2	Active, not recruiting	06-Sep-19		UniQure Biopharma B.V.	IP	Bilateral caudate	miHTT	AAV9
Krabbe Disease	NCT04771416	https://clinicaltrials.gov/ct2/show/NCT04771416	Phase 1/2	Suspended	24-Feb-22		Passage Bio	ICM	Cisterna Magna	GALC	AAVrh10
Krabbe Disease	NCT04693598	https://clinicaltrials.gov/ct2/show/NCT04693598	Phase 1/2	Active, not recruiting	05-Nov-21		Forge Biologics, Inc.	IV	IV	hGALC	AAVrh10
Metachromatic leukodystrophy (MLD)	NCT01801709	https://clinicaltrials.gov/ct2/show/NCT01801709	Phase 1/2	Completed	01-Jun-14	20-Dec-22	Institut National de la Santé Et de la Recherche Médicale, France	IP	12 locatinos in white matter	cuARSA	AAVrh10
MPS I	NCT03580083	https://clinicaltrials.gov/ct2/show/NCT03580083	Phase 1/2	Active, not recruiting	03-Apr-19		REGENXBIO Inc.	IP	Intraparenchymal	IDUA	AAV9
MPS I	NCT02702115	https://clinicaltrials.gov/ct2/show/NCT02702115	Phase 1/2	Terminated	24-May-17	03-Nov-21	Sangamo Therapeutics	IV	IV	IDUA	AAV6-ZFN
MPS II	NCT03566043	https://clinicaltrials.gov/ct2/show/NCT03566043	Phase 2/3	Active, not recruiting	27-Sep-18		REGENXBIO Inc.	IP	Intraparenchymal	IDS	AAV9
MPS II	NCT04571970	https://clinicaltrials.gov/ct2/show/NCT04571970	Phase 1/2	Completed	11-Mar-21	23-May-24	REGENXBIO Inc.	ICM, ICV	Cisterna Magna and Intracerebroventricular	IDS	AAV9

MPS IIIA	NCT03612869	https://clinicaltrials.gov/ct2/show/NCT03612869	Phase 2/3	Unknown status	17-Dec-18		LYSOGENE	IP	12 locations in white matter anterior, medial, and posterior to the basal ganglia [25]	hSGSH-IRES-SUMF1	AAVrh10
MPS IIIA	NCT02716246	https://clinicaltrials.gov/ct2/show/NCT02716246	Phase 1/2/3	Recruiting	25-Apr-16		Ultragenyx Pharmaceutical Inc.	IV	IV	hSGSH	scAAV9
MPS IIIB	NCT03300453	https://clinicaltrials.gov/ct2/show/NCT03300453	Phase 1/2	Completed	17-Sep-13	27-Nov-19	UniQure Biopharma B.V.	IP	16 sites in white matter	NAGLU	AAV5
Multiple Systems atrophy	NCT04680065	https://clinicaltrials.gov/ct2/show/NCT04680065	Phase 1	Recruiting	03-Oct-23		Brain Neurotherapy Bio	IP	Putamen	GDNF	AAV2
Parkinson's Disease	NCT00195143	https://clinicaltrials.gov/ct2/show/NCT00195143	Phase 1	Completed	01-Aug-03	01-Aug-05	Neurologix, Inc.	IP	Unilateral subthalamic nucleus [28]	GAD	AAV2
Parkinson's Disease	NCT01621581	https://clinicaltrials.gov/ct2/show/NCT01621581	Phase 1	Completed	13-Mar-13	04-Feb-22	NINDS	IP	Bilateral putamen	hGDNF	AAV2
Parkinson's Disease	NCT00400634, ext NCT05894343	https://clinicaltrials.gov/ct2/show/NCT00400634	Phase 2	Completed	01-Nov-06	01-Nov-08	Sangamo Therapeutics	IP	Bilateral putamen CED	Neurturin	AAV2
Parkinson's Disease	NCT00985517	https://clinicaltrials.gov/ct2/show/NCT00985517	Phase 1/2	Completed	29-Oct-09	16-Nov-17	Sangamo Therapeutics	IP	Bilateral putamen and substantia nigra	Neurturin	AAV2
Parkinson's Disease	NCT04167540	https://clinicaltrials.gov/ct2/show/NCT04167540	Phase 1b	Active, not recruiting	01-Apr-20		Brain Neurotherapy Bio/UCSF	IP	Bilateral putamen	GDNF	AAV2
Parkinson's Disease	NCT06285643	https://clinicaltrials.gov/ct2/show/NCT06285643	Phase 2	Recruiting	11-Jun-24		AskBio/Bayer AG	IP	Bilateral putamen	GDNF	AAV2
Parkinson's Disease	NCT05603312	https://clinicaltrials.gov/ct2/show/NCT05603312	Phase 1/2	Completed	05-Oct-22	06-Sep-24	Meira GTx	IP	Bilateral STN	GAD	AAV
Parkinson's Disease	NCT00643890	https://clinicaltrials.gov/ct2/show/NCT00643890	Phase 2	Terminated	01-Aug-08	01-Dec-10	Neurologix, Inc.	IP	Bilateral STN	GAD	AAV
Parkinson's Disease	NCT01973543	https://clinicaltrials.gov/ct2/show/NCT01973543	Phase 1	Completed	01-Oct-13	24-Jan-20	Neurocrine Biosciences	IP	Bilateral striatum	hAADC	AAV2
Parkinson's Disease	NCT03562494	https://clinicaltrials.gov/ct2/show/NCT03562494	Phase 1	Completed	17-Oct-18	30-Oct-24	Neurocrine Biosciences	IP	Bilateral	hAADC	AAV2
Parkinson's Disease	NCT03065192	https://clinicaltrials.gov/ct2/show/NCT03065192	Phase 1	Completed	11-May-17	10-Aug-21	Neurocrine Biosciences	IP	Bilateral putamen	hAADC	AAV2
Parkinson's Disease	NCT00229736	https://clinicaltrials.gov/ct2/show/NCT00229736	Phase 1	Completed	01-Nov-04	01-Mar-13	Genzyme (Sanofi)	IP	4 striatal infusions	hAADC	AAV
Parkinson's Disease	NCT04127578	https://clinicaltrials.gov/ct2/show/NCT04127578	Phase 1/2	Recruiting	03-Jan-20		Prevail Therapeutics	ICM	Cisterna Magna	GBA1	AAV9
Parkinson's Disease (GBA1)	NCT07011771	https://clinicaltrials.gov/ct2/show/NCT07011771	Phase 1/2	Not yet recruiting	01-Aug-25		Capsida Biotherapeutics, Inc.	IV	IV	GBA1	AAV
Rett Syndrome	NCT06856759	https://clinicaltrials.gov/ct2/show/NCT06856759	Phase 1/2	Recruiting	14-Jan-25		Guangzhou Women and Children's Medical Center	IT	Lumbar (IT)	MECP2	AAV
Rett Syndrome	NCT05606614	https://clinicaltrials.gov/ct2/show/NCT05606614	Phase 1/2	Recruiting	06-Mar-23		Taysha Gene Therapies Inc.	IT	Lumbar (IT)	miniMECP2	scAAV9
Spinal Muscular Atrophy (SMA)	NCT03505099	https://clinicaltrials.gov/ct2/show/NCT03505099	Phase 3	Completed	02-Apr-18	15-Jun-21	Novartis Gene Therapies	IV	IV	SMN	scAAV9
Spinal Muscular Atrophy (SMA)	NCT06288230	https://clinicaltrials.gov/ct2/show/NCT06288230	Phase 1/2	Recruiting	20-Oct-24		Lantu Biopharma	IV	IV	hSMN1	AAV
Tay-Sachs and Sandhoff Disease (GM2)	NCT04669535	https://clinicaltrials.gov/ct2/show/NCT04669535	Phase 1	Completed	15-Jan-21	16-Dec-24	Terence Flotte	IP/ ICM/IT	Bilateral thalamic (CED) [33], Cisterna Magna [33] and Lumbar intrathecal (IT)	HEXA/HEXB	AAVrh8

1934

1935

Appendix Table 2: Indication Fact Sheet. See interactive presentation of these data in the associated database: https://www.fusfgenetherapy.com

Disease/Indication	Prevalence (US est.)	Genetic Cause Identified	Vector(s) Used in Trials	Typical Delivery Method/Brain Target	FUS Delivery Advantage	Trial Phase(s)	Known Biomarkers	Patient Population Size	FDA Special Designation Precedent	AAV Biodistribution Challenge
AADC deficiency	Ultra-rare; fewer than 50 diagnosed patients in the U.S.	Yes – mutations in DDC (AADC gene), autosomal recessive (monogenic)	AAV2-hAADC (AAV2 vector delivering the AADC gene) used in trials	Intraparenchymal (stereotactic infusion into midbrain; e.g. bilateral putamen/SNc/VTA)	FUS could enable noninvasive BBB opening to target midbrain regions, avoiding brain surgery	Phase 1 trials completed (safety/feasibility)	CSF neurotransmitter metabolites (e.g. 5-HIAA), oculogyric crisis frequency, motor milestones	Ultra-rare (<100 patients); estimated US prevalence <50	Yes – FDA Orphan Drug Designation granted (eladocagene, Upstaza)	Deep brain target; requires localized delivery to the substantia nigra/putamen (BBB prevents IV distribution to these nuclei)
Adrenomyeloneuropathy (AMN)	Rare; X-ALD incidence ~1:17,000 births (~10,000 affected in US/EU; ~2,500 in US)	Yes – ABCD1 gene mutation (X-linked recessive); adult phenotype of X-linked ALD	AAV9-ABCD1 (SBT101) via intrathecal injection	Intrathecal (lumbar) infusion targeting spinal cord and brain (widespread)	FUS could boost widespread gene delivery in the brain introduced by IT injection (also evidence for BSCB opening e.g., O'Reilly paper)	Phase 1/2 ongoing (PROPEL trial of SBT101)	Plasma very long-chain fatty acids (VLCFA) levels, gait velocity, AMN disability scores	Rare disease (~8,000–10,000 men in US+EU with AMN)	Yes – SBT101 granted Orphan Drug & Fast Track by FDA	Needs broad transduction along entire spinal cord (and peripheral nerves); AAV spread is limited from a single lumbar infusion
ALS (SOD1)	Ultra-rare subset (~2% of ALS cases); ~300–500 patients in US	Yes – mutations in SOD1 gene (autosomal dominant); familial monogenic ALS subtype	AAVrh10 vector encoding anti-SOD1 microRNA (e.g. APB-102)	Intrathecal (lumbar) delivery to target spinal cord and brain motor neurons	FUS could improve delivery to motor cortex after IT injection which primarily targets spinal cord (BSCB opening applies)	Phase 1/2 ongoing (e.g. APB-102 trial)	Neurofilament light (NfL) in CSF/serum (axonal injury marker); muscle strength and forced vital capacity	Rare (ALS overall ~20k in US; SOD1 subset ~2%)	Yes – FDA Orphan Drug & Fast Track for APB-102 (SOD1 ALS)	Widespread motor neuron targeting needed (brain & entire spinal cord); intrathecal AAV may not evenly reach all motor neurons (especially upper motor neurons in cortex)
Alzheimer's Disease	Very common; ~6.7 million US patients age 65+	Partially – mostly multifactorial/sporadic. Rare familial forms caused by APP, PSEN1, PSEN2 mutations (autosomal dominant)	AAV2 vectors delivering trophic factors (NGF, BDNF) and AAV2-hTERT have been tested	Intraparenchymal (stereotactic intracerebral injections to basal forebrain) for NGF/BDNF; one trial attempted IV + IT delivery	FUS could boost vector delivery in the hippocampus and cortex (targeted and widespread delivery needed)	Phase 1 and Phase 2 trials (e.g. CERE-110 AAV2-NGF Phase 2)	CSF amyloid-β_{42} and tau (p-tau) levels, amyloid PET imaging, and cognitive scales (ADAS-Cog)	Common disease (>6 million in US; not eligible for Orphan)	No (common indication; Orphan designation not applicable)	Diffuse cortical and hippocampal pathology requires global brain delivery; surgical gene infusion only achieves localized coverage (e.g. basal forebrain)
Batten Disease CLN2	Ultra-rare; US prevalence ~400–500 patients	Yes – mutations in TPP1 (CLN2 gene), autosomal recessive (enzyme deficiency)	AAV2-TPP1 and AAVrh10-TPP1 used in trials (e.g. RGX-181 uses AAV9-TPP1)	Intracerebroventricular or intraparenchymal CNS injections (to deliver enzyme broadly in brain)	FUS could boost vector delivery from widespread transduction resulting from intra CSF injections	Phase 1 and 1/2 trials ongoing (e.g. RGX-181 Phase 1/2)	Enzyme activity (TPP1 level in CSF), brain MRI for atrophy, seizure frequency	Ultra-rare (~0.6–0.7 per million; ~400 US patients)	Yes – Orphan Drug & Rare Pediatric Disease designations for AAV-TPP1 programs (e.g. RGX-181)	Requires broad CNS distribution (enzyme needed in all neurons); current direct injections only cover localized regions of brain (enzyme uptake relies on limited diffusion)
CLN3 (Juvenile Batten)	Ultra-rare; ~200–300 patients in US (juvenile NCL is most common NCL)	Yes – mutations in CLN3 gene, autosomal recessive (monogenic lysosomal disorder)	AAV9-CLN3 in trials (e.g. ABO-201 AAV-CLN3)	Intracerebral or intraventricular injections to target CNS widely (e.g. multiple cortical and ventricular infusions in trial)	FUS BBB opening could enable widespread CNS gene delivery via IV, reaching cortex and deep brain without multiple catheter infusions	Phase 1/2 trial completed (AAV9-CLN3, Abeona)	MRI brain volume, vision (retinal degeneration), behavioral rating scales (if available)	Rare (juvenile Batten ~0.5–1 per million; qualifies as orphan)	Yes – Orphan Drug Designation granted (ABO-201 gene therapy)	Widespread CNS degeneration (cortex, retina); broad brain transduction needed beyond local injection sites (many neurons must receive CLN3 due to limited cross-correction)

CLN6 (Late-infantile Batten)	Ultra-rare; only dozens of US patients (incidence ~1:100,000 births)	Yes – mutations in CLN6 gene, autosomal recessive (monogenic lysosomal disorder)	AAV9-CLN6 (e.g. Amicus AT-GTX-501 program) in Phase 1/2	Intrathecal or intracerebral injections to deliver AAV broadly in CNS	FUS could facilitate diffuse delivery of AAV to brain parenchyma from bloodstream, improving coverage of cortex and cerebellum	Phase 1/2 (Amicus trial completed, n≈13)	Motor and language development scales, seizure frequency, brain MRI changes	Ultra-rare (qualifies as orphan; global NCL incidence ~1/100k)	Yes – Orphan Drug Designation (US & EU) for AAV CLN6 program (AT-GTX-501)	Needs global CNS enzyme delivery; current intrathecal injection may not evenly reach all affected brain regions (multiple sites needed for full coverage)
CLN7 (Late-infantile Batten)	Ultra-rare; only a few dozen US patients (very low incidence)	Yes – mutations in MFSD8 (CLN7 gene), autosomal recessive (monogenic)	AAV9-CLN7 (e.g. TSHA-104) in preclinical stage (no human trial yet; one compassionate use reported)	Likely intrathecal CSF delivery (to reach brain globally via CSF circulation)	FUS could boost vector delivery from widespread transduction resulting from intra CSF injections	No clinical trials yet (preclinical stage)	Seizure frequency, developmental milestones, MRI brain volume (natural history data)	Ultra-rare (<100 patients worldwide; qualifies as orphan)	Yes – Expected (FDA has granted ODD to similar Batten gene therapies; TSHA-104 likely to receive Orphan status)	Extensive CNS involvement; requires whole-brain gene delivery which is difficult with localized injection (current lack of cross-correction means each region must be transduced)
Canavan Disease	Ultra-rare; estimated <1000 worldwide, a few hundred in US	Yes – mutations in ASPA gene (Aspartoacylase deficiency), autosomal recessive	rAAV-Olig001-ASPA (Myrtelle) and AAV9-ASPA (BridgeBio BBP-812) in trials	Intracerebroventricular (neonatal) or intrathecal delivery targeting oligodendrocytes in CNS	FUS could boost vector delivery from widespread transduction resulting from intra CSF injections to transduce oligodendroglia for myelination	Phase 1/2 trials ongoing (Myrtelle and BridgeBio programs)	N-acetylaspartate (NAA) levels in urine/CSF (elevated in Canavan), brain MRI myelination, developmental milestones	Ultra-rare (1 in 100,000 births; qualifies as orphan)	Yes – Orphan Drug, Fast Track, Rare Pediatric Disease granted to AAV-ASPA therapies (Myrtelle rAAV-Olig001)	Diffuse white matter disease; requires near-complete brain coverage (all oligodendrocytes), which is challenging with localized vector delivery (current trials focus on early neonatal delivery for better spread)
Dravet Syndrome	Rare; ~15,000–20,000 patients in US (incidence ~1:15,000 births)	Yes – typically caused by de novo mutations in SCN1A (Na⁺ channel α1 subunit), autosomal dominant (haploinsufficiency)	AAV9 encoding SCN1A enhancer or replacement (gene therapy ETX-101 in development; also ASO therapy STK-001 in trials)	Intracerebroventricular or intrathecal delivery in infancy to broadly distribute vector in brain	FUS could boost vector delivery from widespread transduction resulting from intra CSF injections to transduce cortical and hippocampal regions controlling seizures	Phase 1/2 (antisense oligo therapy ongoing; AAV gene therapy entering Phase 1)	Seizure frequency, EEG improvements, SCN1A expression (e.g. in CSF exosomes), developmental milestones	Rare (~1 in 15,000; qualifies as orphan)	Yes – Orphan Drug Designation granted to multiple Dravet therapies (e.g. STK-001 ASO; expected for SCN1A AAV)	Requires diffuse cortical transduction (to restore interneuron function throughout brain); AAV spread from CSF injection may be incomplete, especially to frontal cortex
Duchenne Muscular Dystrophy (DMD)	Rare; ~10,000–15,000 patients in US (X-linked ~1 in 3,500 male births)	Yes – mutations in the DMD gene (dystrophin); X-linked recessive; very large gene (monogenic)	rAAVrh74-microdystrophin (e.g. SRP-9001) in Phase 3 trials (SRP-9001)	Systemic intravenous delivery (AAV via IV infusion to reach all muscles and heart)	FUS is less critical here (disease is primarily muscular)	Phase 3 completed (SRP-9001); FDA review for approval in 2023	Serum creatine kinase (CK) levels, muscle strength (6-minute walk test), dystrophin expression in muscle biopsy	Rare (~0.002% of male births; qualifies as orphan)	Yes – Orphan Drug Designation for multiple DMD gene therapies (e.g. SRP-9001, Pfizer's fornini-dystrophin)	Body-wide muscle distribution needed; extremely high vector doses required to transduce all skeletal muscles and cardiac muscle. Immune responses and limited vector manufacturing capacity are major challenges (not BBB-limited).
Frontotemporal Dementia (FTD, GRN)	Rare; ~50,000–60,000 Americans with FTD (GRN mutation subset ~5–10% of cases ≈3,000 patients in U.S./EU)	Partially – FTD is heterogeneous; \~20% familial (e.g. GRN haploinsufficiency, MAPT mutations, C9orf72 expansions). AAV target is GRN (progranulin) loss-of-function	AAV9-GRN (Prevail PR006) and AAV1-GRN (Passage Bio PBFT02) in Phase 1/2 trials	Intracisternal or intrathecal CSF delivery (e.g. cisterna magna infusion) to broadly target the brain	FUS could target frontotemporal lobes via BBB opening, boosting widespread AAV delivery to cortex and deep structures, reducing need for high-dose CSF infusion	Phase 1/2 ongoing (Prevail PR006 "PROCLAIM" trial)	Progranulin levels in CSF & plasma (should increase with therapy); neurofilament light (NfL); brain MRI or FDG-PET for frontal/temporal atrophy	Rare (FTD total <200k in US; GRN-FTD ~5k patients; qualifies as orphan)	Yes – Orphan Drug (granted 2019) & Fast Track for PR006 (FTD-GRN)	FTD pathology spans frontal and temporal cortex; achieving sufficient AAV distribution to these lobes via CSF can be inefficient (BBB limits parenchymal uptake, especially in cortex).
Gaucher Disease (neuronopathic)	Ultra-rare (neuronopathic types II/III ~1:100,000; a few hundred patients in US)	Yes – GBA1 gene mutations, autosomal recessive; Type II (acute infantile) and III (chronic) Gaucher are monogenic (deficient glucocerebrosidase)	AAV9-GBA1 (Prevail PR001) intrathecal for Type II/III; also AAV9-GBA1 (same vector) tested in PD-GBA patients	Intrathecal injection (cisternal or lumbar) to deliver AAV to brain (brainstem, cortex, hippocampus, cerebellum) and spinal cord (Prevail); IV dosing in some trials for broad distribution	FUS BBB opening could enhance delivery to specific brain regions (brainstem, thalamus) affected in neuronopathic Gaucher; could boost widespread transduction	Phase 1/2 trial ongoing (PR001 for nGD)	Glucocerebrosidase enzyme activity in CSF, plasma lyso-Gb1 (GL1) substrate levels, liver/spleen volume (systemic disease), neurological exam scores	Ultra-rare (Type II Gaucher <100 US patients; qualifies as orphan)	Yes – Orphan Drug Designation for PR001 (neuronopathic Gaucher)	Wide CNS involvement (cortex, brainstem) plus systemic storage; delivering AAV across BBB to all affected brain regions is challenging. Intrathecal delivery targets spinal cord well but may not uniformly reach cerebral targets like brainstem/cortex.

Giant Axonal Neuropathy (GAN)	Ultra-rare; <100 known cases worldwide (handful in US)	Yes – mutations in GAN (gigaxonin) gene, autosomal recessive; causes intermediate filament accumulation (monogenic)	scAAV9-gigaxonin (TSHA-120) via intrathecal injection	Intrathecal (lumbar) delivery to target both central and peripheral nervous system neurons	FUS could help boost AAV to thalamus, basal ganglia, cerebellum and midbrain after IT injection	Phase 1 trial completed (intrathecal AAV9, NIH-sponsored)	Neurofilament levels, peripheral nerve conduction velocity, developmental milestones (motor function, sensation)	Ultra-rare (qualifies as orphan; NIH compassionate use trial)	Yes – Orphan Drug & Rare Pediatric Disease designations (TSHA-120)	Targets both CNS and peripheral nerves; AAV must reach spinal motor neurons and dorsal root ganglia, plus peripheral nerves. Limited CSF diffusion means distal peripheral nerves receive low vector doses.
GM1 Gangliosidosis	Ultra-rare; estimated ~100–200 patients in US (incidence ~1:100,000)	Yes – mutations in GLB1 (β-galactosidase) gene, autosomal recessive (lysosomal storage disease)	AAV9-GLB1 (AXO-AAV-GM1 by Sio) intravenous and AAV9-GLB1 (Passage PBGM01) intracisternal in trials	Intrathecal or intracisternal injection in infants to broadly deliver vector to brain and spinal cord	FUS could allow IV administration with BBB opening to boost widespread cortical and subcortical transduction	Phase 1/2 trials ongoing (AXO-AAV-GM1, PBGM01)	β-Galactosidase enzyme activity in CSF, GM1 ganglioside levels in CSF or urine, developmental milestones, brain MRI (basal ganglia and white matter)	Ultra-rare (qualifies as orphan; few hundred globally)	Yes – Orphan Drug, Fast Track, RPD designations for AAV9-GLB1 programs (e.g. PBGM01)	Requires enzyme delivery to entire brain and spinal cord; achieving sufficient AAV distribution via CSF is difficult due to the large CNS volume and glycosphingolipid buildup in all regions. IV plus FUS might improve global reach.
Huntington's Disease	Rare (~30,000 symptomatic patients in US; ~10 per 100,000)	Yes – HTT gene CAG trinucleotide repeat expansion (autosomal dominant); monogenic neurodegenerative disorder	AAV5-miHTT (uniQure AMT-130) in trials; other AAV vectors (AAV1/2) for HTT lowering in preclinical development	MRI-guided intraparenchymal infusion into striatum (putamen) for AAV5 (AMT-130); an alternative trial arm delivers AAV into CSF (lumbar intrathecal)	FUS could open BBB at striatum (targeted) and cortex (boost widespread delivery) to deliver gene-silencing AAV throughout affected regions	Phase 1/2 ongoing (AMT-130 trial; both surgical and intrathecal cohorts)	Mutant huntingtin (mHTT) protein in CSF, neurofilament light (NfL) in CSF (neurodegeneration), volumetric MRI of striatum	Rare (~30k in US; meets orphan criteria)	Yes – Orphan Drug, Fast Track, RMAT granted to AMT-130 (first HD gene therapy)	Needs broad distribution to caudate, putamen, and cerebral cortex; direct striatal injection covers limited area, and intrathecal delivery may not sufficiently transduce deep brain nuclei. Achieving widespread cortical HTT silencing remains a challenge.
Krabbe Disease (Globoid cell LD)	Ultra-rare; ~1 in 100,000 births (few dozen new US cases/year; ~200 living US patients)	Yes – mutations in GALC gene (galactocerebrosidase), autosomal recessive; monogenic demyelinating leukodystrophy	AAVrh10-GALC (e.g. gene therapy at Nationwide) in Phase 1; also ex vivo lentiviral HSC gene therapy (used clinically in EU)	Intrathecal or intracerebroventricular AAV delivery in early infancy to target CNS oligodendrocytes (before disease progression)	FUS could boost widespread gene delivery to the entire brain and spinal cord	Phase 1/2 (HUSTLE trial of AAVrh10-cGALC) recruiting (also OTL-200 ex vivo approved in EU)	Galactocerebrosidase activity in CSF, psychosine levels, MRI brain myelination, developmental milestones (motor tone, feeding)	Ultra-rare (qualifies for Orphan; OTL-200 approved EU for Krabbe)	Yes – Orphan Drug Designation (e.g. FDA ODD for FBX-101 ex vivo gene therapy)	Diffuse CNS demyelination; must deliver gene to oligodendrocytes throughout brain and spinal cord. Current approaches use transplant to achieve broad enzyme delivery – in vivo AAV must overcome limited spread from injection sites to cover all CNS.
Metachromatic Leukodystrophy (MLD)	Ultra-rare; ~1 in 100,000 births (few hundred patients in US)	Yes – mutations in ARSA gene (arylsulfatase A), autosomal recessive; monogenic lysosomal leukodystrophy	AAVrh10-ARSA (e.g. in academic trials) and ex vivo lentiviral (OTL-200) used clinically in EU	Intrathecal AAV administration (to achieve CNS enzyme delivery); HSCT or HSC-gene therapy (ex vivo) also used to deliver ARSA via microglia	FUS could boost widespread delivery of ARSA gene to the brain	Phase 1/2 AAV trial in EU (early results); OTL-200 gene therapy approved in EU (2020)	Arylsulfatase A activity in CSF, sulfatide levels in CSF or urine, neurodevelopmental scales, brain MRI white matter changes	Ultra-rare (qualifies for Orphan; OTL-200 has ODD in US/EU)	Yes – Orphan Drug Designation (OTL-200 ex vivo granted ODD; any AAV program would qualify)	Global CNS demyelination; requires enzyme delivery to all brain regions and spinal cord. Ex vivo gene therapy achieves this via engrafted cells – in vivo AAV must overcome limited diffusion to reach the entire CNS.
Multiple System Atrophy (MSA)	Rare; prevalence ~3–4 per 100,000 (≈1,000–1,500 patients in US)	No single genetic cause (sporadic); some rare familial cases but generally multifactorial (α-synuclein protein aggregation)	AAV2-GDNF (CNS10-NPC) tested in Phase 1 (intracranial); other experimental gene therapies (e.g. exenatide gene transfer) in preclinical stages	Intraparenchymal (stereotactic) infusion into putamen (for AAV2-GDNF trial); targeted delivery to nigrostriatal regions	FUS could enable targeted delivery of neurotrophic genes across the BBB to basal ganglia and cerebellum without invasive surgery	Phase 1 trial completed (AAV2-GDNF safety demonstrated)	MRI brain volume (putaminal atrophy), PET imaging of vesicular dopamine transporter, Unified MSA Rating Scale (UMSARS) clinical score	Rare (~<5,000 in US; qualifies as orphan)	No gene therapy ODD (ODDs granted to some drugs like antibodies)	Multifocal neurodegeneration (striatal, olivopontocerebellar regions); multiple deep brain structures need targeting. Currently requires several surgical injections to cover key regions, as AAV does not diffuse far in parenchyma.
Mucolipidosis Type IV (ML4)	Ultra-rare; ~50–100 patients in US (Ashkenazi founder mutation ~1:40,000 births)	Yes – mutations in MCOLN1 (encodes TRPML1 lysosomal channel), autosomal recessive (monogenic)	*No clinical trials yet* (preclinical: AAV9 and AAV.CPP capsid delivering MCOLN1 showed broad CNS correction in ML4 mice)	Future: likely intravenous with BBB-penetrant capsid, or intrathecal if using conventional AAV9 (preclinical used IV AAV.CPP16-MCOLN1)	FUS-mediated BBB opening could enable systemic AAV to reach the entire brain, critical since ML4 requires near-100% CNS transduction (diffuse involvement, no cross-correction)	Preclinical stage (no human trials; 2024 mouse model gene therapy success published)	Visual acuity (retinal degeneration), developmental milestones, MRI brain volume; cellular storage material levels (if biopsy or CSF assay available)	Ultra-rare (~50–100 US patients)	No – no programs started (qualifies for Orphan; none granted yet due to no IND)	Extremely broad CNS (and ocular) involvement; every neuron needs the gene because TRPML1 is not secreted. Requires near-total brain transduction – historically a bottleneck for AAV (standard vectors achieve partial coverage at best).

Parkinson's Disease (idiopathic)	Common; ~1,000,000 patients in US (incidence ~90,000/year)	Partially; ~5–15% have genetic mutations (e.g. LRRK2, GBA1, SNCA) but majority idiopathic	Multiple AAV2 therapies (AAV2-GAD, AAV2-Neurturin, AAV2-hAADC, AAV2-GDNF) tested; one AAV9-GBA1 trial for genetic subset	Intraputaminal (stereotactic bilateral putamen infusions for AAV2 vectors); some trials exploring IV or intrathecal routes for wider delivery	FUS could open BBB at striatum and SNc to allow IV AAV penetration	Multiple Phase 1 and 2 trials completed (e.g. CERE-120 AAV2-Neurturin Phase 2; AAV2-AADC & AAV2-GDNF Phase 1b)	CSF or plasma total and oligomeric α-synuclein levels, dopamine transporter imaging (DAT-SPECT), UPDRS motor scores	Common (~1% of people >60; not orphan-eligible)	No (too common for Orphan; genetic sub-populations considered separately)	Degeneration in widespread regions (substantia nigra, putamen, cortical motor areas); AAV needs to reach deep brain nuclei bilaterally. Current AAV2 infusions cover limited areas (e.g. putamen), leaving other regions (SNc, cortex) untransduced.
Parkinson's Disease (GBA1)	Rare subset (~5% of PD patients carry GBA1 mutations; ~50,000 PD-GBA patients in US, but PD overall is common)	Yes – GBA1 mutation (heterozygous) increases PD risk; not a distinct disease but a genetic risk factor (Gaucher carrier status)	AAV9-GBA1 (Prevail PR001) intrathecal and an engineered AAV capsid (Capsida trial) IV are in trials	Intrathecal (cisterna magna) injection to deliver GBA1 gene to CNS (Prevail); IV dosing with BBB-targeted capsid (Capsida) for systemic route	FUS could target delivery to specific regions affected by PD (e.g. putamen, cortex) in GBA1 carriers, improving enzyme distribution and reducing systemic exposure from IV therapy	Phase 1/2 trials ongoing (Prevail PR001 Phase 1/2 in PD-GBA; Capsida Phase 1/2 IV trial)	Glucocerebrosidase activity in CSF, α-synuclein in CSF/plasma, UPDRS motor scores, cognitive tests for PD dementia	Underlying PD is common (not orphan-eligible as an indication)	No (FDA has not granted Orphan specifically for PD-GBA subset; PD as a whole exceeds orphan criteria)	Pathology similar to idiopathic PD but often more rapid (due to GBA1 heterozygosity). Requires global CNS enzyme delivery (midbrain and cortical regions). Achieving broad distribution remains difficult with intrathecal injection alone – IV + FUS might better reach cortical and limbic areas.
Rett Syndrome	Rare; ~6,000–9,000 females in US (incidence ~1 in 10,000 female births)	Yes – mutations in MECP2 gene on X chromosome (X-linked dominant; usually de novo in girls)	AAV9/PHOENIX vectors encoding MECP2 (truncated or regulated) in trials (e.g. TSHA-102 with miniMECP2)	Intrathecal delivery (lumbar) of AAV to distribute gene through CNS (broad spinal fluid dispersion)	FUS could allow boost of widespread AAV delivery after IT injection (dose reduction important due to MECP2 overexpression risk)	Phase 1/2 trial ongoing (TSHA-102 intrathecal gene therapy)	EEG patterns (abnormal rhythmic activity), breathing irregularity, developmental milestones (motor and communication), brain volume on MRI (cerebral atrophy)	Rare (~1 in 10,000 female births; qualifies as orphan)	Yes – Orphan Drug & Rare Pediatric Disease designations for TSHA-102 (AAV9-MECP2)	Requires gene delivery to essentially all neurons in the brain for efficacy, but MECP2 levels must be tightly regulated. Achieving uniform, safe transduction across the brain is a major challenge (risk of overexpression in some areas, under-delivery in others).
Spinal Muscular Atrophy (SMA)	Rare; ~700 infants born per year in US (prevalence ~1,500–2,000 with treatment)	Yes – deletion or mutation of SMN1 gene, autosomal recessive; monogenic motor neuron disorder (SMN2 copy number modulates severity)	scAAV9-SMN1 (onasemnogene abeparvovec, Zolgensma) approved	Intravenous infusion (one-time IV delivery in infants) for systemic distribution reaching motor neurons via bloodstream	FUS could be useful in older SMA patients by opening BBB to improve AAV delivery to spinal cord and brainstem motor neurons if IV efficacy declines with age (in infants, BBB is more permeable so IV works well)	Approved (Phase 3 completed leading to FDA approval in 2019)	Motor function scales (CHOP-INTEND or Hammersmith), survival/milestone attainment, CMAP (compound muscle action potential), and CSF NfL as a neurodegeneration marker	Rare (~1 in 6,000–10,000 births; qualifies as orphan)	Yes – Orphan Drug designation (Zolgensma received ODD)	Target cells are motor neurons throughout spinal cord and brainstem; in infants systemic AAV9 reaches them before BBB matures. In older or larger patients, BBB limits gene transfer to CNS. Broad CNS delivery in later-onset SMA remains challenging (current therapy relies on early intervention).
Tay-Sachs & Sandhoff (GM2)	Ultra-rare; incidence ~1 in 150,000 (higher in certain populations); <100 patients in US at any time	Yes – mutations in HEXA (Tay-Sachs) or HEXB (Sandhoff) genes, autosomal recessive; causes β-hexosaminidase A deficiency and GM2 ganglioside accumulation	AAVrh8 bicistronic vector (AXO-AAV-GM2) delivering HEXA + HEXB cDNAs used in clinical trial	Combined intrathecal and intracerebral (thalamic) injections to deliver vector broadly in CNS	FUS could allow targeted delivery to thalamus and boost widespread delivery in other areas	Phase 1 (AXO-AAV-GM2) underway; first-in-human gene therapy for GM2 gangliosidosis	β-Hexosaminidase A enzyme activity in CSF, GM2 ganglioside levels in CSF, developmental milestones (motor skills, vision), brain MRI (cerebral atrophy)	Ultra-rare (qualifies as orphan; both Tay-Sachs and Sandhoff are extremely rare)	Yes – Orphan Drug & Rare Pediatric Disease designations (AXO-AAV-GM2)	Entire CNS is affected (storage in cortical neurons, basal ganglia, brainstem, spinal cord); current approach requires both intrathecal and multiple intracranial injections to cover the CNS. Achieving global enzyme distribution remains a key challenge for AAV in GM2 (limited diffusion from injection sites).
MPS I (Hurler syndrome)	Ultra-rare; 1 in 100,000 live births [GARD]	Yes--IDUA gene mutation (autosomal recessive)	AAV9-IDUA (e.g., Regenxbio RGX-111, NCT03071341)	Intracisternal, intrathecal	FUS could allow targeted delivery to hippocampus, thalamus, and ACC, but widespread CNS delivery still required	Phase 1/2 (RGX-111, NCT03071341)	CSF GAG levels, IDUA enzyme activity, neurocognitive scores	Ultra-rare (<300 cases)	Yes (Regenxbio RGX-111)	Requires broad CNS distribution; limited by CSF-mediated vector spread

MPS II (Hunter syndrome)	Ultra-rare; 1 in 100,000 to 150,000 male births [NORD]	Yes-IDS gene mutation (X-linked recessive)	AAV9-IDS (e.g., REGENXBIO RGX-121, NCT03566043)	Intracisternal magna, intrathecal	FUS could allow targeted delivery to striatum, hippocampus and amygda, and boost widespread cortical expression	Phase 1/2 (NCT03566043)	CSF and plasma GAG levels, IDS activity, neurocognitive function	Ultra-rare (<300 cases)	Yes (REGENXBIO RGX-121)	CSF clearance and limited deep brain penetration
MPS III (Sanfilippo A-D)	Rare; 1 in 70,000 births (combined types A-D) [GARD]	Yes-SGSH (A), NAGLU (B), HGSNAT (C), GNS (D); autosomal recessive	AAV10-SGSH, AAV9-NAGLU, AAVrh10-HGSNAT (e.g., NCT02716246, NCT03612869)	ICM, IT, IV, intraparenchymal	FUS could boost IV widespread AAV delivery	Phase 1/2 (Lysogene, Abeona, Allievex)	CSF heparan sulfate, neurocognitive and behavioral scores	Rare (<500 cases)	Yes (multiple developers)	Deep and widespread CNS delivery needed; CED invasive
MPS VII (Sly syndrome)	Ultra-rare; 1 in 250,000 births [NORD]	Yes-GUSB mutation (autosomal recessive)	AAV9-GUSB (preclinical; no active clinical trial)	IV, intrathecal (preclinical)	FUS could allow targeted delivery to striatum, hippocampus and amygda, and boost widespread cortical expression	Preclinical	CSF GAGs, GUSB activity, MRI brain volume	Ultra-rare (<100 cases)	Yes (enzyme replacement precedent)	Need for whole-brain and spinal distribution; CSF route insufficient

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Category	Attribute	Score 1 (Low)	Score 2 (Medium)	Score 3 (High)
Scientific Readiness	Disease Biology & Target Clarity	Multifactorial disease, unclear target	Partial understanding or target unclear	Monogenic, clear target identified
Scientific Readiness	Construct/Target Match	Unknown tropism or poor match	Moderate match with some evidence	Well-matched and validated tropism
Scientific Readiness	Route Suitability	Unvalidated or highly invasive	Validated but invasive route	Validated and non-invasive/localized
Scientific Readiness	Preclinical Model Availability	No relevant models	Surrogate or partial models	Validated and disease-relevant models
Scientific Readiness	Biomarker/Endpoint Predictiveness	Endpoints unknown or not predictive	Exploratory or surrogate only	Validated, predictive endpoints
Operational Feasibility	Platform Readiness	Novel tech, unscalable	Platform exists but limited scale	Scalable, GMP-ready manufacturing
Operational Feasibility	Regulatory Strategy (familiarity)	Unclear pathway, no regulatory expertise	Some precedents or support	Clear path, experienced advisors
Operational Feasibility	Clinical Trial Experience	No experience in indication	Academic experience	Expert team with GT trial record
Operational Feasibility	Eligible Patient Identification	Hard to identify/recruit patients	Registries in development	Well-defined and reachable cohort
Operational Feasibility	Advocacy & Foundation Engagement	No known support	Some awareness	Active support from advocacy/foundations
Operational Feasibility	Market Differentiation	Crowded space, redundant mechanism	Moderately differentiated	Novel MOA or first-in-class
Commercial Readiness	IP Clarity & Risk	High IP risk, unclear ownership	Partial IP coverage	Clear ownership, FTO secured
Commercial Readiness	Funding Availability	No funding identified	Some investment or grant support	Fully backed or partnered
Commercial Readiness	Market & Product Insight	No market data or use case	Limited TAM/SAM info	Well-researched, strong market fit
FUS Suitability	Size of Targeted Delivery Region	Widespread CNS (no specific brain regions)	Regional delivery emphasis but not strictly localized	Transgene expression required in small number of well-defined brain regions
FUS Suitability	Compatibility of Vector Design	Capsid not suitable for systemic delivery and/or not validated with FUS + IV delivery	Moderate compatibility with systemic delivery	Capsid compatible with systemic delivery and/or validated preclinically with FUS

GLOSSARY

Term	Definition	Source
510(k) Premarket Notificaiton	A regulatory submission demonstrating that a new medical device is “substantially equivalent” to a legally marketed predicate device. This is the most common FDA clearance pathway for Class II medical devices.	FDA, 21 CFR 807 Subpart E
Acoustic emissions	The sound waves generated by oscillating microbubbles; analyzed to differentiate modes of cavitation.	
Adeno-associated virus (AAV)	A small, replication-deficient virus commonly used as a vector in gene therapy due to its low immunogenicity and long-term gene expression.	
Antisense oligonucleotide (ASO)	A short, synthetic strand of nucleotides designed to bind RNA and modulate gene expression, typically by promoting degradation or modifying splicing.	
Biodistribution	The distribution of a therapeutic agent throughout the body or within specific tissues following administration. For gene therapy, assessing biodistribution helps evaluate targeting efficiency and off-target expression.	
Biologics License Application (BLA)	A regulatory submission seeking approval to market a biologic product (e.g., AAV-based gene therapy) in the U.S.	
Biomarker-based endpoint	A clinical trial endpoint that relies on a quantifiable biological marker (e.g., CSF protein level, MRI signal change) to indicate treatment effect.	

Biomarkers	Quantifiable biological indicators used to assess disease presence, progression, or therapeutic response.	
Blood-brain barrier (BBB)	A selective physiological barrier formed by endothelial cells lining CNS blood vessels. It restricts passage of most macromolecules, limiting drug and gene delivery to the brain. Targeted BBB disruption via FUS enables delivery of therapeutics otherwise excluded.	
Breakthrough Device Designation	An FDA designation for devices that provide more effective treatment or diagnosis of life-threatening or irreversibly debilitating diseases. Provides prioritized review and interaction to speed development.	FDA, Breakthrough Devices Program Guidance, 2018
Cassette	The full expression construct in the vector, including the promoter, gene, and regulatory elements.	
Cavitation	The formation, growth, and collapse of gas bubbles in a medium exposed to ultrasound. In FUS, stable or inertial cavitation of intravenously administered microbubbles is used to transiently open the BBB. Cavitation dynamics influence safety and delivery efficacy.	Chen & Konofagou, Phys Med Biol. 2014.
Chemistry, Manufacturing, and Controls (CMC)	A regulatory section of an IND or BLA detailing how the gene therapy product is made, tested, and stored.	
Combination Product	A product that combines a drug, device, and/or biologic, regulated based on its primary mode of action (PMOA). In FUS-mediated gene therapy, the combination of a FUS device with a gene therapy vector (e.g., AAV) may be regulated as a combination product, with lead review by CBER (biologic) or CDRH (device), depending on PMOA.	FDA Office of Combination Products
Convection-enhanced delivery (CED)	A method of direct intraparenchymal infusion using a pressure gradient to distribute therapeutics through brain tissue. Often guided by neuronavigation to reach deep brain structures.	

CRISPR/Cas9	A genome editing system used for precise gene knockout, correction, or insertion; delivered via viral or non-viral vectors.	
DaTscan	A radiopharmaceutical-based imaging technique that uses single-photon emission computed tomography (SPECT) to visualize dopamine transporter (DaT) density in the striatum. DaTscan is used to aid in the diagnosis of Parkinsonian syndromes by detecting presynaptic dopaminergic deficits and can serve as a surrogate marker for dopaminergic neuron integrity in clinical trials. Approved by the FDA and EMA for use in evaluating patients with suspected Parkinsonian syndromes.	Booij et al., Eur J Nucl Med Mol Imaging. 2007
Disease-modifying therapy	A treatment that alters the underlying biology of a disease, slowing or halting its progression rather than merely alleviating symptoms.	
Dominant-negative	A mutant protein interferes with the function of the wild-type (normal) version	
Dose-escalation study	A clinical trial design where successive cohorts receive increasing doses to evaluate safety and identify maximum tolerated dose.	
Durability of expression	How long the transgene product is expressed after delivery. Important for assessing long-term efficacy and dosing strategy.	
Ex vivo gene therapy	A technique in which cells are harvested from a patient, genetically modified outside the body (typically using viral vectors), and then reintroduced into the patient. Commonly used in hematopoietic stem cell and CAR-T therapies.	Source: High & Roncarolo, Nat Rev Genet. 2019.
First-in-human (FIH) Trial	The initial clinical trial in which a novel therapy is administered to humans. Often designed for safety, dose-escalation, and early efficacy readouts.	

Fluorodopa Positron Emission Tomography (FDOPA PET)	An advanced imaging technique using [18F]-fluorodopa to assess presynaptic dopaminergic synthesis capacity. Quantifies functional integrity of nigrostriatal terminals. Used in clinical trials to track disease progression or measure target engagement after gene therapy (e.g., AAV-hAADC, AAV-GDNF).	
Gain-of-function	A mutation that causes a protein to be more active, acquire a new function, or become toxic	
Gene editing	A method for directly modifying the DNA sequence within a cell's genome. Tools include CRISPR/Cas9, TALENs, and zinc finger nucleases. Gene editing may be used to correct mutations, knock out genes, or insert therapeutic sequences.	Cox et al., Nat Med. 2015.
Gene replacement therapy	A therapeutic approach involving the delivery of a functional copy of a gene to compensate for a defective or missing gene in a patient. Typically used for monogenic disorders.	FDA CBER; Ginn et al., 2018.
Gene silencing	A strategy aimed at reducing or abolishing the expression of a specific gene. Approaches include antisense oligonucleotides (ASOs), siRNA, shRNA, and CRISPR-based repression systems.	Khorkova et al., Mol Ther Nucleic Acids, 2021.
Gene therapy program	A structured development effort, often within a commercial or clinical framework, focused on advancing a specific gene therapy candidate through preclinical, regulatory, and clinical stages toward approval and commercialization.	
Good Laboratory Practice (GLP)	A quality standard for non-clinical safety studies that are intended to support regulatory submissions. Required for toxicology studies in large animal models.	
Idiopathic	Relating to any disease which arises spontaneously or for which the cause is unknown	

Immunogenicity	The potential of a gene therapy vector, transgene product, or delivery method to trigger an immune response. Impacts safety, redosing potential, and long-term efficacy.	
In vivo gene therapy	A therapeutic approach in which genetic material is delivered directly into the patient's body, typically via systemic or local injection, to target cells within the organism. Delivery vehicles such as viral vectors or nanoparticles are used to transfer the gene to affected tissues without removing cells from the body.	Ginn et al., Gene Ther. 2018.
INTERACT Meeting	An early regulatory interaction with FDA CBER to discuss preclinical strategy and study design before initiating IND-enabling studies for gene therapies.	
Intra-arterial (IA)	A delivery route in which therapeutic agents are infused directly into an artery.	
Intra-cerebroventricular (ICV)	Delivery of agents into the cerebral ventricles, allowing distribution via cerebrospinal fluid. Used in certain AAV and ASO delivery studies.	
Intra-cisterna magna (ICM)	Injection into the cisterna magna, a CSF-filled cavity near the base of the brain. Enables CNS-wide distribution with less systemic exposure than IV delivery.	
Intra-parenchymal (IP)	Direct injection of therapeutics into brain tissue. Provides high local concentrations but is invasive and limited in spatial coverage.	

Intranasal (IN)	An investigational non-invasive delivery route through the nasal cavity, enabling increased BBB crossing abilities . Has limited payload capacity and uncertain targeting efficiency.	
Intrathecal (IT)	Injection into the spinal canal (lumbar region), enabling CSF-mediated distribution of therapies throughout the CNS. Used in approved ASO therapies (e.g., nusinersen).	
Investigational Device Exemption (IDE)	An FDA regulatory mechanism that permits the use of an investigational device in a clinical study to collect safety and effectiveness data. Required prior to initiating a clinical trial if the device is considered “significant risk” and not yet approved for the intended use.	FDA, 21 CFR 812
Investigational New Drug (IND)	A regulatory application submitted to the FDA to begin clinical testing of a novel therapy in humans. Pre-IND or INTERACT meetings guide IND-enabling studies.	
Loss-of-function	A mutation that reduces or eliminates the protein’s normal function	
Mendelian mutation	A mutation that follows classic inheritance patterns (dominant or recessive) and reliably causes disease in carriers	
Microbubbles	Gas-filled, lipid- or protein-shelled microspheres used as ultrasound contrast agents. When exposed to focused ultrasound, they oscillate (cavitate), enabling transient BBB opening.	

microRNA (miRNA)	Endogenous, non-coding RNAs (~22 nucleotides) that regulate gene expression post-transcriptionally by binding to mRNA and promoting degradation or inhibiting translation.	
Modifier effect	A gene that alters the expression, severity, or onset of a disease caused by another gene	
Monogenic mutation	A mutation in a single gene that causes a disease.	
Movement Disorder Society's Unified Parkinson's Disease Rating Scale (MDS-UPDRS)	A standardized, clinician-administered tool used to assess the severity and progression of Parkinson's disease. It is the most widely used outcome measure in PD clinical trials and gene therapy studies and has been validated for sensitivity to therapeutic response and disease progression.	Goetz et al., Mov Disord. 2008.
MRI-guided focused ultrasound (MRgFUS)	A platform that combines MRI imaging with focused ultrasound to deliver thermal or mechanical energy to brain targets under real-time image guidance. Used clinically in essential tremor and under investigation for BBB opening.	
Natural History Studies	Long-term observational research that tracks how a disease progresses in individuals without any experimental treatment. These studies aim to define the typical clinical course, variability, and biological markers of a condition over time.	
Neuronavigation	A computer-assisted surgical guidance system that integrates preoperative imaging (e.g., MRI or CT) with real-time tracking to localize surgical instruments or delivery catheters. Commonly used in stereotactic neurosurgery and CED. Emerging applications include co-registration of FUS devices for targeted BBB opening.	

Neurotrophic factor	A class of proteins (e.g., GDNF, BDNF) that support neuron survival and regeneration. Often used as gene therapy payloads in neurodegenerative disease.	
Non-disease-modifying therapy	A treatment that provides symptomatic relief without affecting the underlying cause or progression of the disease.	
Non-Motor Symptom Scale (NMSS)	A comprehensive rating scale used to evaluate non-motor symptoms in Parkinson's disease across multiple domains, including sleep/fatigue, mood, gastrointestinal, urinary, cardiovascular, sexual function, and cognition. Each symptom is rated based on severity and frequency.	Chaudhuri et al., Mov Disord. 2007
Passive cavitation detection (PCD)	Real-time monitoring of acoustic emissions from cavitating microbubbles to assess safety and efficacy of FUS-induced BBB opening.	
Payers and Providers	Payers refers to entities that finance or reimburse the cost of healthcare. This includes public insurers (e.g. Medicare and Medicaid) and private health insurance companies (e.g. Cigna Healthcare, Aetna). Providers refers to those who deliver medical care to patients, including hospitals, clinics, physicians and specialists.	
Penetrance	The proportion of individuals with a mutation who actually develop the disease. Incomplete penetrance refers to when only a portion of mutation carriers develop the disease.	

Phased-array transducer	An ultrasound array whose elements can be electronically controlled to steer and shape the beam without mechanical movement	
Preclinical gene therapy candidate	A gene therapy approach in the research phase, typically tested in vitro or in animal models, prior to filing an IND or entering clinical trials.	
Premarket Approval (PMA)	The most stringent FDA device review pathway, required for Class III medical devices that support or sustain human life, are of substantial importance in preventing impairment of health, or present potential unreasonable risk of illness or injury. Requires full demonstration of safety and effectiveness, often including clinical data.	FDA, 21 CFR 814
Promoter	A DNA sequence upstream of the transgene sequence that regulates transcriptional activity; may be ubiquitous (e.g., CMV) or cell-specific (e.g., Synapsin for neurons).	
Rare diseases	Diseases that affect fewer than 200,000 individuals in the United States (as defined by the U.S. Orphan Drug Act). Despite low prevalence, many rare diseases are caused by monogenic mutations, making them attractive targets for gene therapy.	
Regenerative Medicine Advanced Therapy (RMAT) Designation	An FDA designation granted to eligible regenerative medicine products—including cell and certain gene therapies—that are intended to treat, modify, reverse, or cure a serious or life-threatening disease or condition. RMAT provides sponsors with expedited development tools similar to Breakthrough Therapy designation, including early and frequent FDA interaction, potential eligibility for priority review, and the ability to use surrogate or intermediate clinical endpoints for accelerated approval.	FDA Guidance for Industry: Expedited Programs for Regenerative Medicine Therapies for Serious Conditions, 2019
Route of administration (ROA)	The path by which a gene therapy or drug is delivered into the body.	

Serotype	A classification of adeno-associated virus (AAV) based on differences in the viral capsid proteins that influence tropism, transduction efficiency, and immune recognition. AAV serotypes exhibit varying affinities for specific tissues or cell types. Serotype selection is a key determinant in designing AAV-based gene therapies, affecting biodistribution, cellular uptake, and species compatibility.	
short hairpin RNA (shRNA)	Artificially designed RNA molecules that fold into a hairpin structure, used to induce RNA interference (RNAi) for gene silencing. Typically delivered via viral vectors.	
small interfering RNA (siRNA)	Double-stranded RNA molecules that promote degradation of complementary mRNA sequences, resulting in gene knockdown. Used in both research and clinical settings.	
Target coverage	In CNS delivery, the volumetric proportion of a target structure (e.g., putamen) that successfully receives the gene therapy payload.	
Target engagement	Evidence that a therapeutic has successfully reached and interacted with its intended molecular or cellular target. Important for proof-of-mechanism studies.	
Transduction	The process by which a viral vector enters a target cell, uncoats, and delivers its single-stranded DNA genome to the nucleus.	Wang et al., Nat Rev Drug Discov. 2019
Transduction Efficiency	A measure of how effectively a gene delivery vector introduces genetic material into target cells and achieves functional expression.	

Transgene	The gene that is delivered into the host cell via a vector; encodes the therapeutic protein or RNA.
Tropism	The natural or engineered preference of a vector (e.g., AAV serotype) for transducing particular cell types or tissues. Critical for optimizing delivery and minimizing off-target effects.
Ultrasound-guided focused ultrasound (USgFUS)	A non-MRI-guided approach to focused ultrasound that uses ultrasound imaging hardware (e.g., linear or phased arrays) to both monitor and deliver therapeutic ultrasound. Allows real-time visualization, cavitation monitoring, and potentially lower-cost, bedside-compatible treatment.
Vector	A vehicle for delivering genetic material into cells. Vectors may be viral (e.g., AAV, lentivirus) or non-viral (e.g., lipid nanoparticles).

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