

Beyond CRISPR Cutting: A Systematic Review of Base Editing and Prime Editing for Therapeutic Precision

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Abstract:

Introduction: Genome editing has advanced considerably, enabling increasingly precise correction of pathogenic variants in human cells. Base editing and prime editing were developed to address important limitations of nuclease-based CRISPR-Cas systems, particularly the risk of Double-Strand Breaks (DSBs) and associated unintended genomic alterations.

Methods: A systematic review was performed to find human-relevant primary studies of base and prime editing using PubMed, Scopus, and CINAHL databases, following the guidelines set forth by the PRISMA group. Two reviewers used predefined inclusion/exclusion criteria; disagreements among reviewers were resolved by consensus, and methodological quality was evaluated according to ROBINS-I principles.

Results: Base editing was very efficient at replacing single-nucleotide transitions, especially when the right PAM sites and an appropriate editing window were present. The key drawbacks of its use were its PAM dependency, limited window of editing and bystander or off-target deamination. Prime editing supported wider ranges of substitutions, insertions, deletions, and regulatory edits with more control over the positions in editing primary human systems, although it was less efficient. In all the disease models studied, therapeutic potential was not only driven by the greatest percentage of editing but also by efficacy of function, delivery potential and safety of the editing approach.

Conclusion: Base editing appears suitable for efficient correction of eligible single-nucleotide transition mutations, whereas prime editing offers greater flexibility and positional precision for complex edits. The most appropriate platform depends on the mutation type, target tissue, route of administration, and disease-specific functional requirements.

Keywords: Prime editing, base editing, CRISPR-Cas system, precision genome editing, human therapeutic models, gene therapy.

1. INTRODUCTION

Since the development of the CRISPR-Cas9 system, genome-editing technology has evolved significantly [1]. The ability to efficiently and accurately cleave targeted DNA with CRISPR-Cas9 ushered in a new era in functional genomics and disease modelling [2]. Double-strand breaks (DSBs), however, may lead to off-target effects, chromosomal breaks and rearrangements, and insertions or deletions, potentially contributing to genomic instability in therapeutic applications [3, 4]. The aim of base editing and prime editing is to minimise these hazards: modification at the nucleotide level without requiring DSB-mediated repair.

For therapeutic purposes, base editing may reduce unwanted repair outcomes because it does not intentionally generate DSBs [5, 6]. Moreover, Joo *et al.* [7] and Li *et al.* [8] discuss how prime editing could correct a broader spectrum of pathogenic variants than that accomplished by conventional CRISPR-Cas9 editing, addressing an important limitation of the latter. Base editing has significant therapeutic relevance since large variations of disease variants are caused by transition mutations in monogenic diseases [9]. Prime editing can introduce all 12 possible base substitutions as well as small insertions and deletions. It uses a Cas9 nickase fused to a reverse transcriptase together with a prime-editing guide RNA

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(pegRNA) containing the template and primer-binding sequences [7].

Yet, in recent studies in cells applicable to humans, three important "translationally relevant" limitations emerge, these are cell-context dependency, repair-pathway interference, and delivery problems. In general, Zhang *et al.* [10] reported decreased editing efficiency in differentiated and patient-derived cells compared to immortalised cells. Hua *et al.* [11] demonstrated that the delivery and cell compatibility issues could not be overcome solely by changing the architecture of the editors. Park [12] also showed that pegRNA design and mismatch-repair activity of prime editing enzymes can constrain prime editing outcomes. All these studies highlight key obstacles to clinical translation, guide design, repair modulation and delivery.

Lee *et al.* [13] highlighted the difficulty of packaging the relatively large prime-editing components into viral vectors, as well as limitations in tissue penetration and duration of expression associated with non-viral approaches. This focus on human-cell and translational models is justified by these limitations, given that only proof-of-concept editing in simplified models has so far been performed.

Many inherited diseases, such as sickle cell disease, cystic fibrosis (CF), and Duchenne muscular dystrophy (DMD), involve pathogenic variants that could, in principle, be targeted using base or prime editing [14]. Because base and prime editing achieve permanent, genome-level genetic correction without intentionally creating DSBs, they hold promise for achieving therapeutic outcomes while avoiding some of the risks associated with DSB-based genome editing [15, 16]. While some studies have demonstrated proof-of-concept success in vitro or in animal models, translation to human therapeutic applications remains challenging. Editing tools that perform effectively in immortalised cell lines may face greater difficulty when applied in primary cells or patient-derived tissues. Comparative studies of clinically relevant human systems are therefore needed to assess the feasibility of therapeutic application.

This review aims to critically assess the accuracy, efficiency, safety, and translatability of base and prime editing in human primary systems or models of diseases. Ex vivo and in vivo human systems, including haematopoietic stem cells, airway epithelial cell cultures, organoids, and early clinical data, are reviewed. It measures on-target editing efficiency, bystander editing, unintended substitutions, indel rates, off-target DNA/RNA effects, genomic instability, cytotoxicity, delivery strategy, scalability, and disease-technology fit. This synthesis is intended to be focused rather than broad, specifically including only those systems relevant to humans and those translation barriers that affect therapeutic application.

2. METHODOLOGY

2.1. Research Design

This review is reported according to the PRISMA 2020 statement [17]. The search for studies was carried out in the

databases from January 1, 2016, to June 26, 2026 (the last search was performed on June 26, 2026). Titles, abstracts and full texts of the articles were independently evaluated by two reviewers, and any conflicts were resolved by discussion and consensus. A prospective protocol was not registered for this review. Study design, type of human cell, editor platform, delivery mode and outcome measures were extracted using a pre-established framework. Because most of the included studies were experimental genome-editing studies, ROBINS-I principles were adapted to evaluate risk of bias and consistency of methodology.

2.1.1. Search Strategy and Selection Criteria

The search was conducted in three databases: PubMed, Scopus, and CINAHL (Appendix, Table 1), which provide comprehensive coverage of research articles in molecular biology, genetics and therapeutic gene editing. Search strings included "base editing", "prime editing", "human cells", "genome editing", "therapeutic application", "disease models" and "clinical translation". Items were screened to establish direct relevance to human systems and therapeutic outcomes with editing. Although the review aimed at providing human-relevant experimental data and did not intend to include reviews or general mechanistic discussions or literature on animal models, 16 primary studies were selected for the final synthesis.

2.1.2. Eligibility Criteria

2.1.2.1. Inclusion Criteria

Studies were included if they reported human clinical data, ex vivo human primary-cell data, patient-derived cells, organoids, or human tissue models involving base editing or prime editing. Eligible studies had to report clinically relevant outcomes such as editing efficiency, precision, safety, functional restoration, or correction of defined single-nucleotide variants or small insertions/deletions. Articles published between 2016 and 2026 were considered (Table 2). The included studies were stratified into three tiers to clarify model relevance:

Tier 1: Clinical/ex vivo primary systems- This group includes studies based on patient-derived cells, primary stem cells, and clinical trials for therapeutic gene editing.

Tier 2: Organoids- Studies involving organoids that serve as human-relevant disease models.

Tier 3: Immortalised cell lines- Studies that use HEK293T or other immortalised cell lines, which primarily serve as proof-of-concept models rather than direct therapeutic models.

This categorisation helps clarify the relevance of different cell models and their clinical applicability.

2.1.2.2. Exclusion Criteria

Studies were excluded if they were animal-only, theoretical, or narrative review papers; addressed diseases outside the scope of the review; reported base- or prime-editing results limited to large insertions and deletions outside the review's focus; were published before 2016; or did not report outcomes

of base editing or prime editing. Studies that did not follow clearly established methods, or that had no clear clinical relevance for therapeutic editing in humans, were also excluded. Sixteen of the primary studies were eligible and used for final synthesis.

2.2. Data Extraction and Synthesis

To reduce bias and improve consistency, data extraction was performed independently by two reviewers using a predefined extraction framework. Extracted variables included study design, editor platform, target gene or mutation, delivery method, human model, on-target efficiency, off-target effects, safety outcomes, and therapeutic or functional endpoints. Discrepancies were resolved by discussion until agreement was reached.

2.2.1. Assessment of Editing Precision and Efficiency

Editing accuracy refers to the ability of a genome-editing platform to introduce the desired genetic change at the target locus while minimizing unintended modifications, whereas editing efficiency refers to the proportion of treated cells in which the intended edit is achieved [18-39]. Safety was assessed in terms of off-target deletions, genomic instability, and unintended mutations. The reviewed studies mainly focused on pathogenic single-nucleotide variants, including variants in genes such as HBB [31] and CFTR [22, 24], with editing outcomes varying according to the editing platform, target sequence, and cellular context. Prime editing exhibited high positional accuracy and fewer bystander edits than base editing, though with lower efficiencies in some human systems [19-21].

2.2.2. Data Analysis

The analysis followed a structured qualitative synthesis. Two reviewers assessed the study characteristics, sampling approach, feasibility of blinding, outcome measures and reporting transparency. Since human genome-editing studies are rarely completely blinded, risk-of-bias interpretation focused primarily on model relevance, delivery variability, donor-to-donor variation, outcome measurement, and completeness of reporting.

2.2.3. Quality Assessment

The primary studies included those reported in references [22-37]. Because no validated risk-of-bias instrument exists specifically for experimental genome-editing studies, ROBINS-I was developed for non-randomised studies of interventions. This study was adapted as a structured framework for appraising study design, outcome reporting, and methodological consistency and should not be interpreted as a validated instrument specifically designed for genome-editing research. Studies were classified as low or moderate risk of bias according to model type, delivery method, sequencing validation, functional testing, and reporting transparency (Table 3). Lower risk was assigned to studies using validated sequencing pipelines, functional assays, and clearly described editor architectures, such as CFTR correction in organoids [22, 23], gamma-globin reactivation [24], and genome-wide fidelity profiling of prime editing [25]. Moderate risk was mainly associated with donor variability, delivery constraints, and primary-cell heterogeneity rather than systematic reporting bias.

3. RESULTS

3.1. Data Screening

Data screening was conducted systematically to ensure transparency and methodological rigour. Initially, 588 records were identified through database searching: Scopus (n = 64), PubMed (n = 347), and CINAHL (n = 177). After duplicate removal and application of the publication-year filter, 24 records were removed before screening. The remaining 564 records were screened by title and abstract, and 446 were excluded because they were unrelated to therapeutic genome editing, involved non-human models, or did not constitute relevant peer-reviewed evidence. Of the 118 reports sought for retrieval, 82 were unavailable because they were conference abstracts, lacked full text, or were incomplete. Thirty-six full-text articles were assessed for eligibility, and 20 were excluded because they did not report eligible primary base- or prime-editing outcomes, lacked methodological clarity, or were review articles. Sixteen primary studies were included in the final synthesis (Fig. 1).

Table 1. Overview of search results for each database.

Database	Search String	Total Results	Key Focus
PubMed	(base editing OR prime editing) AND (human OR human cells) AND (genome editing) AND (therapeutic OR disease)	347	Human-based primary studies evaluating therapeutic genome editing using base or prime editing technologies, including efficiency, precision, and disease relevance.
Scopus	base editing OR prime editing AND human cells AND genome editing AND therapeutic OR disease AND clinical trials	64	Translational and experimental studies in human cells assessing base and prime editing platforms for disease correction and clinical applicability.
CINAHL	base editing OR prime editing AND single-nucleotide mutations AND gene therapy AND human clinical trials	177	Single-nucleotide mutations, gene therapy, and clinical trials in human systems.

Table 2. Study selection criteria.

Criterion	Included	Excluded
Population/model	Human clinical data; human primary or patient-derived cells; HSPC; organoids; human tissues; relevant immortalised human cell lines for proof-of-concept	Animal-only studies or models without a human therapeutic component
Intervention	Base editing or prime editing directed at therapeutic genome modification	Conventional nuclease-only CRISPR, non-genetic interventions, or editing outside the review focus
Outcomes	Editing efficiency, precision, safety, functional restoration, or correction of defined single-nucleotide variants/small indels	No specific base- or prime-editing outcome; insufficient methodological detail
Study type	Primary experimental or clinical evidence with direct relevance to human therapeutic editing	Reviews, theoretical papers, conference abstracts, incomplete reports, or unavailable full text
Publication period	1 January 2016 to 26 June 2026	Published before 2016
Edit scope	Single-nucleotide variants and small insertions/deletions; larger edits only where directly relevant to platform capability	Large insertions/deletions outside the stated therapeutic focus

Table 3. Risk-of-bias mapping for the 16 included primary studies.

Study	Design	Overall Risk of Bias	Justification
Sousa <i>et al.</i> , [22]	Primary airway cells	Low	Quantified functional rescue and optimisation strategy
Anzalone <i>et al.</i> , [23]	Human cell models	Low	Strong controls, precise intervention classification, complete molecular outcomes
Bulcaen <i>et al.</i> , [24]	Patient-derived organoids	Low	Functional validation, comprehensive molecular and phenotypic data
Mbakam <i>et al.</i> , [25]	Human myogenic cells	Moderate	Variable editing efficiency across loci
Chalumeau <i>et al.</i> , [26]	Human HSPC	Moderate	Donor variability and heterogeneity in editing efficiency
Chen <i>et al.</i> , [27]	Human cell lines	Low	Systematic design, controlled comparisons, consistent reporting
Gaudelli <i>et al.</i> , [28]	Human cell lines	Moderate	High efficiency but limited off-target assessment
Habib <i>et al.</i> , [29]	Human stem cells	Low	Whole-genome sequencing and robust fidelity analysis
Halegua <i>et al.</i> , [30]	hiPSCs / HSC	Moderate	Delivery complexity and scalability limitations
Hardouin <i>et al.</i> , [31]	CD34 ⁺ HSPC	Low	Strong functional and lineage integrity validation
Jang <i>et al.</i> , [32]	Patient fibroblasts	Moderate	Multiple editors but heterogeneous outcomes
Koblan <i>et al.</i> , [33]	Human fibroblasts	Low	High editing purity and clear molecular endpoints
Mbakam <i>et al.</i> , [34]	Human myoblasts	Moderate	Delivery limitations in primary cells
Nelson <i>et al.</i> , [35]	Human cell lines	Low	Clear mechanistic improvement and low off-targets
Richter <i>et al.</i> , [36]	Human cell models	Moderate	Expanded PAM scope with limited functional validation
Sürün <i>et al.</i> , [37]	hiPSCs	Moderate	Disease correction was demonstrated, but long-term assessment was limited.

Abbreviation: PAM: Protospacer Adjacent Motif; hiPSCs: human induced pluripotent stem cells; HSC- Haematopoietic Stem Cell; HSPC: Hematopoietic Stem and Progenitor Cells.

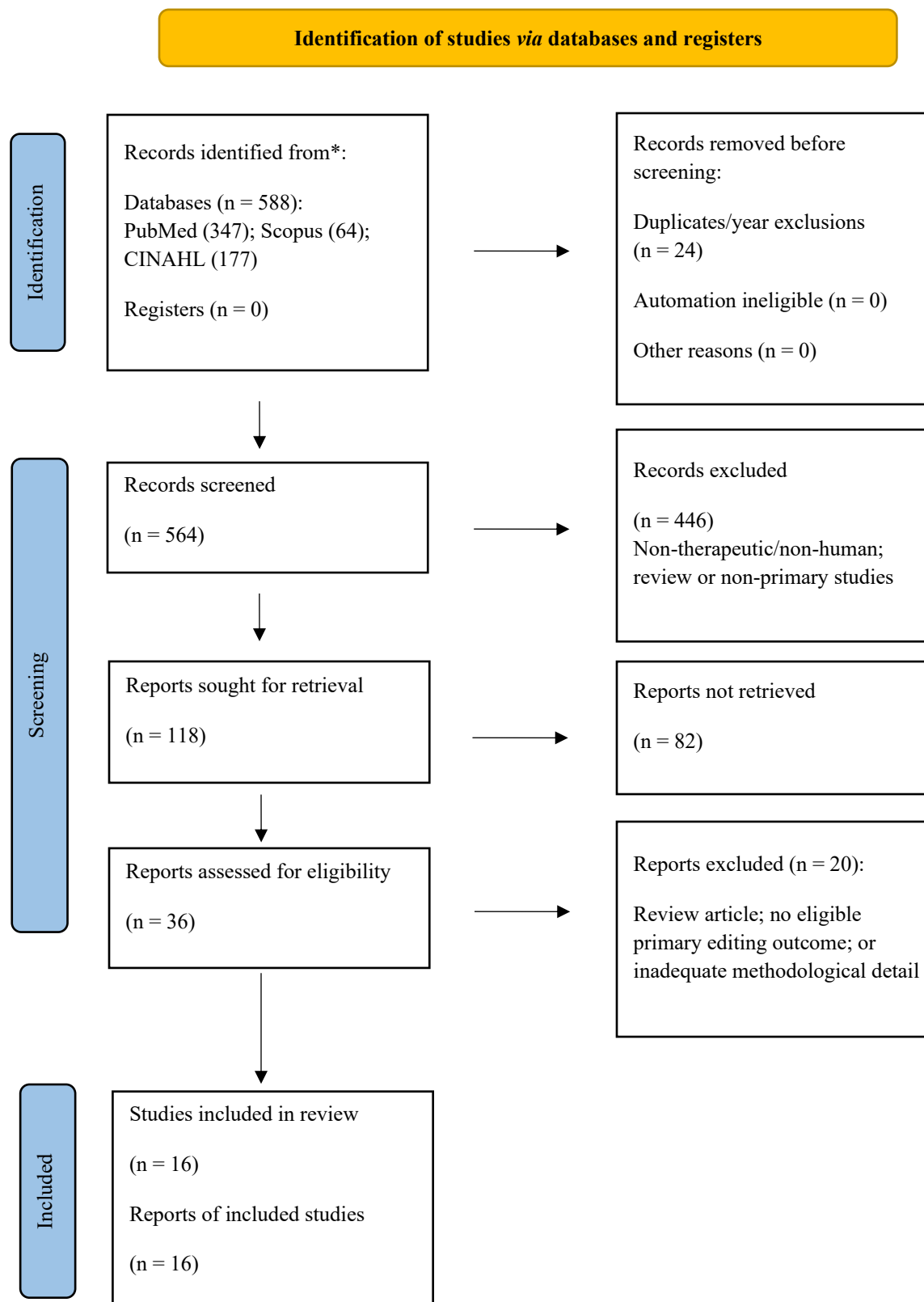


Fig. (1). PRISMA 2020 study-selection flow diagram showing identification, screening, eligibility assessment, and inclusion of 16 primary studies. Adapted from Page *et al.* [17] under CC BY 4.0.

Table 4. Characteristics, methods and outcomes of the 16 included primary studies.

Year	Disease/Model	Target Gene	Editor Platform	Delivery Method	Main Therapeutic Outcome	Reference
2025	Cystic fibrosis	CFTR (F508del)	Prime editor (PEmax/PE6 with epegRNA)	mRNA and RNA electroporation	Functional CFTR restoration; chloride transport recovery	Sousa <i>et al.</i> , [22]
2022	Duchenne muscular dystrophy model; phenylketonuria model	DMD (exon 51); PAH	Twin prime editor (twinPE)	Plasmid transfection	Precise exon deletion and reading-frame restoration; exonic sequence replacement and pathogenic-region recoding; low indels	Anzalone <i>et al.</i> , [23]
2024	Cystic fibrosis	CFTR (L227R, N1303K)	Prime editor (PE2/PE3)	Lentiviral delivery	Genetic correction; restored CFTR processing and function	Bulcaen <i>et al.</i> , [24]
2023	Duchenne muscular dystrophy	DMD (exons 51, 53)	Prime editor (PE2/PE3)	Plasmid transfection / electroporation	Exon skipping; dystrophin expression restored	Mbakam <i>et al.</i> , [25]
2025	Sickle cell disease	HBG1/HBG2 promoters	Prime editor (PE3/PE5max)	Electroporation	HbF reactivation; increased γ -globin expression	Chalumeau <i>et al.</i> , [26]
2021	Genetic disease models	Multiple loci	Prime editor (PE4/PE5/PEmax)	Plasmid transfection	Increased precise editing efficiency; reduced indels in human cells	Chen <i>et al.</i> , [27]
2017	Genetic disease models	Multiple loci	Adenine base editor (ABE7.10)	Plasmid transfection	Efficient A•T→G•C correction; high purity; minimal indels	Gaudelli <i>et al.</i> , [28]
2022	Alpha-1 antitrypsin deficiency	SERPINA1	Prime editor (PE2/PE3)	Electroporation	SERPINA1 (PiZZ) correction in patient-derived iPSCs (precise SERPINA1 correction 0.83%), separate genome-wide WGS in H9-iPE2 hESC showed no significant increase in genome-wide SNVs or indels	Habib <i>et al.</i> , [29]
2025	Genetic disease models	HEK3, RNF2	Prime editor (PEmax, PE-VLP)	Pseudoviral NanoScribes (VLPs)	Efficient precise editing in hiPSCs and HSC; high fidelity	Halegua <i>et al.</i> , [30]
2023	β -thalassemia	HBB (IVS1-110)	Adenine base editor (SpRY-ABE8e)	mRNA electroporation	Precise mutation correction; restored β -globin expression	Hardouin <i>et al.</i> , [31]
2023	Lesch–Nyhan syndrome	HPRT1	Cytidine base editor / Adenine base editor / Prime editor	Plasmid transfection / electroporation	Mutation correction; HPRT activity restored in patient fibroblasts	Jang <i>et al.</i> , [32]
2018	Congenital disorder of glycosylation type 1f; β -globin disorders	MPDU1; HBG1/HBG2 promoters	BE4max/AncBE4max; ABEmax	Nucleofection; plasmid transfection	Pathogenic SNP correction in patient fibroblasts; γ -globin activation and fetal-haemoglobin induction	Koblan <i>et al.</i> , [33]
2022	Duchenne muscular dystrophy	DMD (c.8713C>T)	Prime editor (PE2/PE3)	Plasmid transfection	Point mutation correction; dystrophin expression restored	Mbakam <i>et al.</i> , [34]
2022	Genetic disease models	HEK3, EMX1, RNF2	Prime editor (PE3 with epegRNA)	Plasmid transfection	Increased precise editing efficiency; low off-target activity	Nelson <i>et al.</i> , [35]
2020	Genetic disease models	BCL11A enhancer, HBG promoter	Adenine base editor (ABE8e)	Plasmid transfection	Enhanced A•T→G•C editing; expanded PAM compatibility	Richter <i>et al.</i> , [36]
2020	Aicardi–Goutières syndrome	SAMHD1	Adenine base editor / Prime editor	mRNA nucleofection	Pathogenic mutation correction in patient-derived hiPSCs	Sürün <i>et al.</i> , [37]

3.2. Overview of Included Studies

Table 4 summarises the disease settings, target genes, editor platforms, delivery strategies, and therapeutic outcomes reported across the 16 included primary studies. The evidence shows three main patterns:

1. Base editing generally performed best for eligible single-nucleotide transitions.
2. Prime editing is more adaptable for complex substitutions, insertions, deletions.
3. Regulatory changes while therapeutic success depends on functional restoration, delivery feasibility, and safety rather than editing efficiency alone.

3.3. Engineering Advances Enhancing Editing Precision in Human Cells

3.3.1. Optimisation of Base Editor Architectures in Human Systems

Recent advances in genome editing have focused on improving the accuracy, efficiency, and applicability of base and prime editing in human cells. Gaudelli *et al.*, [28] developed a genome-wide adenine base-editing approach for A•T to G•C conversion by fusing an evolved adenine deaminase to a catalytically impaired Cas9 nickase. In human cell lines, this system generated efficient A•T→G•C conversion with low indel formation, supporting adenine base editing as a high-efficiency platform. However, the study also highlighted persistent limitations, including narrow editing windows and bystander edits within the deaminase activity range.

Expanding these results, Koblan *et al.*, [33] found that intracellular expression level, rather than catalytic activity itself, was the initial and foremost predictor of base-editing efficacy. Nuclear localisation signal optimisation, codon bias, and reconstruction of the ancestral deaminase domains generated BE4max, AncBE4max, and ABEmax. These engineered editors demonstrated substantially improved performance in a variety of human cell types, repairing pathogenic single-nucleotide polymorphisms with higher precision than first-generation editors. Two- to four-fold improvements in editing efficiency were reported compared to ABE7.10, with improved allele-correction frequencies exceeding 70% beyond several disease-relevant sites and reduced bystander editing. These findings extended the work of Gaudelli *et al.*, [28] by demonstrating that editor expression, stability, and host-cell compatibility are important determinants of editing performance.

The remaining drawbacks of base editing were then reduced by Richter *et al.*, [36] through a systematic increase in Protospacer Adjacent Motif (PAM) compatibility and catalytic kinetic optimisation. Using phage-assisted continuous evolution, the authors constructed the ABE8e variant, which had a nearly 590-fold higher deamination rate compared to ABE7.10 and was more broadly compatible with a wider range of Cas proteins. Kinetic studies demonstrated a significantly faster onset of editing, reaching near-maximum activity within

24 h, compared to several days for earlier ABEs. Notably, ABE8e was found to be able to effectively edit genomic loci, including regulatory elements, that were previously inaccessible to base editors. This broader targeting range extended the capabilities described by Koblan *et al.*, [33]. However, increased off-target activity in early ABE8e variants necessitated further specificity-promoting modifications. Genome-wide profiling revealed an increase in off-target editing in both DNA and RNA of early ABE8e variants, thus identifying a measurable trade-off between efficiency and fidelity.

3.3.2. Optimisation of Prime Editing Systems in Human Cells

Chen *et al.*, [27] investigated cellular factors that constrain prime editing in human cells. Using pooled CRISPR-interference screens, they identified mismatch repair as an important suppressor of prime-editing efficiency. The PE4 and PE5 systems, which incorporated transient mismatch-repair inhibition, substantially improved editing outcomes across multiple human loci. These findings show that prime-editing performance is shaped not only by editor design but also by the repair environment of the target cell.

Chen *et al.*, [27] focused on repair-pathway modulation, whereas Habib *et al.*, [29] evaluated prime-editing fidelity in human embryonic stem cells. Using inducible prime-editor platforms, whole-genome sequencing, and a direct comparison with base editing, Habib *et al.*, [29] observed efficient and specific editing with few off-target variants. Genome-wide analysis detected no significant increase in mutations above background rates, supporting the safety and stability of optimised prime editing in therapeutically relevant human cells.

3.4. Expansion of Editing Capability Beyond Single-Nucleotide Variants

3.4.1. Large Insertions, Deletions, and Replacements Using Prime Editing

The ability of genome-editing tools to move beyond single-nucleotide replacement has expanded the therapeutic scope of precision editing in human cells. Anzalone *et al.*, [23] addressed a key limitation of conventional base and prime editing by developing twin prime editing for larger insertions, deletions, inversions, and sequence replacements. This approach uses two pegRNAs to write complementary DNA flaps that can recombine into a defined edited sequence, increasing the range of variants that can be corrected.

Deep sequencing showed precise replacement efficiencies of 20-40% at tested loci and up to 5% for multi-kilobase edits. Comparative genome-wide profiling did not reveal an increase in guide-independent mutations above background. Twin prime editing therefore expanded the ability to perform larger programmed genomic changes, although it required more complex pegRNA design and coordination than canonical prime editing. Efficiency remained dependent on locus, edit size, and genomic context. The capacity for exon-scale correction extends therapeutic scope beyond the variants

accessible to base editing, but it also highlights a practical trade-off between editing breadth and design and delivery complexity.

3.4.2. Regulatory and Promoter Editing for Therapeutic Gene Modulation

In an alternative approach to increased editing capability, Chalumeau *et al.* [26] targeted non-coding regulatory regions rather than protein-coding sequences. They achieved re-expression of foetal haemoglobin by rewriting γ -globin promoters in human haematopoietic cells through prime editing. They achieved approximately 50% accurate editing in human cell lines, and tailorable yet clinically meaningful editing in patient-derived Hematopoietic Stem and Progenitor Cells (HSPC), introducing several mutations associated with hereditary persistence of Foetal Haemoglobin (HPFH) into the HBG1/2 promoters. Interestingly, compounding regulatory edits led to additive action(s) in increased γ -globin expression compared to expression produced by single edits. No significant change in erythroid differentiation markers was observed by RNA sequencing, but levels of gamma-globin transcripts were up to 3x higher than normal. Unlike structural editing strategies, this approach to regulatory editing involves changing regulatory elements instead of directly changing coding elements. In comparison to Anzalone *et al.*, [23], Chalumeau *et al.*, [26] placed emphasis on transcriptional modulation instead of genomic restructuring, which enabled functional rescue through comparatively subtle molecular interventions. However, some editing was more efficient than others depending on the donor, suggesting biologic heterogeneity between primary human stem cell populations.

Regulatory editing was complemented with base editors by Koblan *et al.*, [33]. The authors designed and performed mutations in the promoters to boost adenine and cytidine base editing expression in human cells. Editing levels of over 70% were achieved for selected promoter regions and resulted in uniform upregulation of target-gene expression across all cell types. While base editing is more convenient than the prime-editing approach used by Chalumeau *et al.*, [26] for its ease of guide design and high efficiency in isolated regulatory substitutions, it was not possible to make the same combinations of changes in a single allele. Base editing is thus more efficient for single eligible regulatory variants while prime editing is more versatile for combinatorial regulatory rewriting.

3.5. Disease-Specific Therapeutic Applications in Human Models

3.5.1. Neuromuscular Disorders

Three related but different approaches toward correction were used in these three types of neuromuscular studies: initial correction of the DMD c.8713C>T variant, modifying the splice site(s) to favor exon skipping, and restructuring the gene at the exon level using twin primed editing [23, 25, 34]. The direct correction after RTT was below 20%, and the indel rate was under 5%, while the exon-skipping methods showed an increased level of applicability of the correction method across mutation classes, but results were variable at the level of

pegRNA architecture and modulation of splice-site usage. To enable editing of multi-kilobase deletions and insertions, twin prime editing was developed, but this added new features in the editing procedure involving guide design and delivery. The comparative trend is therefore a compromise between specificity and range: direct editing has the highest mutation specificity, exon skipping can be applied to multiple mutation types, while twin prime can be applied to target a wide range of mutations.

The processes of functional evidence were also varied between these approaches. Exon skipping conditions were optimized and up to 40% of dystrophin transcripts could be represented as exon-spliced isoforms, compared to an improved correlation of sequence repair and dystrophin restoration with direct correction [25, 34]. Comprehensively, though, all strategies tested have been primarily performed in human myogenic in vitro models, where local administration of gene constructs to a small number of cells is more easily achieved than systemic administration to skeletal and cardiac muscle. Therefore, achieving broad, accurate, and safe tissue distribution, along with adequate stability, remains the common challenge for both editing approaches. The length of the target sequence in the reverse-transcriptase template also demonstrates that target sequence design is a crucial factor in the performance of the prime-editing system in a DMD model.

3.5.2. Hemoglobinopathies and Blood Disorders

In all haemoglobinopathies, two therapeutic approaches were observed in clinically relevant HSPC: direct correction of a pathogenic HBB allele and regulatory re-activation of foetal haemoglobin. To correct the mutation (HBB IVS1-110) in these CD34+ HSPC, Hardouin *et al.*, [31] tried using SpRY-ABE8e mRNA electroporation, which resulted in high editing success, restoration of beta-globin expression, and unaltered erythroid differentiation. Chalumeau *et al.*, [26] in contrast designed new HBG1/HBG2 promoters that were then incorporated into the genome at previously established disease-causing alleles, which resulted in an elevated level of gamma-globin, but not in the elimination of the disease-causing alleles themselves. The results demonstrate the potential that base editing possesses in situations where a suitable transition and delivery option exists. Promoter editing is a mutation-independent approach that could benefit genetically diverse patients.

Comparative evidence also makes different risks apparent. Direct HBB correction is allele-specific, restores the physiological beta-globin pathway, and is constrained by the editing window, bystander effects, and PAM availability. Regulatory editing was employed to avoid repeated design cycles for each mutation and led to additive increases in gamma-globin, yet there was variability in the efficiency between donors and combination of promoter edits [26, 31, 33]. In both strategies the maturation of erythroid progenitors was generally maintained, and the ability to produce functional haemoglobin was the most informative translational endpoint.

In comparison to solid tumour disorders, the haemoglobinopathy toolkit is more clinically developed as HSPC can be modified ex vivo, quality controlled and re-

transplanted autologously, thus providing milder treatment side effects. Lineage-specific functional recovery for the IVS1-110 mutation reflects the association between efficient correction and restoration of haemoglobin production in erythroid progeny.

3.5.3. Cystic Fibrosis

In research on cystic fibrosis, the function of prime editing was tested exclusively in primary airway epithelial cells or organoids derived from patients, and the corrected sequence was directly connected to CFTR processing and chloride-channel function. Bulcaen *et al.* [24] found that they could correct drug-ineligible CFTR mutants and restore protein maturation, plasma-membrane localisation, and channel activity for N1303K in CFTR. Sousa *et al.*, [22] targeted the common F508del mutation, and designed a combination of pegRNA, editor-expression and delivery optimisation to restore CFTR chloride transport. Both studies demonstrated significant functional rescue for cells with reasonable correction frequencies, where the corrected cells substantially participated in epithelial ion transport.

Both haemoglobinopathy and cystic fibrosis studies demonstrate excellent phenotype-level validation, but delivery for the CF model is more challenging *in vivo*, and requires repeated targeting of airway epithelia, whereas HSPC delivery, although cumbersome, is more tractable *ex vivo*. Bulcaen *et al.*, [24] recovered N1303K glycosylation, membrane localisation, and channel function, whereas Sousa *et al.*, [22] found that optimisation of the delivery system of the prime edited sequences and enhancement of the prime editor allowed functional repair of F508del. These studies all set functional restoration as a clear go/no-go benchmark for future translation, with only tissue coverage, durability and pulmonary delivery at larger scales remaining to be determined.

3.5.4. Rare Monogenic and Metabolic Disorders

Phenotype-level restoration is, especially in rare-disease models, an essential, translation-relevant endpoint. In hiPSCs, Sürün *et al.*, [37] showed disease-relevant correction, and in patient fibroblasts, Jang *et al.*, [32] correlated sequence correction with biochemical and cellular outcomes relevant to the disease. Therapeutic relevance was assessed by functional recovery, including human system in the reported cell models, as well as by editing frequency, which was kept below 2% in all cell models reported, further emphasizing that the assessing systems need to be suitable for the type of functional benefit sought.

3.6. Delivery Strategies for Human Genome Editing

3.6.1. Non-Viral and Transient Delivery Approaches

Non-viral delivery methods are broadly employed for therapeutic genome editing because of their ability to expose the genome only for a limited time, and their potential for decreasing the risk to the genome in the long term. In human myogenic cells, Mbakam *et al.*, [34] evaluated plasmid-based and electroporation-based prime editing and found that the efficiency of editing was strongly affected by the delivery feasibility. These results showed that the delivery condition should be considered with the editor performance.

Although the efficiency was not high, editing frequencies were still below 10% and the indel rates were consistently below 2% placing the constructs in a favourable range of safety in relation to their inability to achieve high efficiency. By comparison, Hardouin *et al.*, [31] explicitly tackled the effectiveness of delivery by using mRNA electroporation to transfect patient-derived CD34⁺ haematopoietic stem and progenitor cells with adenine base editors. This was employed to correct the IVS1-110 β -thalassaemia mutation; the efficiency of this was around 80% without altering stem-cell characteristics, self-renewal, or erythroid differentiation. When compared to plasmid delivery, mRNA electroporation resulted in sustained expression of the editors and caused less cell stress and toxicity. The properties of these features render *ex vivo* editing feasible, especially in haematological diseases, where autologous stem-cell transplantation is already in use.

Corrective efficiencies of over 75% led to near normal haemoglobin production in differentiated erythroid progeny further reinforcing the functional relevance. To tackle the delivery related constraints, Nelson *et al.*, [35] optimized the molecular cargo. The prime-editing efficiency and the stability of the RNA were increased in human cell lines and primary human fibroblasts by the addition of structured RNA to the 3' end of the pegRNAs, with indel frequencies <1%. While not a direct comparison of delivery platforms, the study demonstrated that the improvements were around 3 to 4-fold in the case of engineered pegRNAs, suggesting that cargo architecture can partially mitigate suboptimal delivery conditions.

3.6.2. Pseudoviral and Advanced Delivery Systems

The purpose of advanced delivery systems is to overcome the efficiency and scalability issues of traditional non-viral delivery systems in specific human stem-cell populations. Halegua *et al.*, [30] created new virus-like particles, called NanoScribes, to deliver prime editing components as ribonucleoprotein complexes. The platform was designed to minimize the delivery of exogenous DNA to ensure that nuclease activity is not sustained. The editing efficiencies had optimized fusogens and stabilized epegRNAs of 68-72% in HEK293T cells and approximately 25% in human iPSCs and haematopoietic stem cells.

When compared to plasmid delivery, delivery of the NanoScribe kept indel frequencies below 1%, and reduced the activity of bystander cells, while similar effect seen upon transient exposure. The system also caused editing in stem cells that were otherwise difficult to transfect and did not affect expression of OCT4 and SOX2 nor the clonogenic capacity, confirming maintenance of identity of the edited cells. However, these limitations of clinical translation are still complicated by the complexity of production, batch-to-batch consistency, scale-up of manufacturing, tissue targeting and regulatory characterisation. Optimised mRNA electroporation was not always superior to VLP delivery across all cell types. It would therefore be advisable to consider this as a complementary platform rather than a universal substitute. Furthermore, it will be dependent on reproducible manufacturing and efficient delivery to the target tissue.

The preservation of stemness markers and clonogenic capacity support the short-term cellular compatibility of NanoScribe delivery [30]. However, translational assessment must extend beyond editing efficiency to include reproducible manufacturing, potency testing, biodistribution, immunogenicity, long-term safety, and compatibility with disease-specific clinical workflows.

3.7. Editing Outcomes, Fidelity, and Translational Readiness

3.7.1. Editing Precision and Safety Profiles

The therapeutic potential of base and prime editing in human systems relies on the precision of editing as well as genomic safety. Gaudelli *et al.*, [28] engineered adenine base editing by combining an evolved Adenine Deaminase with a Cas9 nickase, which can convert A•T to G•C without introducing any DSBs. Efficient editing with fewer indels was achieved using this platform with human cell lines, though its efficacy was limited by the availability of PAM, the position of the editing window and a bystander deamination window of 4-6 nucleotides.

Chen *et al.*, [27] followed up this work to demonstrate that prime-editing efficiency can vary across human cell types and genomic targets. CRISPR-Interference (CRISPRi) screening identified mismatch repair as a key inhibitor of prime-editing efficiency. This comparison demonstrates that prime editing is programmable and is tightly coupled to cellular repair pathways.

Habib *et al.*, [29] took fidelity assessment one step further and tested prime editing in human embryonic stem cells. Minimal rates of guide-independent off-target mutations were observed when expressing the editor under an inducer. Prime editing generated fewer bystander effects and RNA-level edits than base editing but showed lower efficiency in some human systems, which remains a major hurdle for clinical translation.

3.7.2. Translational Readiness and Clinical Applicability

Optimal delivery remains a key translational challenge as the optimal approach varies depending on the cell type, tissue access, editor size, and transient exposure requirement. Ultimately, because the functional potential of modest efficiency in prime editing in patient-derived CFTR models can produce meaningful functional rescue, Bulcaen *et al.*, [24] noted that outcomes at the phenotype level are crucial to consider when evaluating therapeutic potential.

Similarly, Sousa and colleagues [22] showed that model-specific optimization can boost the feasibility of prime editing in human primary airway epithelial cells, highlighting the need for optimal editor design and delivery for different target systems.

Though important, delivery is still a tremendous hurdle in clinical translation, especially in human stem-cell systems. To solve this problem, Halegua *et al.*, [30] proposed to transiently deliver prime-editing components to the cell surface with NanoScribe virus-like particles. This strategy facilitated editing, allowed the editors to be used at low toxicity, enabled

transient exposure to the editors, and was more efficient in human induced pluripotent stem cells and haematopoietic stem cells. The key challenge, however, is to produce these materials in a scalable manner, to target the tissue specifically, and to be able to reproduce their manufacturing.

4. DISCUSSION

A key advancement over the standard CRISPR-Cas9 system is the emergence of base editing and prime editing, which allow for engineered modifications of DNA without the intention to create DSBs. In therapeutic genome editing, minimizing indel formation, chromosomal rearrangements, and genotoxic stress becomes an important factor, while the lack of DSB in their design can abate some negative repair outcomes [5]. The absence of intentional DSB formation does not eliminate other safety and translational concerns, including bystander editing, incomplete repair, and delivery-related toxicity.

Comparative evidence suggests that the two platforms have different strengths. The efficiency of base editing highly depends on eligible mutations like A→G or C→T substitutions including variants associated with haemoglobinopathies [7]. It has been limited by availability of PAM, editing window size, off-target deamination and bystander editing at neighboring bases [29, 32]. In addition to base editing, prime editing also supports small insertions, deletions, and substitutions without the need for a donor DNA template [38]. This is of specific relevance to CFTR correction in 2D airway epithelial and organoid models [22]. Nevertheless, prime-editing efficiency varies across tissues and cell types, with primary muscle, airway epithelial, and fibroblast cells generally presenting greater challenges than immortalised cell lines. These differences are likely associated with delivery conditions, pegRNA design, and cellular repair pathways, but not an inherent stem cell platform limitation [29, 32].

One noteworthy conclusion from these studies is that editing efficiency alone does not determine therapeutic value. Even modest levels of correction can produce clinically meaningful CFTR functional rescue in relevant airway epithelial and organoid models [22, 24]. By comparison, Duchenne muscular dystrophy has much more widespread delivery and tissue coverage needs [34, 25]. Haemoglobinopathies present a comparatively favourable translational context because HSPC can be edited *ex vivo*, characterised before autologous transplantation, and assessed for functional recovery [26, 31]. Thus, clinical readiness depends not simply on the percentage of edited cells but also on the proportion of cells that must be corrected, anatomical distribution, durability of functional rescue, and delivery feasibility.

The delivery aspect is thus still a formidable challenge, especially for tissues that lack *ex vivo* methods for delivery. While the delivery to HSPC and mRNA-based delivery are effective, it is harder to deliver these to skeletal muscle, pulmonary epithelium, and other *in vivo* targets [16]. All three delivery strategies, non-viral, pseudoviral, and viral face

distinct challenges related to immunogenicity, editor size, tissue access, and manufacturing scalability.

Safety and regulatory considerations are also a crucial part of clinical translation. Base-editing and prime-editing technologies lessen dependence on DSB-mediated repair [38-40]. Thus, regulators will expect strong evidence for: genome integrity; off-target activity; long-term safety; and Chemistry, Manufacturing and Controls (CMC) [38, 41]. Considering the implications for future generations from germline editing, as well as other issues regarding ethics, also argues for a somatic-only approach [41].

The two technologies, base editing and prime editing, show therapeutic potential from the 16 systematically selected primary studies, depending on the type of mutation, delivery technology and human cell context (Table 5).

LIMITATIONS OF THE REVIEW

The limited scope of this review reflects the variety of human cell types, delivery methods, outcome measures, and

editor architectures assessed. Few human-relevant studies met the inclusion criteria, further making quantitative meta-analysis difficult.

Further, there are still numerous studies at an early stage included under this umbrella with various endpoints reported such as editing efficiency, specificity, viability and functional restoration [32]. This variability can be a problem in determining the threshold for clinical readiness. Hopefully, in the future, more studies will report standardised safety and functional outcomes on more similar human models, which will strengthen future reviews. As the evidence base expands, transparent machine-learning methods may help integrate heterogeneous molecular, delivery, safety, and functional outcome variables; cross-domain AI studies demonstrate complementary strategies for unstructured-data extraction, prediction from historical and contextual features, and explanation of model outputs [42-44]. Such methods would require biological validation and should supplement, rather than replace, mechanistic and experimental evidence.

Table 5. Decision framework for base editing and prime editing.

Factor	Base Editing	Prime Editing
Mutation Type	Ideal for single-nucleotide transitions (A→G, C→T)	Suitable for complex mutations (insertions, deletions)
Efficiency	High efficiency in target systems (e.g., hematologic disorders)	Lower efficiency in certain primary human systems
Target Range	Limited to specific mutations with PAM sites	Broader range including regulatory sequences
Delivery Systems	Effective with non-viral systems (e.g., electroporation)	More complex delivery systems required
Clinical Relevance	Best for diseases like beta-thalassemia, sickle cell	Best for diseases requiring complex edits like CFTR
Regulatory and Ethical Considerations	Regulatory pathway still evolving; specificity data accumulating	Regulatory pathway still evolving; germline-editing ethical considerations apply as they do for base editing

CONCLUSION

This review illustrates that base editing and prime editing are significant DSB-free alternatives to the conventional CRISPR-Cas9 system. Based on human primary cells, organoids and stem-cell systems, evidence indicates that base editing is most efficient when the target SNP is within the editing window. Base editing performs optimally when bystander editing can be avoided, while bystander editing remains one of its principal limitations. In contrast, prime editing can be more versatile and precise, making it suitable for complex replacements, indels and regulatory changes and generally exhibiting good fidelity in several key human cell systems, albeit context-dependent efficiency in a few. The functional rescue percentage is a better predictor of therapeutic value than simply editing percentage, and this is true of all models of CF, Duchenne muscular dystrophy, haemoglobinopathy and rare monogenic models. Ongoing improvements in pegRNA engineering, mismatch-repair modulation, mRNA/electroporation delivery, and pseudoviral VLP systems may contribute to improved clinical readiness. In conclusion, in the diverse and rapidly evolving field of precision genome medicine, base editing is more powerful for

diseases that require only single-base transition changes, while prime editing is more versatile.

LIST OF ABBREVIATIONS

ABE	=	Adenine Base Editor
CBE	=	Cytidine Base Editor
CF	=	Cystic Fibrosis
CMC	=	Chemistry, Manufacturing and Controls
CRISPRi	=	CRISPR Interference
DMD	=	Duchenne Muscular Dystrophy
HSC	=	Haematopoietic Stem Cell
HSPC	=	Hematopoietic Stem and Progenitor Cells
iPSCs	=	induced Pluripotent Stem Cells
PAM	=	Protospacer Adjacent Motif
PE	=	Prime Editor
pegRNA	=	Prime-Editing Guide RNA
VLP	=	Virus-Like Particle

AUTHOR'S CONTRIBUTION

The author was responsible for study conceptualization, methodology design, data compilation, analysis and interpretation, and manuscript writing.

CONSENT FOR PUBLICATION

Not applicable.

REPORTING GUIDELINES

This study was conducted in accordance with PRISMA guidelines.

AVAILABILITY OF DATA AND MATERIALS

The data will be made available on reasonable request by contacting the corresponding author [A.H.].

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The author declares no conflict of interest.

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DECLARATION OF AI

Author declares usage of ChatGPT for language editing and manuscript refinement. They approve the final manuscript and take responsibility for the published content.

APPENDIX

An official website of the United States government [Here's how you know](#)

NIH National Library of Medicine
National Center for Biotechnology Information

PubMed®

("base editing" OR "prime editing") AND (human cells) AND (genome editing) **Search**

Advanced Create alert Create RSS User Guide

Save Email Send to Sort by: Best match Display options

MY CUSTOM FILTERS 347 results Page 1 of 35

RESULTS BY YEAR

Filters applied: English, Humans. [Clear all](#)

☐ Search-and-replace **genome editing** without double-strand breaks or donor

Fig. (A1). Database search outcomes for PubMed.

Searching: **CINAHL Ultimate**

base editing OR prime editing AND single-nucleotide mutations AND gene therapy AND human clinical

All filters (0) Full Text Peer Reviewed All dates Source type

All results Evidence-based care sheets Quick lessons Video

Results: 177 Show: 10 Relevance

0 selected

1 Peer reviewed | Academic Journal

Fig. (A2). Database search outcomes for CINAHL.

The screenshot shows the Scopus search interface. At the top, there's a search bar with the query 'base editing OR prime editing AND human cells AND genome editing'. Below the search bar, there are options to 'Save search', 'Set search alert', and 'Add search field'. The search results are displayed under the 'Documents' tab, showing '64 documents found'. A table of results is visible with columns for 'Document title', 'Authors', and 'Source'. The first result is 'Kolanu ND. CRISPR-Cas9 Gene Editing: Curing Genetic Diseases by Inherited Epigenetic Modifications. Glob Med Genet. 2024;11(1): 113-22.' followed by its DOI link.

Fig. (A3). Database search outcomes for Scopus.

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