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CRISPR-dependent base editing as a therapeutic strategy for rare monogenic disorders

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Rare monogenic disorders are caused by mutations in single genes and have an incidence rate of less than 0.5%. Due to their low prevalence, these diseases often attract limited research and commercial interest, leading to significant unmet medical needs. In a therapeutic landscape where treatments are targeted to manage symptoms, gene editing therapy emerges as a promising approach to craft curative and lasting treatments for these patients, often referred to as "one-and-done" therapeutics. CRISPR-dependent base editing enables the precise correction of genetic mutations by direct modification of DNA bases without creating potentially deleterious DNA double-strand breaks. Base editors combine a nickase version of Cas9 with cytosine or adenine deaminases to convert C·G to T·A and A·T to G·C, respectively. Together, cytosine (CBE) and adenine (ABE) base editors can theoretically correct ~95% of pathogenic transition mutations cataloged in ClinVar. This mini-review explores the application of base editing as a therapeutic approach for rare monogenic disorders. It provides an overview of the state of gene therapies and a comprehensive compilation of preclinical studies using base editing to treat rare monogenic disorders. Key considerations for designing base editing-driven therapeutics are summarized in a user-friendly guide for researchers interested in applying this technology to a specific rare monogenic disorder. Finally, we discuss the prospects and challenges for bench-to-bedside translation of base editing therapies for rare monogenic disorders.

KEYWORDS

gene therapy, base editing, rare monogenic disease, CRISPR-based therapeutics, mutation correction

1 Introduction

The term "rare disorders" encompasses a group of ~7,000 human diseases affecting less than one in 2,000 people in any given World Health Organization region (Wang et al., 2021; The Lancet Global, 2024; National Institutes of Health, 2023). The worldwide burden is significant: Approximately 300 million people live with rare diseases, 70% of which present in childhood, and such individuals are often neglected and marginalized, especially in low- and middle-income countries (The Lancet Global, 2024).

Around 80% of rare disorders are caused by a single-gene defect, *i.e.*, monogenic (National Institutes of Health, 2023). Despite knowledge of their molecular causes, the low prevalence of rare disorders makes them difficult to recognize, accurately diagnose, and treat, attracting limited research and commercial interest (Zhang and Wu, 2024). The average time for an accurate diagnosis is 4–8 years, and about 30% of children with a rare disease die before the age of five (The Lancet Global, 2024). The limited availability of animal models and clinical specimens has further constrained preclinical studies and

translation of therapies for rare diseases, creating unmet medical needs (Hmeljak and Justice, 2019). As of today, only 500 rare disorders count with available treatments, focusing primarily on symptom management with few curative options (The Lancet Global, 2024; National Institutes of Health, 2023).

Gene therapy has emerged as a promising approach to craft curative treatments for these patients (Kirschner and Cathomen, 2020). Gene therapy modifies a person's genetic material to treat or prevent a disease (FDA, 2018). This umbrella term includes strategies like gene replacement, gene silencing, and gene editing (Zhang and Wu, 2024). While gene replacement and silencing strategies can compensate for protein loss-of-function or inhibit gain-of-function mutation, overexpressing or silencing a gene may be insufficient to correct a specific disease phenotype (Raguram et al., 2022). Gene editing therapies, including CRISPR-dependent base editing, can treat a broader range of genetic diseases, as they can directly correct pathogenic mutations in the genomic DNA. Achieving the same effects as gene replacement/silencing strategies, corrective gene editing approaches offer the potential for long-lasting effects in the form of 'one-and-done' treatments (Raguram et al., 2022).

Here, we provide a user-guide-like review of CRISPR-dependent base editing applications for treating rare monogenic disorders. We discuss current research on base editing as a therapeutic option, including recent advances and potential challenges to provide a reference for future research in this rapidly evolving field. To conclude, we elaborate on the outlook of these therapies and highlight potential actions to streamline bench-to-bedside translation.

2 Gene therapies for rare monogenic disorders: the current landscape

Gene therapies aim to directly correct or modify the genetic cause of a disease. Gene therapies offer the potential for personalized treatments with reduced side effects, leading the transition from symptom management to curative treatments for rare disorders. Gene therapy can be categorized into three distinct approaches: gene replacement, silencing, and correction, each with unique mechanisms and potential advantages (Yan et al., 2024).

Gene replacement strategies deliver an exogenous functional complementary DNA copy of a defective gene into the patient's living cells to restore normal cellular function. This approach is a good candidate for diseases caused by mutations resulting in a specific protein's absence or malfunction advantages (Yan et al., 2024; Petrich et al., 2020). For example, spinal muscular atrophy (SMA) can be treated using adeno-associated virus (AAV)-mediated gene replacement to express the functional *SMN1* gene, as demonstrated by the therapeutic onasemnogene abeparvovec-xioi (Alves et al., 2024).

Gene silencing uses small RNA or DNA molecules, i.e., antisense oligonucleotides (ASOs) or small interference RNA (siRNA), to inhibit the expression of specific genes and reduce the production of a disease-causing protein. ASO and siRNA therapies target the mRNA; they do not alter the patient's DNA, leaving the pathogenic variant uncorrected and requiring lifelong intermittent administration (Alves et al., 2024). ASOs are short oligonucleotides that bind to RNA in a target-specific manner. Their mechanisms of action involve knocking down mRNAs but also modifying pre-mRNA splicing, leading to a reduction, modification, or restoration of a specific protein (Lauffer et al., 2024). They are extremely versatile molecules and can be tailored for individual cases for disorders with only a few known cases, critical to treat rare disorders (Kim et al., 2023; Kim et al., 2019). The Food Drug Administration (FDA) has approved a total of twelve ASO drugs (Vinjamuri et al., 2024), some of which treat rare diseases like Duchenne muscular dystrophy (DMD) and SMA (Alves et al., 2024; Duan et al., 2021). siRNAs are double-stranded RNA nucleotide sequences that bind to and degrade mRNA silencing gene expression (Collotta et al., 2023). To date, the FDA has approved six siRNA-based drugs, including patisiran and lumasiran that treat the rare diseases acute hepatic porphyria and primary hyperoxaluria type 1, respectively (Padda et al., 2025).

By using CRISPR/Cas technologies like CRISPR-Cas9 or CRISPR-dependent base editors, gene editing therapy enables precise modifications to the patient's DNA to delete, replace, or insert genetic material. Noteworthily, in late 2023, exagamglogene autotemcel (Casgevy®) was the first FDA-approved cell therapy utilizing *ex-vivo* CRISPR/Cas9 technology for the treatment of sickle cell disease, a rare inherited blood disorder (Frangoul et al., 2024). Although CRISPR-based gene therapies have not yet received full regulatory approval, clinical trials are underway for diseases including neurological disorders and hypercholesterolemia (Deneault, 2024).

3 CRISPR-dependent base editing: from DNA cutting to direct mutation insertion

CRISPR-based systems are composed of a Cas protein with endonuclease activity and a single guide RNA (sgRNA) that directs the ribonucleoprotein complex to the desired site of the genome (Gaudelli et al., 2017). A protospacer-adjacent motif (PAM) sequence near the target site is required for effective DNA targeting (Hille et al., 2018). Recognition of the target site begins when the Cas-sgRNA complex binds to the matching PAM sequence unwinding the double-stranded DNA (Jiang and Doudna, 2017; Sternberg et al., 2014). The sgRNA protospacer creates an R-loop that exposes the non-target DNA strand, making it accessible to other molecules. In traditional CRISPR-Cas9 systems, Cas9 then undergoes conformational changes that activate its nuclease domains and produce a DNA double-strand break (DSB). These structural changes are hindered by mismatches between the target DNA strand and the sgRNA protospacer, limiting nuclease activation to sequences highly complementary to the sgRNA protospacer (Anzalone et al., 2020). Differentiated mammalian cells largely repair nuclease-induced DSBs through non-homologous end-joining, directly ligating DNA ends, often resulting in nucleotide insertion and deletions around the break site that abrogate gene function (Anzalone et al., 2020; Mali et al., 2013; Xu and Li, 2020; Doench et al., 2014).

While CRISPR-Cas9 is very efficient at selectively disrupting target gene sequences (Mali et al., 2013; Cong et al., 2013), CRISPR-dependent base editing can directly correct genetic mutations by

precise modification of DNA bases (Komor et al., 2016). Base editors combine an engineered Cas9 that inserts single-strand DNA breaks (nickase) fused to cytosine or adenine deaminases. Base editor-mediated mutations occur within a nucleotide activity window defined by the sgRNA, corresponding to the nucleotides in the R-loop that interact with the deaminase (Jiang and Doudna, 2017; Nishimasu et al., 2014). The activity window can be additionally influenced by differences in DNA state, such as chromatin architecture, that can vary by locus or cell type (Gaudelli et al., 2017; Komor et al., 2016). Unlike traditional CRISPR-Cas9 technologies, base editor-induced modifications do not require DSB generation, thus avoiding potential indels at the target locus, chromosomal rearrangements, and p53-driven stress responses (Komor et al., 2016).

Cytosine base editors (CBEs) employ a cytosine deaminase converting cytosines within the R-loop into uracils, which polymerases interpret as thymines (Komor et al., 2016; Komor et al., 2017). CBEs use APOBEC1 deaminase, with later versions incorporating other APOBEC family members (A3A-D, A3F-H) and other deaminases such as CDA or AID with varying kinetic parameters, nucleotide substrate preference, and editing window widths (Ma et al., 2016; Yu et al., 2020; Wu et al., 2019; Yuan et al., 2018). Cytosine deamination to uridine creates a DNA base pair mismatch from the non-deaminated strand that, upon DNA replication, results in a base replacement of the unedited strand. However, uracil is mutagenic and is often rapidly excised by uracil DNA glycosylase (UNG) (Wood, 1996; Radany et al., 2000); CBEs typically incorporate UNG inhibitor proteins (UGIs) to enhance editing efficiency by preventing reversion of the modified base (Komor et al., 2017; Wang et al., 2017). Interestingly, base transversion has been achieved through the fusion of CBEs to UNG (CGBEs) (Wang et al., 2024). UNG excises the deaminated base to generate apurinic/apyrimidinic sites that are repaired by base excision or translesion synthesis leading to a versatile nucleotide replacement (Wang et al., 2024). CGBEs incorporate C-to-A transversions in *E. coli* and C-to-G transversions in mammalian cells (Zhao et al., 2021).

Adenine base editors (ABEs) use a laboratory-evolved mutant of the TadA tRNA deoxyadenosine deaminase (TadA*) that converts adenines within the DNA R-loop to inosines, which are read as guanines by DNA polymerases (Gaudelli et al., 2017). Inosine excision in mammalian cells is less efficient than uracil excision as inhibiting MPG—the glycosylase believed to remove inosine from genomic DNA in eukaryotic cells—failed to enhance editing product purities (Gaudelli et al., 2017). TadA* editing efficiency and Cas9 compatibility were significantly improved by phage-assisted evolution, crafting the ABE8 generation, go-to editors in molecular biology laboratories and therapeutic applications alike (Gaudelli et al., 2017).

4 Testing base editing for rare monogenic disorders: what to consider?

CRISPR-dependent base editors have already been successfully applied in preclinical *in vivo* research for more than twenty-five disorders, including more than ten rare monogenic disorders, compiled in detail in Table 1 (Zhao et al., 2021).

Of note, the progress of base editing technologies in gene therapeutics has already extended beyond preclinical applications. Clinical trials are underway for therapeutics for the common monogenic disorder heterozygous familial hypercholesterolemia (VERVE-101/102) and the rare monogenic disorder alpha-1 antitrypsin deficiency (BEAM-301), with initial results from VERVE-101 showing promising efficacy profiles (Horie and Ono, 2024; Arnaoutova et al., 2024).

Can your rare monogenic disorder of interest disease be targeted with base editing-driven gene therapeutics? Here are some key considerations to explore the application of base editing in preclinical research (Figure 1).

4.1 Sequence context: PAM availability, editing window, and sgRNA design

The availability of a compatible PAM sequence is a key factor in determining whether a target can be edited with a base editor. The initial CBE and ABE variants were developed using SpCas9, which requires an NGG PAM. NGG-targeting CBEs can potentially correct ~26% of annotated pathogenic T·A-to-C·G mutations in ClinVar, while NGG-targeting ABEs could theoretically correct 28% of pathogenic G·C-to-A·T point mutations (Gaudelli et al., 2017; Komor et al., 2016; Landrum et al., 2016; Hu et al., 2018). Engineered Cas9 variants have been developed to recognize alternative PAM sequences and expand the targeting scope of base editors. The VQR, EQR, and VRER SpCas9 variants changed the PAM specificity from NGG to NGA, NGAG or NGCG, respectively (Kleinstiver et al., 2015). Non-G SpCas9s, including SpCas9-NRRH, SpCas9-NRTH and SpCas9-NRCH, collectively recognized NRNH PAMs (where R is A or G and H is A, C or T) (Miller et al., 2020). Cas9-NG/SpG relaxed the PAM restriction from NGG to NGN (Kleinstiver et al., 2015; Miller et al., 2020). SpRY, a near PAM-less SpCas9, has optimal activity on NRN PAMs and low activity over NYN PAMs (where Y is C or T) (Nishimasu et al., 2018; Walton et al., 2020). Base editors have also been assembled with Cas homologs such as SaCas9 versions and Cas12a, further expanding their targeting scope (Kleinstiver et al., 2019; Li et al., 2018; Kim et al., 2017). Together, these variants will theoretically enable CBEs and ABEs to revert ~95% of pathogenic transition mutations in ClinVar (Landrum et al., 2016).

The window of activity of base editors coincides with the R-loop nucleotides in contact with the deaminase and ranges from 5 to 10 nucleotides (Zhang and Wu, 2024). This window width may not be suitable for correcting point mutations in sequence contexts with multiple As or Cs. Mutations engineered to limit deaminase-R-loop contacts have generated editors with narrower windows of activity (e.g., F148A in TadA, and W90Y, R126E, R132E in rat APOBEC1), limiting the insertion of undesired bystander mutations (Zhou et al., 2019; Liu et al., 2020; Doman et al., 2020).

Straightforward sgRNA design can be achieved using web tools that compile information on predicted Cas9 efficiency and specificity (Konstantakos et al., 2022). Users can opt for widely used tools largely designed for knockout approaches (e.g., CRISPick, CRISPOR, and CHOPCHOP) (Doench et al., 2016; Sanson et al., 2018; Concordet and Haeussler, 2018), or more specific tools such as BE-designer, tailored to base editing experiments (Hwang et al.,

TABLE 1 *In vivo* gene editing strategies used in preclinical studies for rare monogenic disorders.

DiseaseDisease	Prevalence	Model	Key organ (cell type)	Targeted gene	Editing strategy	Editing outcome	Editor variant	Delivery method	Administration	Ref
Phenylketonuria	1/10,000	Mice	Liver (hepatocytes)	*Pah* c.835 T>C (p.F263S)	Direct mutation correction	• 10% editing after 4 weeks increasing to 25% after 26 weeks • Return of blood phenylalanine to normal levels	SaCas9KKH-BE3	Intein-based dual AAV8	Systemic (tail vein injection)	Villiger et al. (2018)
		Mice	Liver (hepatocytes)	*Pah* c.835 T>C (p.F263S)	Direct mutation correction	• 23% editing after 8 weeks using AAV • 19% editing 1 week after second LNP dose • Return of blood phenylalanine to normal levels	SaCas9KKH-BE3	Intein-based dual AAV8 and LNP encapsulating mRNA	Systemic (tail vein injection)	Villiger et al. (2021)
		Mice	Liver (hepatocytes)	*Pah* c.835T>C (p.F263S)	Direct mutation correction	• 6% editing and 32% reduced blood phenylalanine	SaCas9KKH-BE3	Intein-based dual AAV8	Systemic (facial vein injection)	Zhou et al. (2022)
		Humanized mice	Liver (hepatocytes)	*PAH* c.1222C>T (p.R408W)	Direct mutation correction	• 26% on-target editing, with 4% bystander editing	ABE8.8-SpRY	LNP encapsulating mRNA	Systemic (retro-orbital injection)	Brooks et al. (2024)
		Humanized mice	Liver (hepatocytes)	*PAH* c.1222C>T (p.R408W)	Direct mutation correction	• 19.1% to 34.6% editing, with 1.4% bystander editing	ABE8e-SpRY	Intein-based dual AAV8	Systemic (tail vein injection)	Yin et al. (2025)
Hereditary tyrosinemia type I	1/100,000	Mice	Liver (hepatocytes)	*Fah* G>A in last nucleotide of exon 8	Direct mutation correction	• 9.5% editing and restoration of mouse body weight	optimized ABE6.3	Hydrodynamic injection of DNA	Systemic (tail vein injection)	Song et al. (2020)
		Mice	Liver (hepatocytes)	*Fah* G>A in last nucleotide of exon 8	Direct mutation correction	• 12.5% editing and restoration of mouse body weight	optimized ABE6.3	LNP encapsulating mRNA	Systemic (tail vein injection)	Jiang et al. (2020)
		Mice	Liver (hepatocytes)	*Fah* G>A in last nucleotide of exon 8	Direct mutation correction	• 58.1% editing in liver tissues with minimal bystander editing.	haA3A-CBE	Intein-based dual AAV8, and LNP encapsulating mRNA	Systemic (tail vein injection)	Yang et al. (2024)
Alpha-1 antitrypsin deficiency	1-5/10,000	Mice	Liver (hepatocytes)	*PiZ SERPINA1* c.1096G>A (p.E342K)	Install a compensatory mutation (p.M374I) that stabilizes AAT protein	• 28.5% compensatory editing in the liver after 1 week, 34.3% after 12 weeks, and 27.2% after 32 weeks	BE4	LNP encapsulating mRNA	Systemic (tail vein injection)	Packer et al. (2022)

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TABLE 1 (*Continued*) *In vivo* gene editing strategies used in preclinical studies for rare monogenic disorders.

DiseaseDisease	Prevalence	Model	Key organ (cell type)	Targeted gene	Editing strategy	Editing outcome	Editor variant	Delivery method	Administration	Ref
					Direct mutation correction	• 12% editing rate in the liver after 1 week, 29% after 12 weeks, and 35.7% after 32 weeks	ngcABEvar9			
Hutchinson-Gilford progeria syndrome	<1/1,000,000	Humanized mice	Heart (vascular smooth muscle cells)	*LMNA* c.1824C > T (p.G608G)	Direct mutation correction	• 30% editing in heart, 20% in aorta • 2.4-fold increase in lifespan	ABE7.10max-VRQR	Intein-based dual AAV9	Systemic (retro-orbital or intraperitoneal injection)	Koblan et al. (2021)
		Humanized mice	Skin cells	*LMNA* c.1824C > T (p.G608G)	Direct mutation correction	• 20.8%–24.1% editing in mice skin cells	ABEmax-VQR	Transient non-integrative MS2-lentiviral particle vector system (LentiFlash®)	Systemic (intraperitoneal injection)	Whisenant et al. (2022)
Duchenne muscular dystrophy	1-9/100,000	Mice	Skeletal muscle (myofibers)	*Dmd* nonsense mutation Q>X in exon 20	Direct mutation correction	• 3.3% local editing • 17% of local myofibers stained for restored dystrophin	ABE7.10	Dual trans-splicing AAV9	Localized (intramuscular injection)	Ryu et al. (2018)
		Mice	Skeletal muscle (myofibers)	*Dmd* exon 51 deletion	Direct mutation correction	• 35% local editing • 96% of local myofibers stained for restored dystrophin	ABE7.10max	Intein-based dual AAV9	Localized (intramuscular injection in the tibialis anterior muscle)	Chemello et al. (2021)
		Humanized mice	Skeletal muscle (myofibers)	*DMD* c.4174C>T, (p.Q1392X)	Direct mutation correction	• 35% editing with 54% dystrophin restoration	RNA base editor mxABE	Single AAV9	Localized (intramuscular injection)	Li et al. (2023)
		Humanized mice	Skeletal muscle (myofibers)	*DMD* exon 51 deletion	Mutation insertion to disrupt DMD splicing sites	• ~15% editing resulting in over 70% exon skipping efficiency • Up to 96% of local myofibers stained for restored dystrophin	ABE8e	Intein-based dual AAV	Localized (intramuscular injection)	Lin et al. (2024)
Spinal muscular atrophy	0.85/100,000	Mice	Brain and spinal cord cells	*Smn2* 6th nucleotide of exon 7	Compensatory base editing of *Smn2* T6>C, a paralogous gene to *Smn1* to restore Smn protein levels	• 87% average T6>C conversion, improved motor function, and extended average lifespan	ABE8e-SpyMac	Intein-based dual AAV9	Localized (intracerebroventricular injection)	Arbab et al. (2023)

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TABLE 1 (*Continued*) *In vivo* gene editing strategies used in preclinical studies for rare monogenic disorders.

DiseaseDisease	Prevalence	Model	Key organ (cell type)	Targeted gene	Editing strategy	Editing outcome	Editor variant	Delivery method	Administration	Ref
		Mice	Brain and spinal cord cells	*Smn2* 6th nucleotide of exon 7	Compensatory base editing of *Smn2* T6>C, a paralogous gene to *Smn1* to restore Smn protein levels	• ~6% editing in brain and ~4% in spinal cord at day 13 • ~10% editing in brain and ~8% in spinal cord at week 12	ABE8e-SpRY	Intein-based dual AAV9	Localized (intracerebroventricular injection) and Systemic (retroorbital injection)	Alves et al. (2024)
Sickle cell disease/ β-thalassemia	1–5/10,000	Humanized mice	Blood (hematopoietic stem cells)	*Hpfh* c.*113A>G	Direct mutation correction	• 30% editing following selection • 21% of blood β-like globins were fetal hemoglobin	ABE7.10max	Adenovirus	Systemic (intravenous)	Li et al. (2021)
		Humanized mice	Blood (hematopoietic stem cells)	*Hpfh* c.*113A>G	Direct mutation correction	• 60% editing with 30% γ-globin of β-globin expressed in 70% of erythrocytes	ABE8e	Adenovirus	Systemic (intravenous)	Li et al. (2022)
Amyotrophic lateral sclerosis	1-9/100,000	Humanized mice	Central nervous system (motor neurons)	*Sod1* c.281G>C (p.G93A)	Direct mutation correction	• 1.2% editing • 11% increase in lifetime, • 85% increase in duration between onset of late-stage disease and death	BE3	Intein-based dual AAV9	Localized (lumbar subarachnoid space injection)	Lim et al. (2020)
Niemann-Pick disease type C	1-9/100,000	Mice	Central nervous system (Purkinje cells)	*Npc1* c.3182T>C (p.I1061T)	Direct mutation correction	• 48% editing in cortex, 42% in Purkinje cells, up to 59% in cortex at test site • 10% increase in lifetime	BE3.9max	Intein-based dual AAV9	Systemic (retro-orbital injection)	Levy et al. (2020)
Hurler syndrome	1-9/1,000,000	Mice	Liver and heart (progenitor cells)	*Idua* G>A (p.W392X)	Direct mutation correction	• ~12.8% editing in liver progenitor cells, ~12.6% in myocytes, ~3.0% in endothelial cells, ~2.3% in fibroblasts	ABE7.10max	*In utero* intein-based dual AAV9	Systemic	Bose et al. (2021)
		Mice	Liver, heart, brain (hepatocytes, cardiomyocites and neurones)	*Idua* G>A (p.W392X)	Direct mutation correction	• ~22.46% editing in hepatocytes, 11.18% in heart, and 0.12% in the brain	ABE8e-SpG	Intein-based dual AAV9	Systemic (temporal vein injection)	Su et al. (2023)
		Mice	Brain (midbrain, hippocampus, forebrain)	*Idua* G>A (p.W392X)	Direct mutation correction	• ~1% editing in midbrain, hippocampus, and forebrain	ABE7.10	C3-LNP encapsulating mRNA	Localized (intracerebroventricular)	Palanki et al. (2023)

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TABLE 1 (*Continued*) *In vivo* gene editing strategies used in preclinical studies for rare monogenic disorders.

DiseaseDisease	Prevalence	Model	Key organ (cell type)	Targeted gene	Editing strategy	Editing outcome	Editor variant	Delivery method	Administration	Ref
Leber congenital amaurosis	1-9/100,000	Mice	Retina (retinal pigmented epithelium)	*Rpe65* c.130C>T (p.R44X)	Direct mutation correction	• 15% editing, restored visual function.	ABE7.10max	Lentivirus	Localized (subretinal injection)	Suh et al. (2021)
		Mice	Retina (retinal pigmented epithelium)	*Rpe65* c.130C>T (p.R44X)	Direct mutation correction	• 14% editing, restored visual function • 89% editing in *Rpe65* cDNA	ABE7.10max-SpCas9-NG	Intein-based dual AAV9	Localized (subretinal injection)	Jo et al. (2023)
		Mice	Retina (retinal pigmented epithelium)	*Rpe65* c.130C>T (p.R44X)	Direct mutation correction	• 54% editing with 40% of visual function restored	NG-ABE	Lentivirus	Localized (subretinal injection)	Choi et al. (2022)
		Mice	Retina (retinal pigmented epithelium)	*Kcnj13* c.158G>A (p.W53X)	Direct mutation correction	• ~20% editing in retinal pigmented epithelial cells	ABE8e	Silica nanocapsules encapsulating mRNA	Localized (subretinal injection)	Kabra et al. (2023)
		Mice	Retina (retinal pigmented epithelium)	*Rpe65* c.130C>T (p.R44X)	Direct mutation correction	• 16.38% editing with ABE8e • 11.33% editing with ABEmax • 9.96% editing with ABE8e-WQ	ABE8e, ABEmax, ABE8e-WQ	Intein-based dual AAV2/9	Localized (subretinal injection)	Lee et al. (2024)
Recessive hearing loss		Mice	Inner ear (hair cells)	*Tmc1* c.545A>G (p.Y182C)	Direct mutation correction	• 2.3% bulk genomic correction, 33% cDNA correction • Increased hearing at 4 weeks that slowly degenerated	AID-BE3.9max	AAV (Anc80 serotype)	Localized (inner-ear injection)	Yeh et al. (2020)
	3% of prelingual deafness in Spain	Humanized mice	Inner ear (hair cells)	*OTOF* c.2485C>T (p.Q829X)	Direct mutation correction	• 80% editing • ~100% OTOF protein restoration of inner hair cells	RNA base editor emxABE	AAV9	Localized (inner-ear injection)	Xue et al. (2023)
Long QT syndrome type 3	1–5/10,000	Mice	Heart (cardiomyocytes)	*Scn5a* c.3908 C>T (p.T1307M)	Direct mutation correction	• ~40% editing • Up to 99% restored *Scn5a* mRNA levels and restored mouse QT and QTc phenotype	ABEmax	Intein-based dual AAV9	Systemic (intraperitoneal injection)	Qi et al. (2024)

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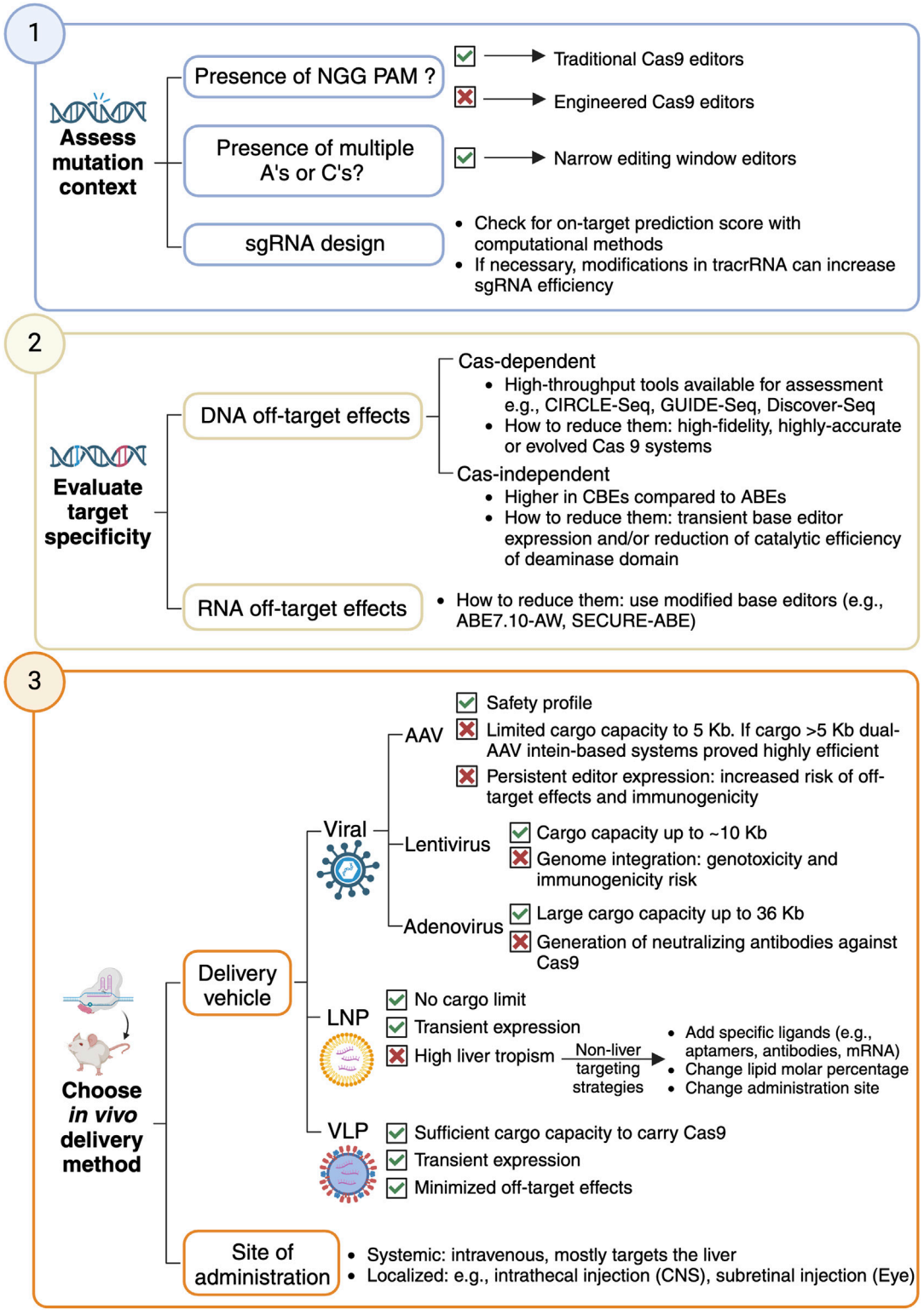

FIGURE 1
Workflow to design a base editing strategy for gene therapeutics for a rare monogenic disorder.

2018). Computational methods have analyzed large-scale CRISPR screens to identify sgRNA and locus-features modulating Cas9 activity (Konstantakos et al., 2022): On-target prediction scores designed for Cas9 are a good surrogate measure for base editing efficiency (Cuella-Martin et al., 2021; Hanna et al., 2021). In addition, machine learning methods trained in base editing

screening data, such as BeHive (Arbab et al., 2020), can provide fine-grained efficiency and editing outcome information for specific base editor-sgRNA pairs. Finally, modification in the tracrRNAs, the part of the sgRNA acting as a scaffold and binding to Cas9, may be considered to increase sgRNA efficiency. Adding mutations to disrupt a natural poly-T tract in the tracrRNA can prevent premature transcription termination resulting in better editing efficiency (Scott et al., 2019; DeWeirdt et al., 2022).

4.2 Target specificity

A major concern for CRISPR-dependent base editing therapies is undesired, off-target editing, both at DNA and RNA sites. Off-target DNA base editing can occur in a Cas-dependent (guided) or a Cas-independent (unguided) manner.

4.2.1 Cas-dependent DNA off-target editing

The Cas9-sgRNA complex may bind to sequences sharing similarity with the target site. A productive off-target base editing requires additional criteria, such as the presence of a targetable base within the editing window and nucleotide sequence context, that are not satisfied for all Cas nuclease-dependent off-target sites (Anzalone et al., 2020). Modification of Cas9-DNA contacts and Cas9 conformation using rational design and systematic screening resulted in high-fidelity (HF), highly accurate (Hypa) and evolved (Evo) systems that minimize Cas-dependent off-target effects (Anzalone et al., 2020; Skeens et al., 2024). High-throughput techniques like CIRCLE-Seq, Discover-Seq, and Guide-Seq can systematically identify candidate loci subject to Cas-dependent off-target base editing; potential off-target sites can then be validated with an orthogonal approach (e.g., amplicon sequencing) *in cellula* or *in vivo* (Wienert et al., 2019; Tsai et al., 2017; Tsai et al., 2015). Of note, the off-target profiles of base editors may differ from that of Cas9 due to additional requirements for productive base editing (e.g., presence of a targetable base in the editing window), prompting the development of high-throughput technologies to specifically detect base editing off-targets, such as CHANGE-seq-BE (Lazzarotto et al., 2024).

4.2.2 Cas-independent DNA off-target editing

Long-term deaminase expression can result in random deamination of nucleotides transiently accessible across the genome (Jin et al., 2019; Lee et al., 2020; Zuo et al., 2019). This type of off-target editing has been observed in mammalian and plant cells expressing CBEs but not in ABEs (Jin et al., 2019; Lee et al., 2020; Zuo et al., 2019). These edits occur at low frequency, with ~5×10^{-8} mutations per base pair in mouse embryos injected with high levels of CBE mRNA, which is below most reported rates of spontaneous somatic cell mutation (Zuo et al., 2019). Using delivery strategies that achieve transient base editor expression limits off-target effects while preserving on-target editing due to a quicker action of BEs at on-target rather than off-target loci (Rees et al., 2017). As an alternative strategy, introducing mutations to the deaminase domain to reduce its catalytic efficiency will slow the kinetics rate at off-target *vs* on-target sites (Liu et al., 2020).

4.2.3 RNA off-target editing

Most widely used deaminase domains in CBEs and ABEs are derived from enzymes that natively deaminate RNA, which can drive RNA off-target base editing when overexpressed (Richter et al., 2020; Rees et al., 2019). This phenomenon occurs at low levels in a random and widespread manner similar to Cas-independent DNA off-target editing. For instance, transient ABE7.10 overexpression in HEK293T cells has been observed to induce ~0.22–0.24% A-to-I deamination across the transcriptome, compared with ~0.14–0.19% A-to-I deamination from endogenous cellular adenosine deaminases (Richter et al., 2020; Rees et al., 2019). RNA off-target editing activity has been mitigated by the development of the ABE7.10-AW (Rees et al., 2019) and SECURE-ABE editors (Grünewald et al., 2019), and the introduction of V106W mutation in both ABE8 and ABE8e showed reduction RNA editing levels similar to those from cellular RNA deamination (Richter et al., 2020).

4.3 Delivery methods

Just like other gene therapy methods, base editor systems rely on a vehicle to reach their target cells (Zhang and Wu, 2024). Gene-editing agents can be delivered as DNA, mRNA or ribonucleoproteins (RNPs), that can be packaged into different vehicles to overcome biological barriers (Raguram et al., 2022), namely,

4.3.1 Viral-based vehicles

Most *in vivo* gene editing applications use AAVs as delivery vehicles, and, to a lesser extent, lentiviruses and adenoviruses (Raguram et al., 2022; Eichler et al., 2024).

4.3.1.1 Adeno-associated virus (AAV)

AAVs are widely used due to their safety profile and the characterized tissue tropism for different serotypes (Costa Verdera et al., 2020) though their limited packaging capacity (5 Kb) restricts delivery of full base editor constructs. To overcome this limitation, dual-AAV strategies, such as intein-mediated protein reconstitution, have been developed (Chemello et al., 2021; Chen et al., 2020), achieving editing efficiencies from 9% to 60% across different therapeutic organs such as liver, eye, and cardiac or skeletal muscle (Alves et al., 2024; Koblan et al., 2021; Levy et al., 2020). Intein-mediated dual-AAV deliveries were successful at introducing ABEs in progeria and SMA mouse models (Alves et al., 2024; Koblan et al., 2021). However, AAV-based delivery results in persistent Cas9 expression, risking off-target effects and immune responses that may lead to edited cell destruction (Raguram et al., 2022). Solutions include self-inactivating AAV systems and conditional base editor activation restricted to on-target loci sites (Ibraheim et al., 2021). AAVs have been a successful delivery system in preclinical research of base editing therapies against DMD (Chemello et al., 2021), amyotrophic lateral sclerosis (Lim et al., 2020), and Hutchingson-Gilford progeria syndrome (Koblan et al., 2021), among others.

4.3.1.2 Lentivirus

Lentiviruses offer larger cargo capacity (~10 Kb), and support multiplex genome editing using CRISPR-based agents but will integrate into the genome, raising risks of genotoxicity and immunogenicity that may limit their clinical potential (Milone and O'Doherty, 2018; Kymäläinen et al., 2014). *In vivo* studies have demonstrated lentiviral efficacy: An ABE delivered to the retina of a mouse model of Leber congenital amaurosis achieved 15% mutation reversion in the *Rpe65* gene and restored visual function (Suh et al., 2021).

4.3.1.3 Adenovirus

Adenoviruses allow large cargoes (up to 36 Kb) and are genetically stable, with well-characterized biology and scalable production (Lee et al., 2017). CRISPR/Cas systems are commonly delivered by adenoviruses, a strategy recently used to deliver an ABE to achieve direct correction of the *Hpfh* c.*113A>G mutation responsible for beta-thalassemia (Li et al., 2021). Despite their proven efficiency for *in vivo* editing, adenoviruses may lead to the generation of neutralizing antibodies against Cas9, potentially due to the immunogenic nature of the vector (Wang et al., 2015).

4.3.2 Non-viral-based vehicles

Although viral vectors have been widely used in clinical trials to deliver CRISPR/Cas systems, the immune response triggered by the vector and/or the CRISPR/Cas components remains a significant concern since it could lead to severe or even deadly side effects (Song et al., 2024). To address the limitations of viral vectors, non-viral alternatives have been developed to deliver CRISPR/Cas machineries.

4.3.2.1 Lipid nanoparticles (LNPs)

LNPs, a fully synthetic option, are FDA-approved for delivery of siRNA and mRNA therapeutics. They provide transient expression with lower immunogenicity than viral vectors and can support repeated dosing (Kenjo et al., 2021). LNP-based ABE delivery showed promising results in disrupting *PCSK9* in the liver with minimal off-target effects, resulting in durable LDL-cholesterol reduction in mice and primate models of hypercholesterolemia (Musunuru et al., 2021; Rothgangl et al., 2021). However, the progress of this therapy to clinical trials highlighted the need for LNP optimization to prevent potential LNP-associated side effects: Despite the high efficiency of the treatment in patients, potential LNP-associated liver abnormalities were observed (Philippidis, 2024). In this respect, base editing therapies can benefit from research on delivery methods for gene therapeutics, such as using sugars like N-acetylgalactosamine (GalNac) to enhance overall drug uptake and delivery. GalNacs are used in FDA-approved gene therapies like eplontersen, givosiran or lumasiran and showed good tolerance and liver targeting using either the asialoglycoprotein or the low-density lipoprotein receptors. GalNac-LNPs are currently undergoing clinical trials for ABE-based treatment of hypercholesterolemia (VERVE-102) (Vafai et al., 2024). An LNP-based base editing therapy is also in clinical trials for alpha-1 antitrypsin deficiency (BEAM-302), highlighting this vehicle as the go-to choice for liver-affecting disorders.

Since LNPs delivered intravenously naturally accumulate in the liver, their effectiveness in targeting non-liver tissues is limited. LNPs can be modified to modulate their tropism by changing their molar percentage, administration site, or adding target-specific ligands in the cargo such as mRNA encoding tissue-targeting proteins, antibodies or aptamers (Loughrey and Dahlman, 2022). Adding anionic lipids successfully redirected liver-targeting LNP containing Cas9-mRNA to the spleen (Cheng et al., 2020); optimized LNPs for nebulized mRNA successfully delivered therapeutic mRNA to the lungs (Lokugamage et al., 2021); adding an mRNA encoding for vascular endothelial growth factor C increased lymphatic specificity (Szőke et al., 2021); and by intravitreal injection of LNP containing less PEG, therapeutic mRNA could arrive to retinal cells (Ryals et al., 2020).

4.3.2.2 Virus-like particles (VLPs)

VLPs offer transient delivery by packaging mRNA, proteins or RNPs reducing the risk of off-target effects and viral integration (Banskota et al., 2022; Mangeot et al., 2019). VLPs are large enough to carry Cas9 and allow controlled delivery, minimizing off-target editing compared to AAVs and LVs (Banskota et al., 2022; Mangeot et al., 2019). They offer key benefits of both viral and non-viral delivery. Recent developments, like the PEG10-based SEND system, use endogenous proteins to improve efficiency and reduce immunogenicity (Segel et al., 2021), with potential for clinical applications if scaled up effectively.

4.4 Site of administration

Many intravenously injected vehicles can efficiently access some tissues, such as the liver, but cannot efficiently access others, such as the central nervous system (CNS), due to intrinsic biological barriers (e.g., the blood-brain barrier) (Daneman and Prat, 2015). Locally injecting vehicles into the CNS (e.g., via intrathecal injection) or eye (e.g., via subretinal injection) can circumvent biological barriers and enable access to certain important cell populations (Bottros and Christo, 2014; Peng et al., 2017).

5 Bench-to-bedside translation for base editing therapies for rare monogenic disorders

CRISPR-dependent base editing can revolutionize the treatment of rare monogenic disorders. The precision and efficiency of base editors provide hope for correcting pathogenic mutations that were once deemed untreatable. Yet, the transition from preclinical innovation to clinical implementation faces some hurdles.

Despite extensive base editor optimization, unintended off-target effects are still a primary concern. The observed targeting events with current base editors suggest high on-target and minimal off-target editing. However, base editors and the methods used for detecting their off-targets still require technical innovation, rigorous *in vivo* validation, and long-term studies for comprehensive assessment of safety profiles. The delivery of base editors also remains a bottleneck. Viral vectors, the most common delivery tools, have limited packaging capacities and immunogenic risks, whereas non-viral methods still require optimization for targeting specific tissues like the brain. Finally, base editing strategies can only

be designed for diseases caused by transition mutations. This gap can be filled with newer, more versatile nickase-based gene editing technologies like prime editing (PE) or click editing (Ferreira da Silva et al., 2024). For instance, PE uses nickase Cas9 fused to reverse transcriptase (RT) and bound to a prime editing guide RNA (pegRNA) that can theoretically install precise insertions, deletions, and all twelve types of point mutations. Of note, advances in prime editors are directed to reduce their size, increase their efficiency, and reduce indel ratios, enhancing their therapeutic potential (Testa and Musunuru, 2023; Lee et al., 2023; Doman et al., 2023; Chen et al., 2021). Although still in the early stages of clinical application, PEs have been used *in vivo* in preclinical studies to correct pathogenic variants in liver diseases, such as tyrosinemia (Jiang et al., 2022) and phenylketonuria (Böck et al., 2022).

Beyond technical limitations, the therapeutic application of base editing must address complex regulatory and ethical considerations with evidence of efficacy, safety, and reproducibility before approval. This is particularly challenging for rare monogenic disorders: the lack of available treatments prompts shorter preclinical development times. Further, the small patient populations—often including primarily children—pose additional challenges to clinical trial design and implementation. The transition of base editing into clinical trials for a prevalent disease like heterozygous familial hypercholesterolemia establishes a strong precedent for rare disease therapeutics to follow. Optimized base editor configurations could be applied across multiple disorders, and learnings on safety and efficacy in broader patient populations (e.g., by determining optimal delivery vehicles or immunogenicity profiles) are critical to streamlining the progress of such therapies to the clinic, paving the way to design base editing therapeutics for rare monogenic disorders.

Finally, equitable access to these advanced therapies should remain a priority. Without proactive measures, the high costs associated with gene editing could exacerbate existing healthcare disparities, limiting the global impact of these innovations. Another key to advancing CRISPR-dependent base editing lies in collaborative efforts between academia, industry, and regulatory bodies. Public-private partnerships could accelerate the development of scalable manufacturing processes and standardized clinical protocols. By addressing the above challenges, the rare disorder field can navigate the complexities of translating CRISPR-dependent base editing from bench to bedside, paving the way for a new era of personalized medicine.

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J-JC-R: Data curation, Investigation, Writing – original draft. RC-M: Conceptualization, Supervision, Funding acquisition, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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