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Building CRISPR Gene Therapies for the Central Nervous System
A Review

Sally E. Salomonsson, PhD; Claire D. Clelland, PhD, MD

IMPORTANCE Gene editing using clustered regularly interspaced short palindromic repeats (CRISPR) holds the promise to arrest or cure monogenic disease if it can be determined which genetic change to create without inducing unintended cellular dysfunction and how to deliver this technology to the target organ reliably and safely. Clinical trials for blood and liver disorders, for which delivery of CRISPR is not limiting, show promise, yet no trials have begun for central nervous system (CNS) indications.

OBSERVATIONS The CNS is arguably the most challenging target given its innate exclusion of large molecules and its defenses against bacterial invasion (from which CRISPR originates). Herein, the types of CRISPR editing (DNA cutting, base editing, and templated repair) and how these are applied to different genetic variants are summarized. The challenges of delivering genome editors to the CNS, including the viral and nonviral delivery vehicles that may ultimately circumvent these challenges, are discussed. Also, ways to minimize the potential in vivo genotoxic effects of genome editors through delivery vehicle design and preclinical off-target testing are considered. The ethical considerations of germline editing, a potential off-target outcome of any gene editing therapy, are explored. The unique regulatory challenges of a human-specific therapy that cannot be derisked solely in animal models are also discussed.

CONCLUSIONS AND RELEVANCE An understanding of both the potential benefits and challenges of CRISPR gene therapy better informs the scientific, clinical, regulatory, and timeline considerations of developing CRISPR gene therapy for neurologic diseases.

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Gene therapies based on clustered regularly interspaced short palindromic repeats (CRISPR) hold great promise for curing not only diseases caused by genetic variants but also acquired viral, bacterial, and oncological diseases. Most CRISPR-based gene editing uses CRISPR-associated protein 9 (Cas9), a protein derived from the bacterial adaptive immune system, to cut DNA.[1,2] What makes the CRISPR system so powerful is that pairing Cas9 with a single guide RNA (gRNA) homes the endonuclease to a specific genomic site with single-nucleotide fidelity.[2]

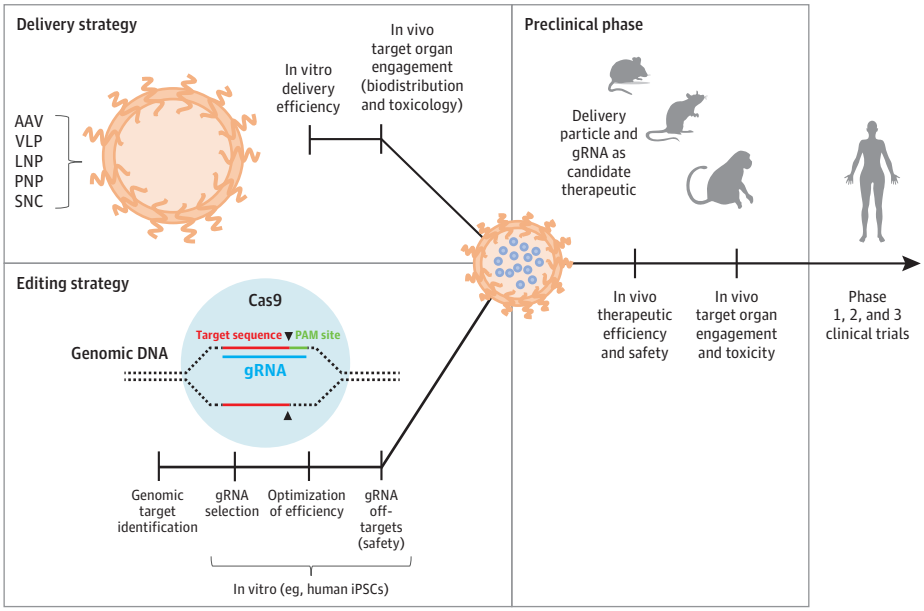
Although CRISPR gene therapies have been in clinical trials since 2016 for cancer, blood disorders, and liver disorders,[3-6] no clinical trials, to our knowledge, are currently recruiting or ongoing for central nervous system (CNS) disorders because of the unique and significant challenges involved. These challenges include delivering CRISPR reagents across the protective blood-brain barrier (BBB), achieving proper distribution in the brain parenchyma, and avoiding neurotoxic effects due to the CRISPR cargo or its packaging, alongside uncertainties around best practices for the preclinical assessment of the risk for off-target genome editing in vivo. The traditional pipeline to bring novel therapies from conception through cell and animal studies to human trials is depicted in **Figure 1**.

Herein, we review the state of progress of in vivo CRISPR therapeutics and lay out a map of roadblocks and milestones for their successful development for neurologic diseases. Furthermore, we delineate the methodology and limitations of classic CRISPR (herein defined as the canonical ribonucleoprotein complex of Cas9 and gRNA) in the clinical setting and contrast it with newer programmable CRISPR approaches, such as base and prime editing. Our goal is to outline the clinical relevance of the rapidly evolving scientific landscape, previewing the roadmap for successful CNS CRISPR therapeutics.

The New Landscape of In Vivo Gene Editing Strategies

CRISPR-based editing strategies have largely replaced their gene editing predecessors, such as transcription activator–like effector nucleases and zinc fingers, because they are more efficient, less costly, and easier to redesign and deploy against novel targets.[7] CRISPR editing also builds on advances in transient gene disruption or silencing approaches, such as small interfering RNAs and antisense oligonucleotides, which provide important proof-of-concept data that genetic targeting can treat disease[8,9] but typically require redosing and may increase the risk of immune response and chemical meningitis over a lifetime of therapy. CRISPR-based strategies are dis-

Figure 1. Overview of the Development Pipeline for In Vivo Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Therapeutics



The candidate therapeutic for preclinical studies contains the selected CRISPR gene editing cargo, packaged in a particle with suitable properties for safe and efficient delivery to the target organ. Both the cargo and the particle undergo thorough testing and optimization before the preclinical phase. In classic CRISPR editing, CRISPR-associated protein 9 (Cas9) is directed to a specific genomic site by a guide RNA (gRNA) binding to a unique sequence in the target gene, where it makes a double-stranded DNA break. This type of editing poses the risk of unintended insertions or deletions at other genomic loci with similarity to the target sequence. Since such off-target editing may cause cancer or other abnormalities, safety concerns are addressed at all stages of development. First, candidate gRNAs ought to be validated for minimal off-target events by 2 independent genome-wide sequencing methods. Second, the delivery particle should ideally be broken down through endogenous mechanisms after editing has occurred to avoid integration and continuous expression of the gene editing complex. Moreover, the immunogenicity of the particle is a particular concern with virus particles or viruslike particles (VLPs). Beyond the safety aspect, editing and delivery efficiencies are considerable obstacles at all stages of development.
AAV indicates adeno-associated virus; iPSCs, induced pluripotent stem cells; LNP, lipid nanoparticle; PAM, protospacer adjacent motif; PNP, polymeric nanoparticle; SNC, silica nanocapsule.

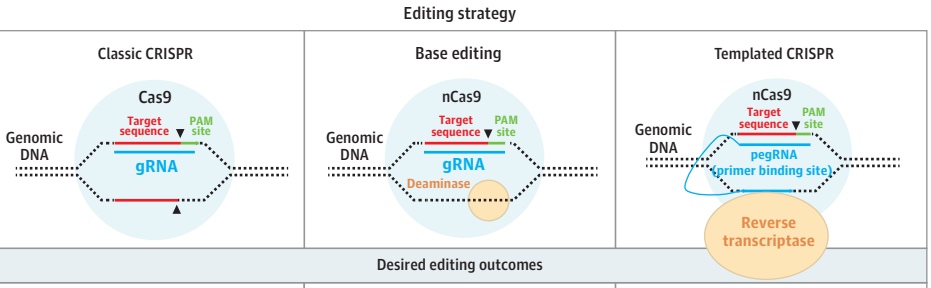
tinct in that they hold promise as a long-term and curative therapy after a single dose.

For in vivo gene editing, a critical concern is to make a desired change at the genomic target (on-target editing) without inducing changes at other genomic loci (off-target editing). In this regard, classic CRISPR editing has inherent limitations that raise safety concerns and reduce its effectiveness in postmitotic cells. First, classic CRISPR hinges on the presence of a 2- to 6-nucleotide sequence (protospacer adjacent motif) immediately adjacent to the gRNA's target sequence for the DNA to be cut at the desired site.[2] Many desirable editing sites do not have an adjacent protospacer adjacent motif. This restricts the number of candidate cut sites within a target gene, in some cases leaving only suboptimal sites that have a high risk of off-target editing or are located far from the nucleotide(s) to edit. Second, classic CRISPR relies on the cell's DNA repair pathways to reseal the DNA after a Cas9-induced cut. Although the cut itself is highly controlled, the repair is not. Repair can involve a variety of mechanisms (eg, nonhomologous end joining, homology-directed repair, or microhomology-mediated end joining),[10] leading sometimes to the desired outcomes (for instance, the inactivation of a disease-causing allele) and other times to deleterious events (for instance, chromosomal rearrangements).[11-13] Last, repair mechanisms vary across cell types. For example, postmitotic cells, such as neurons, are unlikely to carry out the more accurate homology-directed repair.[14,15] Recent advances using modifications to the Cas9-gRNA complex may overcome these limitations of classic CRISPR (**Figure 2**).

Base editing and templated CRISPR approaches opened the possibility of correcting genes rather than just silencing or removing them. Importantly, base editing and some forms of templated CRISPR do not make double-stranded DNA breaks like classic CRISPR does but instead nick 1 strand, in theory increasing the editing precision as off-target single-stranded breaks are repaired with high fidelity.[16-19] Base editing allows single–base-pair conversions.[18] Templated forms of CRISPR, namely prime editing,[16] twin prime editing,[19] and programmable addition via site-specific targeting elements,[17] allow insertions and deletions with higher precision than classic CRISPR, in part because they use a DNA template to instruct the cell how to repair the cleaved DNA. The template is produced by a reverse transcriptase integrated into the gRNA. Some forms of templated repair can facilitate very large insertions (up to 36 000 base pairs to date).[17]

Still, both base editors and templated CRISPR strategies have problems that limit their clinical utility. Base editors are relatively promiscuous and can cause off-target editing throughout the genome.[20-22] Prime editing has been prone to undesired insertion-deletion formation across the genome.[23] Both can induce conversions from single-stranded DNA breaks to double-stranded breaks,[19]

Figure 2. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Editing Strategies



Type of variant	Examples	Desired editing outcomes		
Recessive	*Frataxin* (FA) *CLN* (BD) *Parkin* (PD) *CSTB* (EPM1)	1. Double-stranded break Deletion of 1 to millions of bp 2. With optional donor DNA repair template GTACCATCAGGTTAGTCATGCG CATGGTAGTCCAATCAGTACGC Insertion of new sequence	From C to T Precise single-base conversion	1. Precise deletion of 1 to millions of bp 2. GTACCATCAGGTTAGTCATGCG CATGGTAGTCCAATCAGTACGC Insertion of new sequence 3. A T Precise single-base conversion
Dominant loss of function	*SMN1* (SMA) *GRN* (FTD) *TARDBP* (ALS) *HTT* (HD)			
Dominant gain of function	*MAPT* (FTD) *TARDBP* (ALS) *HTT* (HD)			
Limitation and notes		NHEJ: high risk of unintended edits (insertions and/or deletions) HDR: low integration efficiency in postmitotic cells like neurons HITI: low integration efficiency	Limited to specific bases: C to T, T to C, G to A, or A to G	PE: cannot make large insertions or deletions (≤40-bp insertions and ≤80-bp deletions) TwinPE and PASTE: can make large insertions but are not optimized for in vivo delivery

Several notable advancements have improved the gene editing efficiency and fidelity of CRISPR. Base editing allows correction of disease-causing point mutations or therapeutic modulation of gene expression. The templated forms of CRISPR can be used for precise deletions (prime editing [PE], twin prime editing [twinPE], and programmable addition via site-specific targeting elements [PASTE]), large insertions (twinPE and PASTE), and single-base conversions (PE, twinPE, and PASTE). Importantly, base editing and templated CRISPR strategies do not cause double-stranded DNA breaks, thus potentially reducing the risk of unintended edits and DNA damage compared with classic CRISPR. Templated CRISPR can be used to inactivate an allele carrying a dominant gain-of-function variant, correct a point mutation, replace a defective part of a gene, or upregulate a compensatory gene. ALS indicates amyotrophic lateral sclerosis; BD, Batten disease; bp, base pair; Cas9, CRISPR-associated protein 9; EPM1, progressive myotonic epilepsy type 1; FA, Friedrich ataxia; FTD, frontotemporal dementia; gRNA, guide RNA; HD, Huntington disease; HDR, homology-directed repair; HITI, homology-independent targeted insertion; nCas9, CRISPR-associated protein nickase; NHEJ, nonhomologous end joining; PAM, protospacer adjacent motif; PD, Parkinson disease; pegRNA, prime-editing guide RNA; SMA, spinal muscular atrophy.

which may lead to no more precise outcomes than classic CRISPR and potential genotoxic effects. Moreover, the complex required for templated CRISPR is too large for the available in vivo delivery systems.[24] Last but not least, neither base editing nor templated CRISPR has so far proven more efficient than classic CRISPR in postmitotic cells.

The target genes also present their own challenges. In particular, many diseases are caused by different variants at different locations within the same genetic locus. A strategy that aims to correct these variants would require different gRNAs for different patients, a costly proposition as each gRNA needs to be thoroughly tested before clinical use.

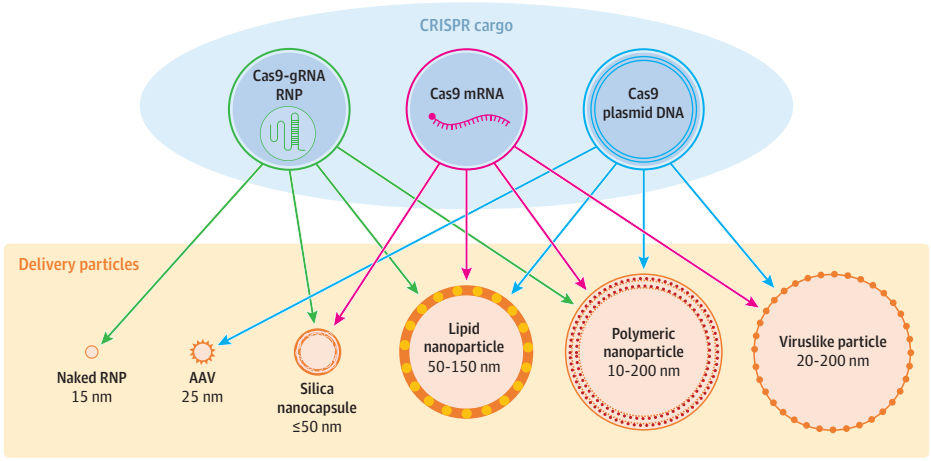
Instead, most researchers aim for broader strategies. For example, for dominant genetic variants leading to toxic gain of function, a small cut that knocks out the allelic variant but leaves the other allele intact might be made. Loss-of-function variants, on the other hand, may benefit from a strategy that upregulates a compensatory gene or the normal allele if the variation is heterozygous or that turns on a silenced gene (CRISPR activation).[25,26] A notable example of such a broadly applicable CRISPR editing strategy is reactivation of fetal hemoglobin for the treatment of both sickle cell disease (caused by 1 specific variation in the adult hemoglobin gene) and β-thalassemia (caused by several variants in the adult gene).[27] Reactivation of the fetal gene is achieved by silencing its repressor (*BCL11A*) via CRISPR.[6]

Challenges and Solutions for CNS Delivery

Challenge 1: Crossing the BBB and Achieving Brain Biodistribution

After selecting the CRISPR editing strategy, the next step is to pair the optimal Cas9 and gRNA combination with a suitable delivery particle. The particle must shuttle the CRISPR cargo to the target organ effectively and protect it from premature degradation. Achieving sufficient CRISPR cargo biodistribution in the CNS is arguably the greatest present hurdle toward applying CRISPR gene editors for neurologic diseases. Of all possible routes of administration, transdermal, enteric, and intramuscular are unlikely to allow enough particles to reach the CNS. Injection into brain tissue is invasive, risks surgical adverse effects, and does not guarantee effective biodistribution.[28] Delivery into the cerebrospinal fluid would achieve high cargo concentration but currently has limited distribution within the CNS[29,30] and would increase the cost of CRISPR therapy. Intra-

Figure 3. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Cargo Formats and Potential Modes of Delivery to the Nervous System



The CRISPR machinery can be packaged as plasmid DNA, messenger RNA (mRNA), or preassembled CRISPR-associated protein 9 (Cas9)–guide RNA (gRNA) ribonucleoprotein (RNP). RNPs can be delivered naked, but other cargo formats rely on a delivery particle to enter target cells. The size and biophysical properties of a potential delivery particle determine what types of cargo it can hold (arrows). Potential delivery particles for CRISPR therapeutics belong to 2 broad classes: (1) virus or viruslike particles and (2) engineered nanoparticles. Adeno-associated virus (AAV) is one of the most well-researched viral delivery particles and has been used in gene therapy clinical trials, eg, for spinal muscular atrophy. However, AAVs have a relatively small cargo capacity and a propensity to integrate and cause immunogenicity. Viruslike particles bypass some of these limitations, although more preclinical research is needed. Lipid nanoparticles have been successfully used for delivery of SARS-CoV-2 mRNA vaccines and show a relatively good safety profile due to degradation after cargo release. Nanoparticles, including lipid nanoparticles and silica nanocapsules, share the potential for surface modification with targeting ligands or stealth coatings, which can facilitate blood-brain barrier passage or alter circulation time and biodistribution. Polymeric nanoparticles can be made of a range of cationic nanomaterials, including poly-L-lysine, poly-L-ornithine, chitosan, and gelatin.

venous administration is attractive because it is inexpensive and accessible worldwide. However, particles must pass the BBB.

To become a BBB-crossing therapeutic, a particle must fulfill several criteria. First, the particle must be small enough (approximately ≤60 nm)[31-33] to cross the BBB, be distributed within the brain parenchyma, and reach remote CNS structures. Second, it must avoid rapid sequestration by the liver.[34] Third, it must escape degradation on its journey through the blood, the endothelial-pericyte-astrocyte system, the brain parenchyma, and the target cells' endolysosomal system so that it enters the cell's nucleus with its CRISPR cargo functionally intact.

CRISPR delivery approaches to the CNS have so far focused on viral particles and nanoparticle encapsulation strategies (**Figure 3**). While viral particles, such as adeno-associated virus (AAV), have been used in clinical trials for gene replacement, recent safety concerns have dampened enthusiasm for their use. The most widely used clinical-grade nanoparticles are lipid nanoparticles, such as those used for the SARS-CoV-2 messenger RNA vaccines.[35-37] Lipid nanoparticles have proven to be safe and effective at delivering CRISPR machinery in clinical trials targeting the liver[5] but have not yet been successfully engineered to cross the BBB, in part because of their larger size (approximately 100 nm). This large size may nevertheless be one of their main advantages, as it allows for larger cargo. Their other advantage is that they are readily degraded by the body after cargo release,[38] which limits the risk of off-target edits and immune response to the bacterial Cas9. Other forms of nanoparticles have been manufactured that pass the BBB in animal studies thanks to their small size, their positive charge, or their decoration with ligands engineered for receptor uptake.[39,40] The efficacy and safety of novel viral particles and nanoparticles, both in vitro and in vivo, are ongoing and promising avenues of research.

Challenge 2: Risks of Toxic Effects With CRISPR and Delivery Particles

While AAV is the gene therapy vehicle most used in the clinic, it poses significant risks. At least 13 deaths have been reported from 1999 to 2023 due to high-dose AAV for diseases such as ornithine transcarbamylase deficiency, *SOD1*-associated amyotrophic lateral sclerosis, myotubular myopathy, and Duchenne muscular dystrophy.[41-43] Recently, nonfatal liver damage, kidney failure, and cardiopulmonary failure were attributed to AAV vector toxic effects in clinical trials for Duchenne muscular dystrophy and spinal muscular atrophy.[44] The risk increases at higher doses,[44] which is particularly galling for CNS targets since they require high systemic doses to achieve adequate local dose. With AAV delivery of CRISPR to the CNS, a big concern is the persistent expression of the CRISPR-associated (Cas) enzyme off the AAV vector after cell entry.[45,46] Persistence of the Cas enzyme could lead to the accumulation of off-target genomic editing over time,[47] leading to cell death or increasing cancer risk.

Synthetic particles, such as lipid nanoparticles or polymer particles, may prove safer and tolerable at higher, more effective doses than AAV particles. In addition, in such particles, the Cas enzyme is delivered as protein or RNA, which readily degrades within cells within days, in contrast to viral DNA, which is expected to persist for years.[48-50] Such transient delivery is attractive as the Cas enzyme is present only long enough to make the desired edit. However, nanoparticles are much larger than AAV (Figure 3), which inhibits their BBB penetrance and brain distribution. While there is significant enthusiasm for engineering smaller nanoparticle technologies, they are not yet ready for CNS clinical trials.

Another impetus for transient Cas delivery is to limit the immune response to expression of a bacterial protein. Approximately 80% of people appear to have antibodies against the common bac-

teria *Staphylococcus aureus* and *Streptococcus pyogenes*, from which the most common Cas variants are derived, and T cells against the 2 most common Cas variants have recently been detected in human donor serum samples.[51] An immune response neutralizing Cas is more likely to render the treatment inactive than to cause overt toxic effects, although an immune response to a cell harboring the Cas protein could lead to the killing of the very cells targeted for therapy.[52] To date, data from clinical trials and from animal models suggest that transient Cas delivery is well tolerated without appreciable immune response.[5,53,54] Still, screening for a Cas-induced immune response prior to CRISPR therapeutic treatment may identify individuals at risk for an adverse event.

Genotoxic events are a serious concern with CRISPR editing. These events include small changes at off-target genomic loci or even large gene rearrangements or rare chromosomal breakage.[11-13] They could alter normal cellular function or even cause cell death if DNA damage exceeds the cell's repair capacity. The bigger concern is that rare off-target editing at a proto-oncogene or tumor-suppressor gene could trigger a cancer or that DNA damage induced by CRISPR would hasten a cancer in cells predisposed to it (such as those lacking tumor protein p53).[55] An unresolved question in the field is how off-target editing risk should be measured at the preclinical stage. Animals have traditionally been used to derisk new therapies before clinical trials. However, preclinical animal models cannot accurately predict off-target events in human patients because of sequence differences between human and animal genomes. Instead, bioinformatic and in vitro assays in human cells have been developed to quantify the risk of off-target events[56-60] and new quantification tools are rapidly evolving, although there is no consensus over best practices.

Despite the risks inherent to CRISPR gene editing and delivery, CNS CRISPR therapeutics are currently being developed for the most debilitating, chronic, and fatal diseases, and the field is rapidly evolving new models and tools to measure and mitigate known and theoretical risks. Thus, once a therapeutic passes preclinical biosafety assessments, the benefits may outweigh the risks for patients eligible for clinical trial participation.

Clinical Trials: Promise of CRISPR Therapies for Blood and Liver Disorders

Currently, to our knowledge, no CRISPR-based therapeutics have reached approval by the US Food and Drug Administration (FDA) and no CNS-targeting CRISPR-based clinical trials have been announced. However, promising clinical trials for cancer, blood disorders, and liver disorders demonstrate the safety and efficacy of CRISPR.[5,6,61,62] Most CRISPR clinical trials to date target cancers and blood disorders because these diseases are relatively common, severe, and suitable for ex vivo CRISPR editing, which bypasses the delivery challenges. More recent in vivo trials are targeting chronic diseases in organs accessible by lipid nanoparticles, such as the liver. Until recently, all CRISPR clinical trials had used classic CRISPR, but 3 new clinical trials use base editing: 2 ex vivo trials for sickle cell disease[63] and leukemia,[61] and 1 in vivo trial for familial hypercholesterolemia.[64] Despite promising preclinical animal studies,[17,65-67] no trials for templated CRISPR have been announced.

The curative potential of CRISPR-based therapies for genetic disease was demonstrated recently in a successful clinical trial commenced in 2018 by Vertex Pharmaceuticals and CRISPR Therapeutics for sickle cell disease and β-thalassemia.[6] The ex vivo CRISPR/Cas9 therapy exagamglogene autotemcel (exa-cel) led to clinically significant improvements in most participants and a stable proportion of edited target cells over a 12-month period. No signs of off-target editing have been detected so far. Exa-cel was approved by the FDA for the treatment of sickle cell disease in December 2023, a historic first for CRISPR gene therapy.[68] A milestone was also reached regarding the in vivo safety of CRISPR therapeutics when Intellia Therapeutics and Regeneron Pharmaceuticals shared promising interim results of the NTLA-2001 clinical trial for transthyretin amyloidosis.[5,50,69,70] More recently commenced trials of CRISPR to target HIV[71] and as a novel antibiotic[72,73] demonstrate the phenomenal potential for diverse applications of single-dose curative CRISPR therapies and the cascade of treatment possibilities that may follow once the first effective and safe CRISPR strategies and delivery particles have been approved for the clinic. Although no direct CNS editing has yet been achieved, the clinical trials for sickle cell disease and transthyretin amyloidosis, which can both result in significant neurologic disease burden, demonstrate the promise of CRISPR for neurologic diseases.

Ethical Considerations: Germline Editing

In late 2022, the FDA placed a clinical hold on the VERVE-101 in vivo base editing trial for familial hypercholesterolemia due to theoretical safety concerns, including the concern for germline editing.[74] The risk of germline editing is perhaps of greater concern to promiscuous base editors but is not unique to base editing. Rather, it is an inherent risk of most in vivo CRISPR-based therapeutics, even CNS therapies, as wayward editors could potentially edit ova or sperm.[75] Most researchers agree that germline editing, which could persist in subsequent generations, requires special consideration.[76] So far, calls for a moratorium on germline editing have been respected, save 1 clinical experiment in 2018, which resulted in international condemnation and incarceration of the lead scientist.[77,78]

The question arises as to how to address possible germline editing as an unintended consequence of somatic cell targeting. It should be noted that germline mutagenesis, which spurred the current FDA hold on VERVE-101, is a known and accepted risk of other therapeutics, such as platinum-based chemotherapies and radiation therapy for cancer.[79,80] It is possible that fatal neurologic diseases, such as genetic amyotrophic lateral sclerosis, frontotemporal dementia, and Huntington disease, will engender ethical consideration similar to that for fatal cancers. We argue that a putative curative CRISPR therapeutic should not be delayed because of unknown germline editing risk alone. However, steps should be taken to quantify and mitigate germline editing and its impact. This mitigation can include family planning counseling and offering ova and sperm freezing prior to a CRISPR treatment. Given that in vivo CRISPR clinical trials are now commencing and proceeding around the world, spurred by the urgency of curing life-threatening diseases, data on actual germline editing rates and outcomes will likely be released eventually and preclinical reports are so far encouraging.[81] Such data will allow development of safer in vivo

CRISPR therapeutics and inform future clinical practice and appropriate applications of such therapeutics.

Unique Challenges of Derisking In Vivo CRISPR Therapeutics

Beyond the risk of unintended germline editing, off-target genomic editing remains one of the most serious concerns in vivo, both within and outside the target organ. In the brain, off-target genomic editing could, in theory, nucleate glioblastoma or cause death of the cell the CRISPR therapeutic was attempting to save. Since 1938, FDA regulations had required preclinical biosafety testing in animals prior to clinical trial, although this was recently lifted.[82] Animal testing is poorly equipped to derisk human off-target genomic editing owing to the differences between genomes. That is, gRNAs are species specific. How, then, do we derisk cutting-edge, species-specific biologics such as CRISPR in the modern age?

As far as predicting off-target CRISPR edits, cell-based, biochemical, and in silico human models not only are feasible but also appear more reliable than animal models.[56-60,83] Such prediction methods are already integrated in the CRISPR therapeutic development pipeline, at both the strategy development and preclinical stages. On the other hand, animals remain good models to evaluate particle delivery and Cas enzyme expression in nontargeted organs. Such evaluations routinely determine dosing, biodistribution, and toxicity parameters for both small molecules and biologics alike. Animal models of disease may also provide insight on the efficacy of the editing strategy, although many variants and diseases lack an animal model.

Our hope is that as preclinical and clinical trials demonstrate the safety of individual CRISPR and delivery particle components, we may be able to mix and match the components without derisking each new combination through the entire preclinical and clinical trial pipeline, which is slow and expensive. For example, if a CNS-targeting delivery particle and the Cas enzyme have been thoroughly tested through clinical trial, it may be possible to safely combine them with a novel gRNA whose editing efficacy and off-target data were obtained in human cells and functional efficacy data were obtained in an animal model, without repeating years-long dose-finding, toxicity, and biodistribution studies. Such an approach may adequately evaluate risk while also accelerating the clinical testing of potentially life-saving therapies.

Conclusions

The time is right for CNS gene therapy. Promising results from ongoing clinical trials demonstrate the safety and efficacy of CRISPR for clinical use. Delivering this technology to the CNS remains the biggest barrier, with nonviral delivery approaches holding the greatest potential to meet clinical criteria for a successful CNS CRISPR therapeutic. Regulation will also need to evolve to ensure the safety of these human-specific novel therapies.

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