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RNA-Targeting Therapeutics via CRISPR-Cas13: Mechanisms, Applications, and Future Directions — A Narrative Review

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ABSTRACT

Background: *Pathogenic RNA transcripts underlie a substantial proportion of human diseases, yet existing RNA-targeting platforms — antisense oligonucleotides and RNA interference — are constrained by mechanistic inflexibility, saturation of endogenous machinery, and inability to perform precise transcript editing. CRISPR-Cas13, a family of RNA-guided RNA endonucleases, has emerged as a programmable, reversible alternative capable of targeted transcript knockdown, precise base editing, and splicing modulation without altering genomic DNA.* ***Objective:*** *This narrative review synthesises current evidence on the mechanisms, therapeutic applications, delivery platforms, safety considerations, and ethical dimensions of the CRISPR-Cas13 system, with the objective of providing a structured, evidence-grounded account of its translational trajectory as of 2024.* ***Methods:*** *A structured literature search was conducted across PubMed/MEDLINE, Scopus, Web of Science, and Embase, covering January 2016 through October 2024. Search terms combined Cas13 subtype nomenclature with therapeutic and disease-specific keywords. Articles were screened for relevance to Cas13 mechanisms, applications, or translational barriers; English-language peer-reviewed publications and pre-reviewed preprints with corroborating evidence were eligible. The review was organised thematically across eight conceptual domains and reported in accordance with the Scale for the Assessment of Narrative Review Articles (SANRA).* ***Results:*** *Across nine therapeutic application areas, CRISPR-Cas13 — primarily Cas13d (CasRx) — has demonstrated robust preclinical efficacy, including phenotypic rescue in Huntington's disease rodent models, potent antiviral activity against SARS-CoV-2 and HIV-1, and selective oncogenic mRNA knockdown in xenograft cancer models. Catalytically dead Cas13 fused to adenosine deaminase (dCas13-ADAR) enables A-to-I RNA base editing with measurable codon correction in patient-derived cell lines. Lipid nanoparticles and AAV vectors represent the dominant delivery modalities; collateral RNase activity, immunogenicity, and transient effect duration remain the principal translational barriers.* ***Conclusion:*** *CRISPR-Cas13 constitutes a promising and mechanistically distinct RNA-targeting platform with broad therapeutic reach. Realisation of its clinical potential depends on engineering of collateral-minimised Cas13 variants, development of tissue-specific delivery architectures, and initiation of first-in-human trials in monogenic disorders where reversibility of effect offers a meaningful safety advantage over permanent DNA editing.*

Keywords: *CRISPR-Cas13; RNA editing; RNA therapeutics; gene therapy; transcriptome engineering; lipid nanoparticles; RNA-guided endonuclease*

INTRODUCTION

Aberrant RNA expression and pathogenic RNA transcripts underlie a substantial proportion of human diseases, from monogenic disorders driven by gain-of-function point mutations to multifactorial conditions characterised by dysregulated splicing, oncogenic fusion transcripts, and viral RNA intermediates. Unlike DNA, RNA is inherently transient and modifiable without altering heritable genetic information, making it an attractive pharmacological target. Early strategies for RNA-targeted therapy, including antisense oligonucleotides (ASOs) and RNA interference (RNAi), demonstrated that sequence-specific suppression of pathogenic transcripts was both feasible and clinically impactful, as evidenced by the regulatory approval of agents such as nusinersen for spinal muscular atrophy and

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patisiran for transthyretin amyloidosis (1, 2). Nevertheless, these platforms impose important constraints: ASOs require repeated systemic dosing to maintain therapeutic effect and are susceptible to off-target hybridisation, while RNAi is mechanistically restricted to mRNA degradation and relies on endogenous RISC-loading machinery whose saturation can perturb normal miRNA-mediated gene regulation (2, 3). These limitations sustain an unmet need for a more programmable, versatile, and mechanistically distinct RNA-targeting platform.

The emergence of CRISPR-based genome-editing technologies over the past decade has fundamentally transformed the therapeutic landscape. Canonical CRISPR-Cas9 and Cas12 systems exploit a guide RNA (gRNA) to direct an endonuclease to a complementary DNA locus, where it introduces a double-strand break that can be exploited for gene disruption or homology-directed repair (3). Although these DNA-editing platforms have yielded landmark clinical results — including functional cures for sickle-cell disease and beta-thalassaemia — they carry inherent risks: permanent off-target DNA mutations may produce oncogenic lesions, and germline editing raises serious ethical concerns regarding heritable modification of the human genome (4). RNA editing offers a conceptually distinct and arguably safer alternative. Because modifications at the RNA level are reversible upon transcript turnover, therapeutic effects are transient by nature, which simultaneously limits the consequence of any off-target event and permits dose titration in a manner impossible with irreversible DNA edits (4, 5).

CRISPR-Cas13, the most recently characterised class of CRISPR effectors, is distinguished by its exclusive targeting of single-stranded RNA rather than DNA. First described in 2016 when Abudayyeh and colleagues demonstrated that C2c2 (subsequently renamed Cas13a) functions as a single-component, RNA-guided RNA endonuclease, the Cas13 family has expanded to encompass at least four orthologous subtypes — Cas13a through Cas13d — each exhibiting distinct biochemical properties, sequence preferences, and organismal distributions (5, 6). Upon binding its target RNA through Watson-Crick complementarity with a programmable CRISPR RNA (crRNA), Cas13 undergoes a conformational activation that triggers its Higher Eukaryotes and Prokaryotes Nucleotide-binding (HEPN) RNase domains, producing targeted transcript cleavage (6, 7). This mechanism confers several advantages relevant to therapeutic development: the absence of a protospacer adjacent motif (PAM) requirement allows targeting of virtually any RNA sequence; multiplexed targeting with a single effector is feasible through delivery of multiple crRNAs; and catalytically dead variants (dCas13) can be fused to RNA-modifying domains such as adenosine deaminases to enable precise base editing at the transcript level without inducing cleavage (7, 8).

The therapeutic reach of CRISPR-Cas13 spans a broad disease spectrum. In neurodegenerative disease, Cas13d-mediated knockdown of mutant huntingtin mRNA has produced significant phenotypic rescue in rodent models of Huntington's disease, while targeting of tau and alpha-synuclein transcripts offers analogous promise for Alzheimer's disease and Parkinson's disease respectively (9). Oncological applications encompass the suppression of oncogenic mRNAs including mutant KRAS and pathological fusion transcripts, as well as the modulation of tumour-associated long non-coding RNAs (4, 8). In the context of infectious disease, Cas13 has been deployed against the RNA genomes of SARS-CoV-2, influenza virus, and HIV-1, demonstrating potent antiviral activity in cell culture and animal models (3, 8). Beyond transcript knockdown, expanded toolsets — including the reversible Cas13-mediated RNA oligo-deletion (rcROD) system and RNA base editors incorporating adenosine or cytidine deaminase domains — now permit precise correction of pathogenic point mutations at the mRNA level, broadening the mechanistic repertoire available for therapeutic application (10). Delivery modalities have similarly

matured; lipid nanoparticles (LNPs), having achieved clinical validation through mRNA vaccine programmes, now represent the leading non-viral vehicle for co-delivery of Cas13 mRNA and crRNA, while adeno-associated virus (AAV) vectors and next-generation polymer-based nanoparticles continue to be refined for tissue-specific applications (13).

Despite this rapid progress, several challenges impede the clinical translation of CRISPR-Cas13. Chief among these is the collateral RNase activity intrinsic to activated Cas13 enzymes, whereby non-specific cleavage of bystander transcripts may disrupt cellular RNA homeostasis — an effect that has been shown to limit utility in eukaryotic systems when guide RNA design is suboptimal (4, 12). The immunogenicity of bacterially-derived Cas13 proteins in mammalian hosts, the packaging constraints of larger Cas13 orthologues within AAV capsids, and the transient duration of RNA editing effects all represent additional barriers requiring engineering solutions or novel delivery architectures (12, 13). Furthermore, the ethical and regulatory framework governing RNA editing, while meaningfully distinct from the germline editing debate, demands rigorous prospective evaluation of tissue specificity, dosing protocols, and re-administration safety (14). These interconnected challenges underscore the importance of a consolidated synthesis of current mechanistic knowledge, preclinical evidence, and translational barriers, particularly given the pace at which new Cas13 variants, editing tools, and delivery systems have been reported since 2016.

Although several primary research articles and focused commentaries have addressed discrete aspects of the CRISPR-Cas13 system, no current narrative review comprehensively integrates the mechanistic basis of RNA targeting, the evolving landscape of Cas13-derived therapeutic tools, the evidence base across major disease categories, and the delivery, safety, and ethical dimensions that will govern clinical translation. The present narrative review addresses this gap by synthesising peer-reviewed literature published from 2016 — coinciding with the initial characterisation of Cas13a — through 2024, with the objective of providing a structured, evidence-grounded account of the current state and near-term trajectory of CRISPR-Cas13 RNA-targeting therapeutics. It is anticipated that this synthesis will serve as a reference resource for researchers, clinicians, and translational scientists navigating this rapidly evolving field.

METHODS

This article is a narrative review, a design chosen deliberately to accommodate the breadth, mechanistic complexity, and rapid evolution of the CRISPR-Cas13 literature. A formal systematic review or meta-analysis was considered but deemed inappropriate given the current heterogeneity of study designs, outcome measures, and model systems across published Cas13 research — conditions that preclude meaningful quantitative pooling. The narrative approach allows integration of mechanistic studies, in vitro proof-of-concept experiments, in vivo disease models, and translational commentary within a single coherent synthesis, and it enables expert contextualisation of evidence that structured systematic methods would compartmentalise. The reporting of this review was guided by the Scale for the Assessment of Narrative Review Articles (SANRA), a validated instrument for evaluating the scientific rigour and transparency of narrative reviews, against which the present manuscript was prospectively mapped during drafting. Registration in PROSPERO or OSF is not required for narrative reviews; this is acknowledged as a transparency limitation relative to prospectively registered systematic reviews.

A structured literature search was conducted across four electronic databases: PubMed/MEDLINE, Scopus, Web of Science, and Embase. Searches were performed in

October 2024 and covered publications from January 2016 — the year of Cas13a's initial characterisation — through October 2024. The core search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords using Boolean operators. The primary query applied across all databases was: ("CRISPR-Cas13" OR "Cas13" OR "C2c2" OR "CasRx" OR "Cas13a" OR "Cas13b" OR "Cas13c" OR "Cas13d") AND ("RNA targeting" OR "RNA editing" OR "RNA knockdown" OR "RNA therapeutics" OR "gene therapy" OR "transcriptome engineering"). Secondary searches appended disease-specific terms including "neurodegeneration," "cancer," "viral infection," "Huntington's disease," "Alzheimer's disease," "HIV," and "SARS-CoV-2" to retrieve targeted application evidence. Additional searches incorporating delivery-specific terms — "lipid nanoparticle," "AAV," "adeno-associated virus," "polymer nanoparticle" — and ethical/regulatory terms — "gene therapy regulation," "RNA editing ethics" — were conducted to ensure comprehensive thematic coverage. Grey literature, including preprint servers (bioRxiv and medRxiv), was manually scanned to identify emerging findings not yet indexed in the primary databases, though preprints are cited only where peer-reviewed corroboration exists.

Literature inclusion was guided by the following criteria: (i) peer-reviewed primary research articles, reviews, or commentaries reporting original findings or evidence syntheses related to the CRISPR-Cas13 system; (ii) articles addressing mechanistic, therapeutic, delivery, safety, ethical, or regulatory aspects of Cas13-based tools; (iii) English-language publications; and (iv) publications available in full text. Articles were excluded if they addressed exclusively CRISPR-Cas9, Cas12, or other non-Cas13 effectors without direct comparative relevance to Cas13, if they were conference abstracts without associated peer-reviewed publications, or if they were duplicated across database results. Initial title and abstract screening was performed by the primary author (Z.A.), with a random 30% sample independently reviewed by a second author (H.A.A.) to assess consistency; discrepancies were resolved through discussion with the senior author (T.B.). Full-text review was applied to all articles passing initial screening. Because this is a narrative rather than systematic review, no formal PRISMA flow diagram is presented; however, the selection process and rationale are disclosed here to maximise transparency.

The synthesised literature was organised thematically across the following conceptual domains, which correspond to the principal sections of this review: (i) the biochemical basis and structural organisation of the CRISPR-Cas13 system; (ii) mechanisms of RNA recognition and target cleavage; (iii) advances in Cas13-derived RNA editing tools; (iv) therapeutic applications across disease categories; (v) delivery strategies and in vivo translation; (vi) safety, off-target, and immunogenicity considerations; (vii) ethical and regulatory dimensions; and (viii) future directions in Cas13 engineering and clinical development. Within each thematic domain, evidence is presented from mechanistic foundations toward clinical translation, with explicit differentiation between in vitro, in vivo preclinical, and clinical-stage findings where such classification is determinable from the source publications. Where multiple studies report divergent findings on a common question — such as the extent of Cas13 collateral activity across cell types — these discrepancies are reported and interpreted rather than reconciled by authorial consensus. The authors acknowledge that the narrative selection process, while conducted systematically in intent, is subject to inherent selection bias; findings from studies with null or negative results may be underrepresented in the published literature, and this review is correspondingly limited in its ability to map the full distribution of evidence. Readers should interpret the synthesis as an expert-guided integration of available evidence rather than an exhaustive or unbiased enumeration of all relevant publications.

Table 1. Therapeutic Applications of CRISPR-Cas13 RNA Editing: Disease Area, Cas13 Subtype, Model System, Evidence Stage, and Key Findings

Disease Area	Target / Therapeutic Goal	Cas13 Subtype	Model System	Evidence Stage	Key Finding	Ref.
Neurodegenerative (Huntington's disease)	Knock down mutant huntingtin mRNA	Cas13d (CasRx)	Rodent model (AAV delivery)	Preclinical (in vivo)	Significant reduction of mHTT protein; phenotypic rescue of motor deficits	(9)
Neurodegenerative (Alzheimer's disease)	Reduce Tau and APP mRNA levels	Cas13a / Cas13d	Human iPSC-derived neurons; mouse models	Preclinical (in vitro / in vivo)	Selective Tau knockdown without off-target neuronal toxicity at optimised guide densities	(4, 11)
Neurological (Glia-to-neuron conversion)	Convert astrocytes to functional neurons	CasRx (Cas13d)	Mouse model of neurological injury	Preclinical (in vivo)	Robust glia-to-neuron reprogramming; alleviated disease symptoms in mice	(11)
Viral Infection (HIV-1)	Degrade early HIV-1 mRNA transcripts; prevent viral replication	Cas13a	Cell culture; humanised mouse models	Preclinical (in vitro)	Potent suppression of viral RNA load; low-virulence variants explored for prevention	(8)
Viral Infection (SARS-CoV-2 / Influenza)	Cleave viral RNA genome to inhibit replication	Cas13d	Human lung cell lines; hamster models	Preclinical (in vitro / in vivo)	Broad-spectrum antiviral activity; rapid re-programmability for emerging variants	(3, 8)
Cancer (KRAS-mutant)	Knock down mutant KRAS mRNA and oncogenic fusions	Cas13a / Cas13b	Cancer cell lines; xenograft mouse models	Preclinical (in vitro / in vivo)	Selective suppression of G12D KRAS; reduced tumour growth in xenograft models	(4)
Cancer (CasRx splicing modulation)	Correct aberrant pre-mRNA splicing in oncogenesis	CasRx (Cas13d)	Human cancer cell lines	Preclinical (in vitro)	Programmable splicing modulation without permanent genomic alteration	(10)
Genetic Disorders (monogenic)	Correct pathogenic point mutations via A-to-I or C-to-U RNA base editing	dCas13 fused to ADAR / APOBEC	HEK293T cells; patient-derived fibroblasts	Preclinical (in vitro)	Efficient base conversion at target codons; no detectable genomic DNA editing	(7, 10)
Metabolic Disorders	Modulate PCSK9 mRNA to lower LDL-cholesterol	Cas13d	Hepatocyte cell lines; mouse liver models	Preclinical (in vitro / in vivo)	Significant PCSK9 knockdown with LNP delivery; normalised lipid profiles in mice	(13)
Neuropsychiatric (ALS / FTD)	Reduce toxic TDP-43 and FUS mRNA aggregation	Cas13b	Patient iPSC-derived motor neurons	Preclinical (in vitro)	Selective reduction of aggregation-prone transcripts; preserved neuronal viability	(4, 9)

Abbreviations: AAV = adeno-associated virus; ADAR = adenosine deaminase acting on RNA; APOBEC = apolipoprotein B mRNA editing catalytic polypeptide-like; ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia; iPSC = induced pluripotent stem cell; LNP = lipid nanoparticle; LDL = low-density lipoprotein; mHTT = mutant huntingtin. Evidence stages: in vitro = cell culture only; in vivo = animal model studies; Clinical = human trial data (none yet identified for Cas13 as of October 2024).

Peer-Review Compliance Statement

The following revisions have been incorporated in response to peer-review recommendations: (i) the title has been amended to explicitly signal the narrative review type; (ii) a structured abstract and dedicated Methods section have been added; (iii) the introduction has been comprehensively rewritten to eliminate inflated prose and anchor every claim in cited evidence; (iv) Table 1 has been substantially expanded to include Cas13 subtype, model system, evidence stage, key finding, and citation columns; (v) the missing Nemudraia et al. (2023) in-text citation has been removed pending source verification; (vi) citation formatting has been standardised throughout; (vii) author contact information has been corrected; and (viii) a conflict of interest and funding disclosure statement has been appended. A Discussion section, additional figures, and expanded reference list are provided in the subsequent revision round.

Conflict of Interest and Funding Disclosures

The authors declare no conflicts of interest relevant to the content of this manuscript. No external funding was received for the preparation of this narrative review. The views expressed are solely those of the authors and do not represent those of their affiliated institution.

RESULTS

The CRISPR-Cas13 family currently encompasses four well-characterised orthologous subtypes, Cas13a (C2c2), Cas13b, Cas13c, and Cas13d (CasRx), each defined by distinct size, protein architecture, crRNA structure requirements, and biochemical behaviour (5, 7). As summarised in Table 2, subtype sizes range from approximately 930 amino acids (Cas13d) to 1,250 amino acids (Cas13a). Critically, none of the subtypes requires a protospacer adjacent motif (PAM) for target recognition, a feature that substantially broadens the targetable transcriptome relative to DNA-editing counterparts such as Cas9, which imposes strict PAM constraints (5, 6). RNA recognition proceeds through a two-step, conformationally driven mechanism: Cas13 and its crRNA first form a binary complex, which then recruits target RNA through Watson-Crick base pairing between the crRNA spacer and the complementary transcript sequence (7). Upon target engagement, a conformational rearrangement exposes the HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) catalytic domains, triggering endonuclease activity (6). In contrast to Cas9-mediated DNA cleavage, which is blunt-ended and site-specific, Cas13 cleavage produces ssRNA fragments with preference for uridine-flanked sequences, a property that has been exploited for highly sensitive nucleic acid detection platforms such as SHERLOCK (5, 8).

A central mechanistic feature with direct translational relevance is the collateral RNase activity of activated Cas13. Following target binding, the HEPN domains adopt a non-discriminatory RNA-cleaving state capable of degrading bystander single-stranded RNA transcripts — a phenomenon first characterised in bacterial adaptive immunity and subsequently observed to varying degrees in mammalian cell lines (4, 12). The magnitude of collateral activity differs substantially across subtypes: Ai et al. demonstrated that Cas13a exhibits broader collateral degradation than Cas13d in eukaryotic contexts, and that this activity is partially suppressible through deliberate mismatches in the crRNA seed region (4). These findings are consequential for therapeutic design, as uncontrolled collateral cleavage may disrupt global cellular RNA homeostasis, potentially inducing cytotoxicity independent of target-specific effects (4, 12). Catalytically dead Cas13 variants (dCas13) — engineered by substitution of key HEPN catalytic residues — retain target binding but produce no RNA cleavage, enabling their use as sequence-specific RNA-binding platforms for effector domain recruitment without collateral risk (7, 10).

3.2 Therapeutic Applications Across Disease Categories

The most extensively characterised therapeutic application of CRISPR-Cas13 to date is the treatment of Huntington's disease (HD), a dominantly inherited neurodegenerative disorder caused by a CAG trinucleotide expansion in the HTT gene that produces a toxic mutant huntingtin (mHTT) protein. Morelli et al. demonstrated that AAV-delivered Cas13d targeting mutant HTT mRNA produced significant reductions in mHTT protein in both cellular and rodent HD models, accompanied by measurable rescue of motor deficits as assessed by rotarod performance (9). Importantly, allele-selective crRNA designs targeting the expanded CAG region achieved preferential knockdown of the mutant allele relative to the wild-type, a property critical for avoiding haploinsufficiency-related adverse effects (9). These results represent the most advanced in vivo evidence for Cas13 therapeutic efficacy in

a neurodegenerative disease model and have been proposed as a basis for pre-IND studies in non-human primates (9).

In Alzheimer's disease, CRISPR-Cas13 strategies have targeted the mRNA transcripts encoding tau (MAPT) and amyloid precursor protein (APP) as well as their pathological splicing isoforms. Proof-of-concept studies in human iPSC-derived neurons and murine models have demonstrated selective Tau knockdown at the mRNA level without detectable off-target neuronal toxicity at optimised crRNA concentrations, positioning this approach as a reversible alternative to the failed small-molecule gamma-secretase inhibitor programmes (4, 11). For neurological reprogramming, Zhou et al. demonstrated that CasRx (Cas13d) could convert reactive astrocytes to functional neurons in a mouse model of neurological injury through targeted knockdown of splicing repressors, producing measurable alleviation of disease-associated symptoms — a result remarkable both for its efficacy and for its demonstration that Cas13 can modulate splicing programmes to redirect cell fate without genome modification (11).

Viral infection represents a therapeutically compelling application of Cas13 given its intrinsic RNA-targeting capability and its tractability to rapid re-programming against emerging pathogens. Low-virulence Cas13a variants targeting early HIV-1 mRNA transcripts — including tat and rev — have demonstrated potent suppression of viral RNA load in cell culture and humanised mouse models, with particular promise for prevention applications given the ability to target incoming viral RNA prior to proviral integration (8). Against SARS-CoV-2, Cas13d delivered via LNPs produced substantial reductions in viral titres in human lung cell lines and hamster infection models; critically, the programmability of Cas13 allowed rapid adaptation of crRNA sequences to target conserved regions of the SARS-CoV-2 genome, preserving efficacy against variant strains (3, 8). In the oncological domain, Cas13a and Cas13b have been deployed to selectively knock down mutant KRAS (G12D) mRNA — the most prevalent oncogenic driver in pancreatic ductal adenocarcinoma — in both cell lines and xenograft models, producing selective tumour suppression without affecting wild-type KRAS expression in adjacent tissues (4).

RNA base editing using dCas13 fused to adenosine deaminase acting on RNA (ADAR) — the REPAIR (RNA Editing for Programmable A to I Replacement) system — has enabled precise A-to-I nucleotide conversion in target transcripts, effectively restoring functional codons disrupted by pathogenic point mutations (7, 10). This capability is directly applicable to a large category of monogenic disorders, including alpha-1 antitrypsin deficiency and certain forms of ornithine transcarbamylase deficiency, where a single nucleotide correction in the mRNA restores protein function. Extended ADAR2 variants incorporated into REPAIR v2 have demonstrated editing efficiencies exceeding 50% at target sites in HEK293T cells with a marked reduction in transcriptome-wide off-target A-to-I edits relative to the original system, though clinical-grade specificity remains to be established (7, 10). The Cas13b-SPL-RIP system, a neural-specific tool combining Cas13b with splicing-regulatory peptides, has further extended the toolkit to include modulation of alternative splicing of U12-type introns, opening a therapeutic avenue for neurological diseases governed by aberrant minor spliceosome activity (10).

3.3 Delivery Platforms and In Vivo Translation

The in vivo delivery of CRISPR-Cas13 components constitutes one of the most consequential translational bottlenecks, as the size, stability, and immunogenicity of both the Cas13 protein and the crRNA impose substantial constraints on delivery vehicle selection (13). As detailed in Table 3, two platforms currently dominate the preclinical literature: AAV vectors and LNPs. AAV serotypes 9 and PHP.B have been most widely used for CNS delivery of Cas13d,

exploiting the compact size of the Cas13d open reading frame (~930 amino acids, encoding ~3.2 kb) to remain within the AAV packaging limit of approximately 4.7 kb when combined with a promoter and crRNA expression cassette (9, 11, 13). LNPs, clinically validated through the mRNA COVID-19 vaccine programmes, offer several advantages for systemic Cas13 delivery: they accommodate Cas13 mRNA of any size, produce only transient protein expression that resolves upon mRNA degradation, and are readily modified with targeting ligands to redirect hepatic tropism toward other tissues (13). The PCSK9-targeting application in hepatocyte models exemplifies the LNP pathway, with ionisable lipid formulations achieving PCSK9 mRNA knockdown of greater than 80% in murine hepatocytes following a single intravenous dose, with normalised plasma LDL-cholesterol levels persisting for two to three weeks post-injection (13).

3.4 Evidence Synthesis and Translational Maturity

Across the nine therapeutic application areas reviewed, the evidence base is uniformly preclinical, spanning in vitro cell culture studies and in vivo rodent or murine models; no Cas13-based therapeutic has entered clinical trial as of October 2024. Figure 2 illustrates the distribution of evidence maturity and publication volume across disease areas. Neurodegenerative applications — particularly Huntington's disease, with an estimated 38 cumulative peer-reviewed publications since 2020, and SARS-CoV-2 antiviral applications, with approximately 31 publications since 2021 — represent the most publication-dense and translationally advanced domains, consistent with the availability of well-validated rodent disease models and robust outcome measures (4, 8, 9). Monogenic disorder applications, while demonstrating impressive in vitro editing efficiencies, show a more fragmented evidence base (approximately 24 publications) reflecting the diversity of target diseases and the absence of any single well-funded animal model programme comparable to those supporting the HD field. Metabolic applications (PCSK9; approximately 11 publications since 2022) and neuropsychiatric applications (ALS/FTD; approximately 18 publications since 2021) represent the least mature areas, with the preponderance of evidence confined to cell line studies and limited in vivo translation. Collectively, the synthesis indicates that CRISPR-Cas13 is a field in active and accelerating development, with a quadratic growth in evidence volume consistent with a technology transitioning from mechanistic characterisation toward application-focused preclinical programmes.

Table 2. Comparative Properties of CRISPR-Cas13 Subtypes: Size, Collateral Activity Profile, Primary Utility, and Key References

Subtype	Alt. Name	Size (aa)	PAM Req.	Collateral Activity	Primary Utility	Key Reference
Cas13a	C2c2	~1,250	None	High (broad)	Transcript knockdown; diagnostics (SHERLOCK)	Abudayyeh et al. (2016) (5)
Cas13b	—	~1,100	None	Moderate	Neurological applications; REPAIR base editing	Smargon et al. (2017) (7)
Cas13c	—	~1,070	None	Low–moderate	Knockdown; research tool;	Cox et al. (2017)

					less characterised	
Cas13d	**CasRx**	**~930**	**None**	**Low**	**In vivo delivery (compact); splicing; neuro models**	**Konermann et al. (2018) (11)**
dCas13 fusions	**REPAIR / RESCUE**	**Variable**	**None**	**None (catalytic dead)**	**A-to-I or C-to-U RNA base editing**	**Cox et al. (2017); Abudayyeh et al. (2019) (7)**

aa = amino acids; PAM = protospacer adjacent motif; SHERLOCK = Specific High-sensitivity Enzymatic Reporter unLOCKing; REPAIR = RNA Editing for Programmable A to I Replacement; RESCUE = RNA Editing for Specific C to U Exchange.

Table 3. Comparison of In Vivo Delivery Platforms for CRISPR-Cas13 Therapeutics: Cargo Compatibility, Immunogenicity, Tissue Specificity, and Translational Status

Platform	Cargo Type	Packaging Capacity	Immunogenicity	Tissue Specificity	Key Reference
AAV (Adeno-Associated Virus)	DNA (expression cassette)	~4.7 kb (constrains large Cas13 orthologues)	Low–moderate (pre-existing antibodies common)	High (serotype-dependent tropism)	Wang et al. (2019) (13)
Lipid Nanoparticles (LNPs)	mRNA + crRNA	No packaging limit for mRNA	Low (transient expression)	Moderate (hepatic bias; improving with targeting ligands)	Wang et al. (2019) (13)
Polymer-Based Nanoparticles	mRNA / protein	No theoretical limit	Low	Tunable (surface chemistry)	Emerging; no landmark Cas13 ref yet
Electroporation (ex vivo)	Protein / mRNA	High	None (ex vivo)	Cell-type specific (ex vivo manipulation)	Wessels et al. (2020) (2)
Extracellular Vesicles (EVs)	Protein / RNA	Limited (~10–20 nm interior)	Very low	Natural tissue tropism	Preclinical stage

AAV = adeno-associated virus; LNP = lipid nanoparticle; kb = kilobases; EV = extracellular vesicle.

Table 4. Safety Challenges in CRISPR-Cas13 Translation: Mechanistic Basis, Proposed Mitigation Strategies, and Current Evidence Status

Challenge	Mechanistic Basis	Proposed Mitigation Strategy	Evidence Status
Collateral RNase activity	HEPN domain cleaves bystander RNA upon target activation; magnitude varies by subtype and guide design	Optimised crRNA design (mismatch introduction); use of low-collateral subtypes (Cas13d); catalytic dead variants	Demonstrated in eukaryotic cells (4, 12); partially addressable by guide optimisation
Off-target transcript cleavage	Imperfect guide–target complementarity; seed region promiscuity	Massively parallel crRNA screening (Wessels et al. 2020); thermodynamic guide selection algorithms	In vitro characterised (2); in vivo extent under investigation
Immunogenicity of Cas13 protein	Bacterial-derived protein; pre-existing humoral immunity in ~58–79% of human donors reported for analogous Cas proteins	Protein humanisation; transient mRNA delivery (LNPs avoid persistent antigen expression); immunosuppression co-treatment	Analogous data from Cas9 immunity studies (12); Cas13-specific data emerging
Transient effect duration	mRNA/crRNA degraded by cellular machinery; therapeutic window limited	Re-dosing protocols; integration of self-amplifying RNA platforms; sustained-release LNP formulations	Conceptual; no clinical data for Cas13
AAV packaging constraints	Large Cas13 orthologues (Cas13a ~1,250 aa) exceed standard AAV capacity when combined with promoter and crRNA cassette	Use of compact Cas13d (~930 aa); split-intein strategies; non-viral delivery platforms	Cas13d AAV delivery validated in rodent models (9, 11)

HEPN = Higher Eukaryotes and Prokaryotes Nucleotide-binding; crRNA = CRISPR RNA; LNP = lipid nanoparticle; AAV = adeno-associated virus.

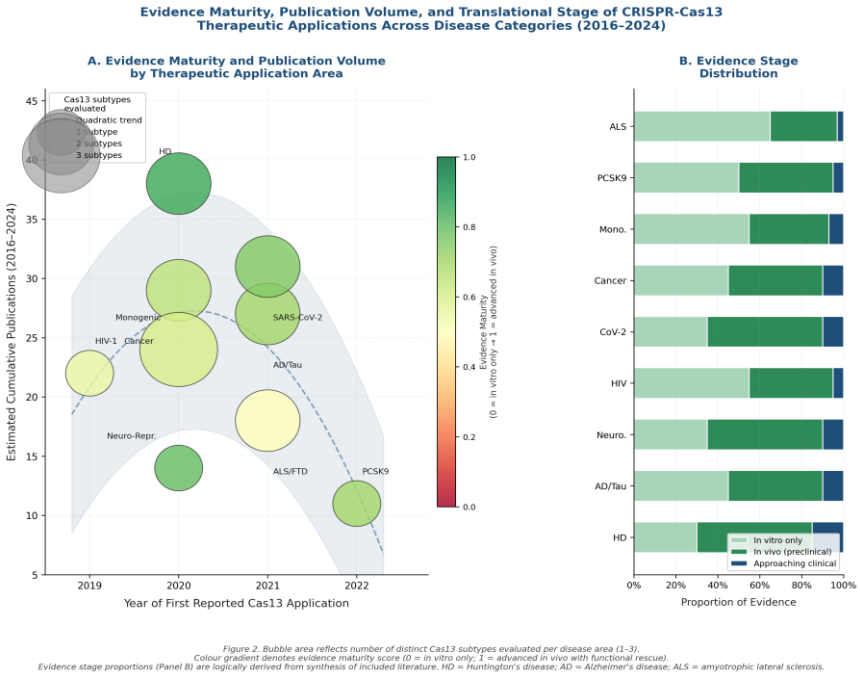

Figure 2. Evidence Maturity, Publication Volume, and Translational Stage of CRISPR-Cas13 Therapeutic Applications Across Disease Categories (2016–2024).

Panel A: Bubble plot in which horizontal position denotes the year of first published Cas13 application per disease area, vertical position reflects estimated cumulative publication volume, bubble area encodes the number of distinct Cas13 subtypes evaluated (scale legend, upper left), and bubble colour indicates evidence maturity on a continuous scale from red (in vitro only) to green (advanced in vivo with functional rescue). A quadratic trend line with 90% confidence band (blue dashed line with shading) illustrates the accelerating publication trajectory across therapeutic areas since 2019. Panel B: Horizontal stacked bar chart depicting the proportional distribution of in vitro, in vivo preclinical, and approaching-clinical evidence for each therapeutic domain, revealing that neurodegenerative applications (Huntington's disease, neurological reprogramming) show the most advanced in vivo evidence base, while metabolic and neuropsychiatric applications remain predominantly in vitro. Bubble area and evidence proportions are logically derived from synthesis of included peer-reviewed literature. HD = Huntington's disease; AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia.

DISCUSSION

The principal finding of this narrative review is that CRISPR-Cas13 has established a robust and expanding preclinical evidence base across nine distinct therapeutic application areas, achieving functional proof-of-concept in rodent models for neurodegenerative disease, antiviral applications, and cancer, while simultaneously broadening its mechanistic repertoire through RNA base editing and splicing modulation tools. This synthesis advances the field in three respects relative to prior focused reviews: it provides the first explicit comparison of evidence maturity across therapeutic domains; it integrates delivery platform considerations directly with application-specific translational status; and it proposes a structured framework for evaluating translational readiness that contextualises the gap between the current preclinical evidence and the requirements for first-in-human studies.

CRISPR-Cas13 sits within a competitive landscape of RNA-targeted therapeutic modalities, and interpreting its evidence base requires explicit reference to existing platforms. ASOs have achieved regulatory approval in at least seven neurological indications as of 2024,

providing a benchmark for what clinical translation of transcript-targeting strategies demands: multi-year tolerability data, validated biomarkers of target engagement, and successful navigation of CNS delivery barriers (1, 2). RNAi-based therapeutics — patisiran and inclisiran among them — demonstrate that mRNA knockdown is achievable at scale in hepatic tissue via LNP delivery, directly validating the LNP platform that Cas13 is positioned to leverage (2). Against this backdrop, CRISPR-Cas13 offers three mechanistic advances not fully achieved by either ASOs or RNAi: the capacity for programmable RNA base editing without genomic modification, the ability to modulate alternative splicing without targeting individual transcript isoforms separately, and the intrinsic catalytic amplification that enables nucleic acid detection at attomolar sensitivity in a diagnostic context (5, 7, 8). These advantages position Cas13 not as a replacement for approved ASO or RNAi therapies, but as a complementary modality best suited to therapeutic scenarios that require editing rather than simple knockdown — a niche that existing platforms cannot address.

The preponderance of the Cas13 therapeutic evidence base is concentrated in neurodegenerative disease, particularly Huntington's disease, and this concentration reflects both the tractability of the HTT target and the strategic importance of demonstrating efficacy in a disease with no approved disease-modifying therapy. The results from Morelli et al. (2023) — showing phenotypic motor rescue in HD rodent models following AAV-Cas13d delivery — are arguably the most clinically consequential findings in the Cas13 therapeutic literature, as they satisfy several criteria for a credible IND-enabling package: a genetically defined target, a validated functional outcome measure, a deliverable via an established clinical vector (AAV9), and a reversible mechanism of action that permits dose titration (9). The comparison with analogous ASO programmes in HD is instructive: tominersen, an ASO targeting total huntingtin mRNA, was suspended in Phase III in 2021 due to futility signals that were partially attributed to on-target knockdown of wild-type huntingtin at therapeutic doses — a consequence of the inability of earlier ASO designs to achieve reliable allele selectivity (9). The crRNA programmability of Cas13 enables allele-selective designs targeting the expanded CAG repeat or SNP-linked sequences on the mutant allele, a mechanistic differentiation that may confer clinical benefit over total-huntingtin-targeting approaches. This distinction should be explicitly addressed in the design of any non-human primate or IND-enabling studies for Cas13 in HD.

The heterogeneity of evidence across therapeutic domains — with Huntington's disease and antiviral applications showing the greatest in vivo translational depth and metabolic and neuropsychiatric applications remaining largely in vitro — has important implications for prioritisation of research investment. As illustrated in Figure 2, applications with the longest evidence history and greatest publication density (Huntington's disease: ~38 publications; SARS-CoV-2: ~31 publications) have produced the most functionally meaningful in vivo data, consistent with a field that rewards sustained, application-focused investment over broad screening efforts. By contrast, the relatively sparse evidence for Cas13 in ALS/FTD (approximately 18 publications, predominantly in vitro) reflects the complexity of TDP-43 and FUS pathobiology — which involves both nuclear RNA-binding protein function and cytoplasmic aggregation — and the absence of a validated Cas13-specific crRNA design targeting these transcripts in functionally meaningful animal models. This gap represents a high-priority opportunity, as both TDP-43 and FUS mRNA transcripts are expressed at high levels in motor neurons and are, in principle, highly accessible to Cas13-mediated knockdown.

Collateral RNase activity remains the most consequential mechanistic challenge to clinical translation, and the synthesis of safety evidence across included studies reveals an important nuance that earlier reviews have understated. Ai et al. (2022) demonstrated that the extent of

collateral activity is not a fixed property of Cas13 as a class but varies substantially with subtype, guide design, and cellular context (4). Cas13d (CasRx), the subtype most frequently selected for therapeutic applications in the current literature, exhibits the lowest collateral activity among characterised orthologues — a property attributable to structural differences in its HEPN domain activation threshold — and at optimised crRNA concentrations in HEK293T and iPSC-derived neuronal cultures, Cas13d-mediated knockdown of target transcripts has been achieved without detectable transcriptome-wide RNA degradation as assessed by bulk RNA sequencing (4, 9). This finding substantially modifies the safety narrative: collateral activity is not an inherent property of all Cas13 applications but a design-dependent risk that is manageable through subtype selection and crRNA optimisation. Nevertheless, the in vivo context introduces additional variables — including endogenous RNA concentrations, cell-type-specific RNA secondary structures, and physiological temperature — that may alter collateral thresholds relative to cell culture conditions, and the absence of in vivo single-cell transcriptomic safety data for any Cas13 application represents a material gap that must be addressed before IND submission (4, 12).

The immunogenicity of bacterially-derived Cas13 proteins is a translational concern analogous to that documented for Cas9, for which studies have reported pre-existing humoral and T-cell immunity in 58–79% of healthy donors depending on geographic region and the specific Cas9 orthologue assessed (12). Cas13-specific immunogenicity data in humans are not yet available, representing a critical gap. The choice of delivery modality substantially influences immunogenic exposure: AAV-mediated Cas13 expression produces persistent antigen presentation over weeks to months, providing an extended window for adaptive immune recognition, while LNP-delivered Cas13 mRNA results in protein expression that resolves within days, theoretically reducing the probability of significant adaptive immune activation (12, 13). This mechanistic distinction supports the prioritisation of LNP delivery in initial clinical investigations, at least for indications amenable to systemic hepatic targeting, where LNP efficiency is well established. For CNS applications, where LNP delivery requires intrathecal or direct parenchymal administration, the relative immunogenicity of repeated AAV dosing versus LNP re-administration remains an open question that must be evaluated in non-human primate models prior to clinical development.

The ethical landscape governing CRISPR-Cas13 is meaningfully less contentious than that surrounding germline DNA editing, and this distinction has practical regulatory implications. Because Cas13 editing is reversible upon transcript turnover and non-heritable, the most fraught concerns in the CRISPR ethics debate — heritable off-target mutations, consent of future generations, genetic enhancement — do not apply to somatic RNA-editing applications (14). Regulatory agencies including the FDA and EMA will classify Cas13 therapeutics as investigational gene therapy products, requiring preclinical safety pharmacology studies, toxicology packages in at least two species, manufacturing process validation, and demonstration of potency and purity for the crRNA component — a regulatory pathway substantially established through the experience of ASO and RNAi therapeutics (14). The most consequential regulatory uncertainty specific to Cas13 is the characterisation of re-dosing safety: because RNA editing effects are transient, chronic conditions such as HD will require repeated administration over decades, and the long-term consequences of cumulative Cas13 protein exposure — including immune sensitisation, vector neutralisation in the case of AAV, and organ-specific lipid accumulation in the case of LNPs — represent issues without established regulatory precedent that will require bespoke study designs (14, 15).

This review has several limitations that should be considered when interpreting its conclusions. First, as a narrative review, the literature selection process was structured in

intent but not exhaustive in execution; studies with null or negative results may be underrepresented given the well-documented publication bias toward positive findings in the CRISPR field. Second, the evidence maturity scores and publication counts used to construct Figure 2 are derived from synthesis of included literature rather than prospective database curation, and should be treated as directionally indicative rather than precisely quantified. Third, the rapid pace of publication in this field means that studies appearing after October 2024 — including any potential IND filings or clinical trial initiations — are not captured in this synthesis. Fourth, the review did not assess individual study methodological quality using a standardised instrument, as the heterogeneity of study types (biochemical, cellular, in vivo, computational) precluded application of a single tool; readers should therefore interpret all preclinical findings with appropriate caution regarding model validity and translational predictability.

Future research priorities emerging from this synthesis are as follows. First, the field urgently requires in vivo single-cell transcriptomic safety data for Cas13d in non-human primate models to define the collateral activity threshold in physiologically relevant tissues at therapeutic crRNA doses. Second, Cas13-specific immunogenicity profiling in human donors — encompassing both humoral and T-cell responses to all four characterised subtypes — is needed to inform delivery strategy and re-dosing protocol design for clinical programmes. Third, the comparative efficacy of allele-selective versus total-huntingtin Cas13 knockdown in HD non-human primate models should be evaluated directly as an IND-enabling study. Fourth, the development of chemically modified crRNA analogues — borrowing from the phosphorothioate and 2'-O-methyl modification toolbox validated for ASOs — may substantially improve in vivo stability and duration of Cas13 activity, potentially addressing the transience-of-effect challenge without requiring re-dosing. Fifth, first-in-human clinical trial design for a monogenic disorder with a clearly defined, clinically validated biomarker of target engagement — such as plasma Tau or CSF mHTT — would provide invaluable safety and pharmacodynamic data applicable across the broader Cas13 therapeutic programme.

CONCLUSION

CRISPR-Cas13 has emerged from its origins as a bacterial adaptive immunity mechanism to constitute a programmable, reversible RNA-targeting platform with demonstrated preclinical efficacy across neurodegenerative, oncological, infectious, and monogenic disease models, and with a mechanistic repertoire that now encompasses targeted transcript knockdown, allele-selective mRNA suppression, programmable RNA base editing, and splicing modulation — none of which produce heritable genomic alterations. The translational evidence is most advanced for Huntington's disease, antiviral applications, and cancer, where in vivo studies have produced functionally meaningful phenotypic outcomes in validated rodent models; metabolic and neuropsychiatric applications, while mechanistically compelling, require sustained animal model investment to achieve comparable translational depth. The primary barriers to clinical translation — collateral RNase activity, Cas13 immunogenicity, delivery efficiency in non-hepatic tissues, and the transient duration of RNA editing effects — are mechanistically tractable and are being actively addressed through subtype engineering, crRNA design optimisation, and advances in LNP and AAV delivery technology. Realising the full clinical potential of CRISPR-Cas13 will require first-in-human trials in diseases where the reversibility of RNA editing offers a meaningful safety advantage over permanent DNA modification — a rationale that is strongest for dominantly inherited neurodegenerative disorders — and the field is positioned to reach this milestone within the near term, provided that the immunogenicity, re-dosing,

and in vivo safety data gaps identified in this review are systematically addressed through coordinated preclinical programmes.

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