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Perspective

Emerging clinical applications of ADAR based RNA editing

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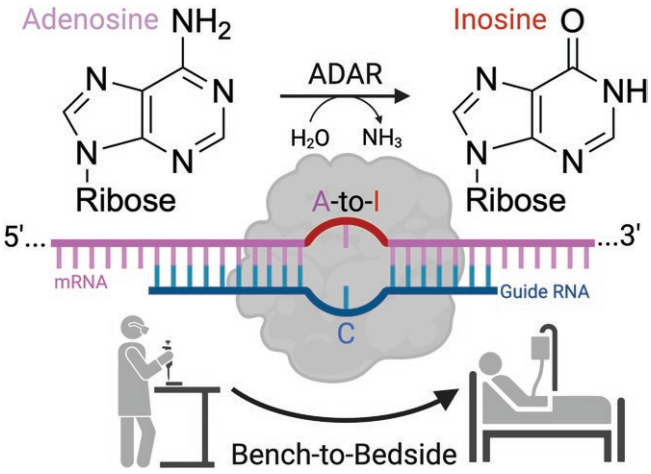
Abstract

RNA editing via adenosine deaminases acting on RNA (ADARs) offers precise and reversible modifications at the RNA level, complementing traditional DNA-targeting therapies. ADAR enzymes catalyze the conversion of adenosine to inosine within double-stranded RNA, influencing critical cellular processes such as translation, splicing, and RNA stability. Utilizing endogenous ADARs guided by exogenous guide RNAs enables site-specific RNA editing without the need for transgenic editor expression, minimizing immunogenicity, and enhancing control over gene expression. Towards addressing the challenges in ensuring specificity, optimizing delivery methods, and navigating regulatory landscapes, ongoing innovations in guide RNA design, delivery technologies, and computational modeling are propelling the field forward. Already, initial clinical advancements are demonstrating the potential of ADAR-mediated RNA editing in treating human diseases. Collaborative efforts among researchers, clinicians, and industry partners are overcoming existing hurdles, progressively positioning ADAR-mediated RNA editing to revolutionize personalized medicine and provide effective treatments for a wide array of historically intractable diseases.

Graphical Abstract



ADAR enzymes catalyze the A-to-I conversion in RNA, a promising tool for precise, reversible RNA modifications. By using guide RNAs to recruit endogenous ADARs, targeted RNA editing is achieved without transgenic editors, reducing immunogenicity and enhancing control. This approach holds therapeutic potential, transitioning from bench to bedside, for treating various diseases through personalized, site-specific RNA interventions.

Significance statement

This article examines the therapeutic promise of RNA editing using naturally occurring enzymes known as ADARs, which can precisely and reversibly modify RNA to correct disease-causing genetic errors. By guiding these enzymes to specific RNA sequences, it is possible to restore normal gene function without permanently altering DNA. This strategy offers a safer and more adaptable alternative to conventional gene therapies. This perspective review highlights key advances in targeting, delivery, and clinical development of ADAR-based RNA editing, emphasizing its potential to transform personalized medicine and provide new treatment options for genetic diseases.

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Introduction

Advancements in genomic sequencing have unveiled the genetic underpinnings of numerous human diseases, propelling biotechnological innovations and the development of DNA and RNA therapeutics.[1,2] While DNA-centric treatments offer the promise of long-lasting cures, RNA-focused strategies present unique advantages due to their adaptability and reversibility, thereby avoiding permanent off-target effects.

This review explores cutting-edge RNA editing methodologies, particularly those leveraging adenosine deaminases acting on RNA (ADARs), to facilitate precise endogenous RNA regulation beyond conventional gene manipulation techniques. ADAR enzymes are pivotal in converting adenosine (A) to inosine (I) within double-stranded RNA (dsRNA),[3-6] affecting critical cellular processes such as translation,[7] splicing,[8] RNA stability,[9] R-loop resolution,[10] and regulatory mechanisms.[11] Although the concept of A-to-I RNA editing dates back to the mid-1980s,[3,4] recent years have witnessed a resurgence of interest in its therapeutic potential.[12] Researchers have demonstrated the feasibility of site-specific A-to-I editing using guide RNAs (gRNA) to target messenger RNA (mRNA) sequences in both cellular and animal models.[13,14] Furthermore, the use of endogenous ADAR enzymes has simplified targeted RNA editing by utilizing the cell's natural machinery,[15,16] making it highly therapeutically relevant and advancing its opportunity for clinical translation.[17] Exogenous ADAR therapies have also demonstrated significant promise, enabling precise RNA editing and expanding the scope of emerging therapeutic applications.[18-20]

Approximately 58% of human genetic variants associated with disease are caused by point mutations.[21] Notably, 28% of pathogenic single-nucleotide variants (SNVs) are G-to-A missense and nonsense mutations, making them attractive targets for ADAR-mediated restoration of the wild-type sequence.[22] Moreover, the significance of adenosines in functional RNA sites underscores the therapeutic capacity of RNA editing in modulating protein expression and splicing, complementing existing strategies such as antisense oligonucleotide (ASO) therapies. The versatility of A-to-I changes allows for diverse amino acid substitutions, enabling the modulation of protein function and interactions. Natural ADAR activity influences numerous proteins,[23] with conserved editing sites identified across species.[24] Despite the immense potential of RNA editing, several challenges persist, including ADAR promiscuity, off-target effects, and the necessity for precise gRNA design. Addressing these challenges is crucial for realizing RNA editing's clinical promise and ensuring its successful translation into therapeutic applications.

This review delves into ADAR-mediated RNA editing, its therapeutic applications, and the obstacles and opportunities in the field, facilitating a deeper understanding of this promising therapeutic modality.

The RNA editing toolbox

An initial question arises: Why target RNA instead of DNA? First and foremost, RNA-based edits are transient and reversible. This transience reduces the risk of permanent off-target effects and allows for controlled, reversible therapeutic interventions, where dosing strategies such as escalation or periodic administration can be tailored to patient needs. In contrast, DNA editing results in irreversible changes,[25,26] which can have unintended consequences. Additionally, multiplexed targeting of RNAs is readily feasible, unlike multiplexed DNA edits where there is the possibility for inducing cytotoxic DNA damage responses. Recent advancements in CRISPR engineering, such as epigenome editing with tools like dCas9 fused to epigenetic modifiers, aim to circumvent some of these risks by enabling precise, and potentially reversible regulation of gene expression without altering the underlying DNA sequence.[27] However, epi-editing is unable to repair disease-causing mutations, or alter specific protein residues such as those involved in protein-protein interactions or active sites of enzymes.

Recognizing these constraints, several RNA editing technologies have emerged, each with unique mechanisms and applications, contributing to a growing toolbox for transcriptome engineering. By leveraging programmable RNA targeting systems like CRISPR-Cas13,[28] tethered with RNA modifying enzymes or domains, one can specifically edit desired RNA transcripts.[18,29] Pentatricopeptide repeat (PPR) proteins can also edit RNA when connected to endogenous deaminases[30-32] and apolipoprotein B mRNA editing catalytic polypeptide (APOBEC) can make C-to-U RNA edits.[33-35] However, these RNA editing technologies face challenges as they approach therapeutic use. CRISPR-Cas13 involves large exogenous and potentially immunogenic proteins[36]; APOBECs lack intrinsic programmability and require a second protein (ACF) to bind to RNA[37,38]; and engineering PPR proteins to bind specific RNA sequences remains complex.[39]

Among RNA editing systems, ADAR enzymes, endogenous to the human genome, have emerged as especially promising for therapeutic applications. ADARs catalyze the deamination of adenosine to inosine (A-to-I) within dsRNA, which is interpreted as guanosine (G) during translation. When guided to target transcripts by exogenously delivered gRNAs that induce dsRNA structures, ADARs can achieve precise and high-fidelity correction of pathogenic point mutations. To enhance their targeting capabilities, several groups have developed strategies that tether ADAR domains to RNA-binding motifs, enabling programmable RNA editing via engineered gRNA scaffolds. For instance, SNAP-tag–ADAR fusion proteins can be covalently linked to gRNAs modified with benzylguanine, supporting stoichiometric and transcription-independent RNA editing.[40-42] Similarly, RNA-binding proteins such as λN, which binds to boxB stem-loops,[20,43,44] and MS2 coat protein (MCP), which binds MS2 RNA hairpins,[45-47] have been used to recruit ADARs to target transcripts. Additional designs, such as the CRISPR–Cas-Inspired RNA-Targeting System (CIRTS), use only human-derived protein components for guide RNA recruitment,[19] while others like AI-REWIRE eliminate the need for gRNAs entirely by fusing ADAR to Pumilio and FBF homology (PUF) domains for direct RNA binding.[48] While compact in design and amenable to delivery as ribonucleoprotein complexes or via viral vectors, all these approaches still require the introduction of engineered, exogenous protein components.

In contrast, strategies that harness endogenously expressed ADAR enzymes, redirected by chemically stabilized or structured gRNAs, eliminate the need for exogenous protein delivery altogether. This approach leverages the widespread and natural expression of endogenous ADARs across human tissues, reducing immunogenicity complications and simplifying therapeutic delivery. Importantly, the use of user-engineered gRNAs enables tunable, transcript-specific modulation of RNA function across a broad range of targets,

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providing a modular and programmable platform for therapeutic RNA editing. As a result, directing endogenous ADAR editing has emerged as the primary clinical strategy for RNA editing, offering an optimal balance of precision, flexibility, and clinical practicality for addressing a diverse set of therapeutic targets.

Progress in understanding the biological functions of ADAR enzymes

Recent advancements have significantly deepened our understanding of the biological functions of ADAR enzymes. ADARs are crucial for the A-to-I RNA editing process, which alters RNA sequences post-transcriptionally and can impact RNA splicing, localization, stability, and translation.

There are three main ADAR isoforms in mammals - ADAR1 (ADAR), ADAR2 (ADARB1), and ADAR3 (ADARB2)[6,49-51] - each with distinct expression patterns and functions (Figure 1A). ADAR1 exists in two isoforms, p110 and p150, generated through alternative promoter usage. The p110 isoform is constitutively expressed and primarily localized to the nucleus, while p150 is interferon-inducible and shuttles between the nucleus and cytoplasm via a nuclear export signal on its N-terminus, consistent with its function in interferon-mediated immune responses.[52-54] ADAR1 is ubiquitously expressed in all tissues and cells (Figure 1B, C).[55,56]

ADAR2 is predominantly expressed in heart, vessels, and neuronal tissues and is essential for editing transcripts involved in neurotransmission. These include GluA2, the subunit of glutamate receptors, where ADAR edits alter ion channel properties for proper synaptic transmission and prevention of excitotoxicity.[57-59] ADAR3, although mainly expressed in the brain similar to ADAR2, lacks catalytic activity and is thought to modulate RNA editing by competing for RNA binding sites.[51,60]

ADAR enzymes interact with various forms of endogenous dsRNA, including circular RNAs (circRNAs),[61] DNA/RNA hybrids,[62] and microRNAs (miRNAs).[63] CircRNAs can form dsRNA structures that are substrates for ADAR-mediated editing, influencing their stability and function.[64,65] DNA/RNA hybrids, formed during transcription, are also known to interact with ADARs, where the protein helps to resolve hybrid structures such as R loops preventing genotoxic stress common in some cancers.[10,66,67] ADARs can also edit miRNAs, altering their sequences and affecting maturation and target specificity, which in turn modulates post-transcriptional gene expression.[68,69]

Physiologically, ADARs play vital roles in both editing-dependent and editing-independent mechanisms (Figure 2). In the immune system, ADAR1 prevents inappropriate activation of innate immune responses by binding and editing

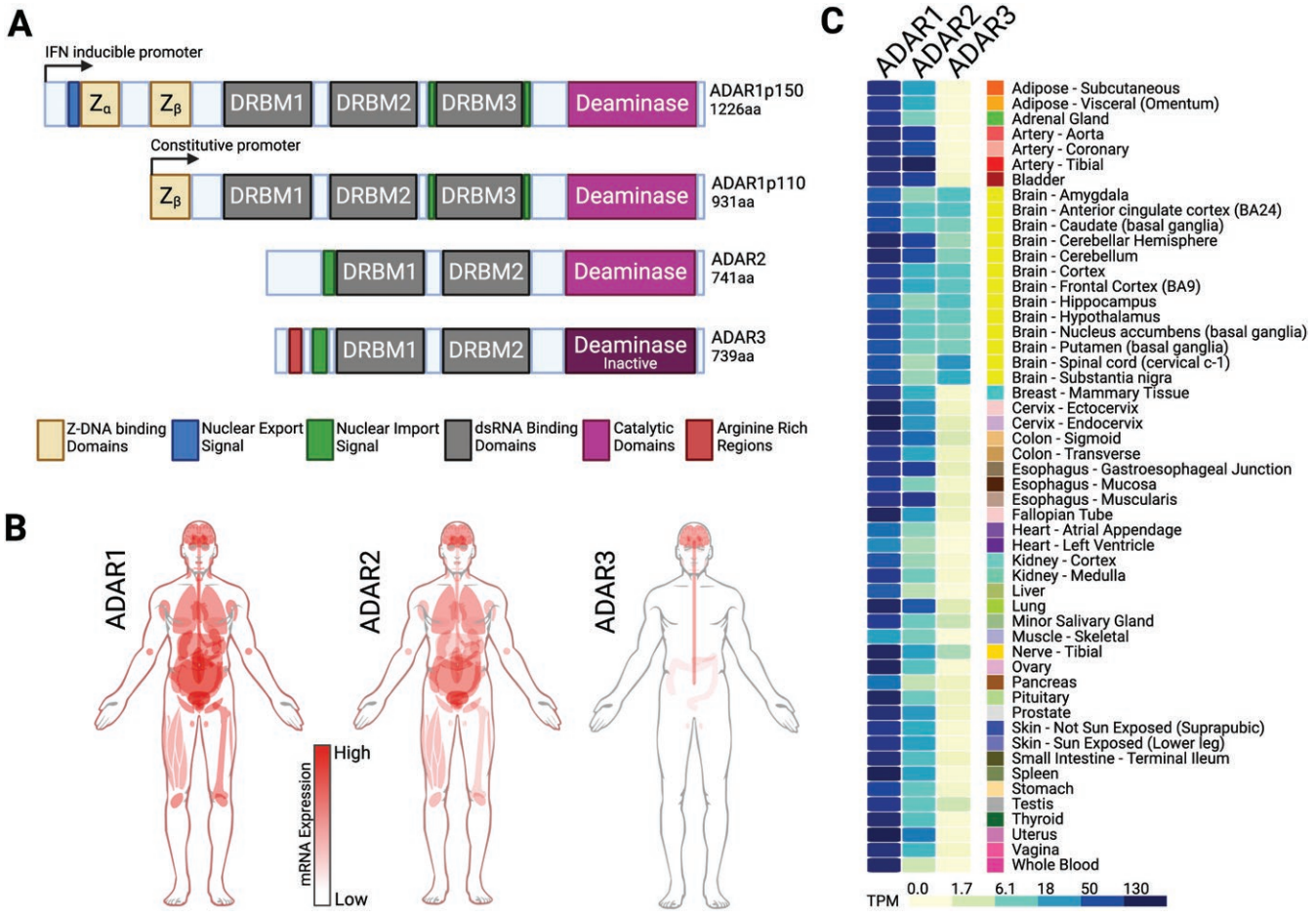

Figure 1. ADAR domain and tissue expression profiles. (A) Schematic of domains in ADAR isoforms, ADAR1p150, ADAR1p110, ADAR2, and ADAR3 provided by uniprot.org. (B) Anatomogram of ADAR1, ADAR2, and ADAR3 mRNA expression across male human tissue provided by proteinatlas.org. (C) Heatmap of ADAR1, ADAR2, and ADAR3 mRNA expression across human tissues provided by gtexportal.org. Tissue colors were assigned to conform to the GTEx publication convention. TPM = transcript per million.

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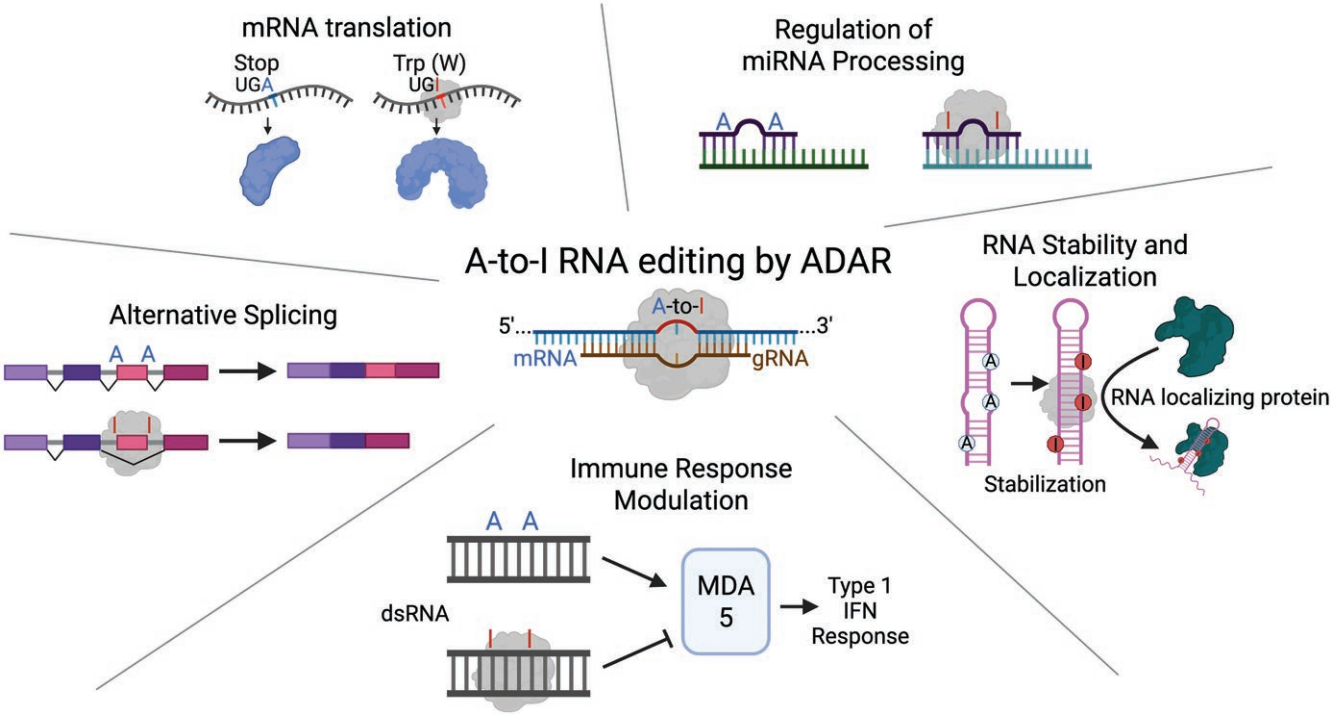

Figure 2. Functional roles of ADAR. The central ADAR activity, A-to-I RNA editing, influences several key functions, including mRNA translation, where inosine is interpreted as guanosine, miRNA processing and binding, immune regulation through actions like preventing dsRNA recognition by sensors like MDA5, alternative splicing through changes in RNA structure, and RNA stability and localization.

endogenous dsRNA, thereby averting recognition by cytoplasmic RNA sensors like MDA5[70-72] that could mistake self-RNA for viral RNA. The p150 isoform of ADAR1 is particularly important during interferon responses to viral infections, fine-tuning the immune reaction to prevent autoinflammatory consequences in the cellular cytosol.[53,54]

Alterations in ADAR function have been linked to various pathologies. Mutations in the ADAR1 gene can lead to autoinflammatory disorders such as Aicardi-Goutières syndrome, characterized by excessive interferon production due to unedited endogenous dsRNA activating immune sensors.[73,74] In neurological diseases, impaired ADAR2 function is linked with conditions like encephalopathy, intellectual disability, seizures, and amyotrophic lateral sclerosis (ALS), where deficient editing of neuronal transcripts results in neuronal dysfunction.[55,75,76] Additionally, dysregulated ADAR expression and RNA editing patterns have been observed in several cancers, affecting tumor growth, metastasis, and immune evasion strategies.[77]

The intricate mechanisms by which ADARs influence RNA editing, and consequently gene expression, protein function, and cellular responses, highlight their potential as targets for treating a range of pathologies. This growing body of knowledge has spurred the development of RNA editing therapeutics that leverage ADAR activity to correct genetic mutations at the RNA level.

Current scope of RNA editing applications in human therapeutics

Since Woolf and colleagues first proposed the potential of ADAR-based therapeutics in 1995,[78] the field of RNA editing has made significant strides. A significant milestone was achieved in 2019 when researchers demonstrated that both simple long antisense oligonucleotides[15,16] and oligonucleotides containing ADAR-recruiting dsRNA domains could effectively harness endogenous ADAR enzymes to perform precise and programmable RNA edits.[15,16] These advancements have propelled RNA editing therapies to the forefront of medical research, with several oligonucleotide-based drugs now entering clinical trials. By harnessing the body's own ADAR enzymes, researchers are developing innovative therapies that can edit mRNA transcripts with high precision.[14,16,40,64,79-81] This approach offers the possibility to correct genetic mutations at the RNA level, providing treatments that are both specific and reversible without permanently altering the genome.

Clinical advancements and leading therapeutics

Wave Life Sciences has initiated RNA editing therapies with their lead drug candidate WVE-006, currently undergoing Phase1 (NCT06186492) and Phase 1b/2a (NCT06405633) clinical trials, initiated in 2023 and 2024 respectively, for alpha-1 antitrypsin deficiency (AATD). AATD is a hereditary disorder caused by mutations in the SERPINA1 gene, most commonly the E342K mutation, which results from a single G-to-A nucleotide substitution converting the normal M-allele to the Z-allele.[82] This mutation impairs the folding and function of the alpha-1 antitrypsin (AAT) protein, affecting an estimated 1 in 2000 to 1 in 5000 individuals.[83] Without functional AAT secreted by the liver, the lungs become susceptible to chronic inflammation due to unneutralized neutrophil elastase activity, often resulting in chronic obstructive pulmonary disease (COPD). Existing treatments, such as protein replacement therapy, have limitations, making RNA editing a promising comprehensive solution by addressing both the genetic defect and its clinical manifestations. WVE-006 is designed to recruit endogenous ADAR enzymes to correct the specific G-to-A

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missense mutation in the *SERPINA1* mRNA. By converting the mutated adenosine back to inosine, the therapy restores the production of functional AAT protein. Preclinical studies have demonstrated encouraging results, achieving up to 75% correction of the mutant protein in disease models,[17] potentially alleviating both hepatic and pulmonary symptoms associated with the disease. The ongoing trial, RestorAATion-2, includes both single ascending and multiple ascending dose arms to evaluate the safety, tolerability, and efficacy of WVE-006, a tri-antennary GalNAc-conjugated oligonucleotide delivered subcutaneously, while closely monitoring off-target effects. Recent initial results showed that a single subcutaneous dose of WVE-006 (200mg) in two patients with homozygous "ZZ" AATD was well-tolerated, with no serious adverse events, and led to a durable increase in plasma AAT levels to approximately 11 micromolar, with over 60% being functional wild-type M-AAT—levels consistent with the low-risk heterozygous "MZ" genotype. Results from the Phase 1b/2a trial's multidose arm are expected in 2025. These findings mark a significant milestone for RNA editing therapeutics, demonstrating proof-of-mechanism for ADAR-based RNA editing in patients and highlighting the opportunity RNA editing therapies have to transform the treatment of genetic disorders.

Other companies like AIRNA Bio, Korro Bio, ProQR, and Shape Therapeutics are also contributing to the rapidly evolving RNA editing landscape. While several firms are developing proprietary platforms that utilize endogenous ADAR enzymes to edit RNA transcripts via delivery of ASOs, similar to Wave, some firms, like Shape Therapeutics, are utilizing adeno-associated virus (AAV) vectors to deliver their ADAR guides to different tissues, resulting in sustained guide expression without the need for repeat dosing. This approach contrasts with current ADAR-based editing therapies in or nearing clinical trials, where repeated administration of guide therapies is often required. Additionally, Shape and other groups are exploring novel guide designs, incorporating elements like the SmOPT U7 hairpin system to enhance stability and efficiency.[84] These innovations aim to improve editing precision and broaden the therapeutic potential of RNA editing technologies.

The growing interest in RNA editing has attracted collaborations with major pharmaceutical companies. GSK, Eli Lilly, Novo Nordisk, and Roche have entered partnerships with biotech startups Wave Life Science, ProQR Therapeutics, Korro Bio, and Spark Therapeutics respectively, to accelerate the development of RNA editing therapies. These alliances provide valuable resources and expertise, facilitating the translation of innovative research into clinical applications.

Disclaimer: information in this section is available in the public domain through aforementioned company press releases or publications as of March 31, 2025.

Challenges facing ADAR therapeutics

The advancement of RNA editing therapies utilizing ADAR enzymes places this technology at the forefront of medical innovation. However, the journey from laboratory research to clinical application involves navigating complex regulatory landscapes and addressing unique challenges to ensure both efficacy and safety.

Molecular

A central concern is achieving exceptional specificity in RNA editing to minimize off-target effects and reduce transcriptomic risks. Precise targeting is crucial for ensuring desired therapeutic outcomes while avoiding unintended edits that could lead to deleterious consequences. Researchers employ deep RNA sequencing[57,85] to comprehensively assess both on-target and off-target editing events, interestingly finding that the introduction of an exogenous ADAR increases the rate of off-target A-to-I editing[15,18,86-88] above that of redirected endogenous ADAR which have minimal off-target effects.[14,17,64] A key aspect of this assessment is the characterization of the "editing signature," which refers to the pattern of transcriptomic changes at targeted and non-targeted sites. Understanding the editing signature across different cell and tissue types is key for predicting clinical safety profiles. Recent innovations, such as the fusion of Cas13 or gRNA binding domains with ADAR deaminase domains for directed base editing, exemplify efforts to enhance selectivity.[40,47,64,89] Thorough evaluation of these editing patterns is particularly important because off-target effects can vary widely, from benign synonymous codon changes to harmful non-synonymous mutations that disrupt protein function. These considerations highlight the need for meticulous, case-by-case analyses to ensure therapeutic safety.

In tandem with specificity concerns is the challenge of preserving normal ADAR activity. The human transcriptome naturally contains millions of A-to-I editing sites across thousands of RNA substrates.[90,91] While introducing a therapeutic substrate may not significantly disrupt overall ADAR function, certain gRNA designs with recruitment domains that independently bind ADAR could pose risks if overexpressed. Long-term interference with natural ADAR functions might lead to immunological consequences and unintended impacts on cellular pathways. Therefore, careful gRNA design and thorough preclinical evaluation are essential. Moreover, the differential expression and substrate preferences of ADAR1 and ADAR2 add another layer of complexity.[92] Tailoring therapeutic strategies requires an understanding of these differences, for example, liver-targeted therapies primarily engage ADAR1, whereas brain-targeted treatments must account for higher ADAR2 expression.

The method of delivering gRNAs further complicates the regulatory landscape. Chemically modified oligonucleotides delivered without a carrier offer a straightforward approach but are quickly cleared from the body, and often necessitate repeated dosing due to their transient presence and pharmacokinetic limitations. For certain therapeutics, longer intervals between doses may be advantageous for patient well-being and compliance. Developing safe and effective methods to extend the duration of treatments, such as chemical modifications or sustained-release formulations, could improve therapeutic outcomes. Insights from antisense oligonucleotide (ASO) trials have been instrumental in informing these developments, particularly in optimizing oligonucleotide stability, reducing immunogenicity, and improving delivery efficiency. RNA editing platforms leveraging endogenous ADAR enzymes have begun to adopt these strategies, demonstrating how foundational knowledge from ASO therapeutics can accelerate the clinical application of RNA editing.

Alternatively, viral vectors like AAV can provide persistent expression from a single administration by delivering DNA-encoded gRNAs. However, transitioning an ADAR cassette to an AAV backbone introduces limitations, such as the loss of flexibility for chemical modifications that could enhance potency, selectivity, or reduce off-target effects. Unlike chemically modified oligonucleotides, AAV-delivered constructs

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cannot be readily adjusted post-administration, making initial design and optimization critical to achieving therapeutic efficacy and minimizing risks. While promising, AAV vectors also introduce challenges such as immune responses, limitations on repeat dosing, and potential adverse events at high doses. To address these issues, researchers are exploring strategies like immunosuppression protocols,[93] development of immune-orthogonal AAVs,[94] and capsid engineering to enhance targeting efficiency and reduce dosing requirements.[95]

In parallel, the immune response to the delivered RNA editing tools themselves must also be considered. Although properly modified ADAR gRNAs alone have generally not been associated with strong immunogenicity, other components from the RNA editing toolbox may raise greater concern. For example, PPR proteins, many of which are plant-derived, may be particularly immunogenic in humans. CRISPR-Cas13 systems, being large and bacterial in origin, have also been shown to trigger immune responses, limiting their suitability for repeated or systemic delivery.[96,97] Conversely, exogenous ADAR deaminase-based designs tethered to small human-originated RNA-binding proteins, such as CIRTS, may pose lower immunogenic risk, enable more localized editing to reduce off-target effects, and offer improved compatibility with AAV or other gene therapy vectors due to their compact size. This comparative immunogenicity profile is an important consideration when choosing a therapeutic platform for *in vivo* RNA editing.

Additionally, non-viral delivery methods, including targeted lipid nanoparticles and tissue-specific vehicles, are being developed to expand the reach of RNA editing therapies to tissues beyond the liver.[98,99] Effective delivery methods are crucial for treating diseases affecting various tissues and organs, ensuring that therapeutic agents reach their intended targets. Addressing molecular challenges of specificity and normal ADAR activity, along with optimizing delivery methods and treatment duration, is central for the successful translation of ADAR-based therapeutics into clinical applications.

Regulatory

Navigating the technical challenges of RNA editing therapies is deeply intertwined with the complexities of regulatory approval for these novel treatments. Unlike conventional therapies, RNA editing interventions are tailored to specific molecular mechanisms and diseases, necessitating individualized regulatory assessments. This is particularly evident in the development of treatments for orphan diseases (conditions affecting small patient populations where extensive clinical trials may not be feasible). Regulatory agencies often provide more flexible and adaptive pathways for these rare diseases, making them attractive candidates for RNA editing interventions. While less stringent Investigational New Drug (IND) evaluations for orphan diseases can expedite development and approval, they still require rigorous attention to safety and efficacy. Proactive collaboration with regulatory bodies becomes vital to align safety considerations, establish clear expectations, and adapt approval pathways suitable for these cutting-edge therapies.

Overall, to effectively address the multifaceted challenges in regulation, an integrative approach is crucial. Leveraging insights from multiple model systems, including healthy animal models, disease-specific models, and emerging humanized systems, provides a robust foundation for developing tailored drug strategies. However, species-specific differences in RNA transcript conservation and endogenous ADAR expression present significant translational challenges. RNA editing therapies designed for human transcripts may not function effectively in standard animal models like mice, often necessitating the use of non-human primates (NHPs) to more accurately replicate human disease states. NHPs are particularly valuable in cases where variations in transcript sequences or ADAR tissue expression profiles hinder the use of human-directed therapies in smaller models. Furthermore, even when on-target RNA edit sites are conserved between model species and humans, differences in the whole transcriptome can result in distinct off-target effects, adding another layer of complexity to preclinical evaluation. To address these challenges, researchers are increasingly integrating preclinical studies in NHPs with advanced humanized systems, such as organoids or human-derived cells. This comprehensive strategy not only bridges the gap between preclinical and clinical studies but also aligns with regulatory requirements, ensuring the development of safe and effective interventions across diverse biological systems.

Future directions

Building on the initial success of ADAR-based RNA editing therapies in monogenic diseases, researchers are exploring broader applications to address a wider array of genetic disorders and conditions. This expansion holds promise not only for diseases currently under investigation but also for many other disorders that lack effective treatments. Advancements in technology, understanding of genetic mechanisms, and collaborative efforts are essential to realizing the full potential of ADAR therapeutics. The following subsections highlight key areas where ADAR-based therapies are poised to make significant strides.

Expanding applications to polygenic diseases

Treating polygenic diseases requires a nuanced approach that can modulate several genetic factors simultaneously. By designing gRNAs that target multiple RNA transcripts associated with a disease pathway, it is possible to adjust the expression levels of several genes in a coordinated manner. This multiplexed editing approach could help restore balance in dysregulated networks, potentially ameliorating disease symptoms or progression. The reversibility and controllability of RNA editing make it particularly suitable for polygenic diseases, where dynamic regulation of gene expression is often necessary. Additionally, first clinical trials involving genetic perturbations in polygenic diseases carry greater risk—a challenge that RNA editing can alleviate due to its capacity for reversal. However, this strategy demands sophisticated gRNA design and delivery methods to ensure specificity and minimize off-target effects across the transcriptome.

Enhancing computational guide RNA design

Advancements in computational biology and machine learning are significantly improving the design of gRNAs, which are required for directing ADAR enzymes to specific RNA targets.[100] By optimizing gRNA sequences for higher specificity and efficiency, researchers can increase precision and reduce off-target effects. These technological innovations facilitate the development of more effective RNA editing strategies by enabling rapid screening and prediction of gRNA-target interactions. Enhanced computational tools allow for personalized approaches tailored to individual

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genetic profiles, accelerating the development process and improving therapeutic outcomes.

Broadening editable targets beyond A-to-I conversion

Expanding the scope of editable targets represents a significant avenue for advancement in RNA editing. Currently, endogenous ADARs are limited to adenosine-to-inosine (A-to-I) conversions. Developing methods to specifically edit other nucleotides could significantly broaden therapeutic applications, enabling the correction of a wider range of genetic mutations.

There are several strategies emerging which could help achieve this goal. One approach leverages engineering ADAR variants through rational design or directed evolution to recognize alternative RNA substrates to edit, beyond mispaired adenosines.[101] Another promising avenue in synthetic biology is the creation of hybrid systems by fusing ADARs or other deaminases with other RNA-interacting or modifying enzymes, potentially enabling novel types of nucleotide editing and RNA substrate binding.[35,79,102,103] Alongside these strategies, researchers are investigating the use of chemically modified gRNAs to redirect existing ADAR activity toward alternative nucleotide changes.[104,105] Advances in this area could extend the potential of RNA editing therapies to treat genetic disorders not addressable with current technologies, thereby broadening the impact of RNA therapeutics.

Reprogramming nonsense mutations

One promising, yet still preclinical, application of ADAR-mediated RNA editing is the reprogramming of nonsense mutations (UAG, UGA, UAA) into tolerable tryptophan residues (UGG). This approach could expand the prospective therapeutic strategies of ADAR systems by restoring full-length protein translation in cases where premature stop codons drive disease pathology. While studies have demonstrated the feasibility of such edits *in vitro*,[43,86,87] their clinical translation has yet to be realized.

RNA editing in cancer therapy

In oncology, RNA editing emerges as both a challenge and a promising opportunity for cancer treatment. Abnormal RNA editing patterns have been implicated in cancer transformation and tumorigenesis through misregulating cell proliferation, differentiation, invasion, migration, stemness, metabolism, and drug resistance.[106] By precisely targeting these aberrant editing events, it is possible to correct the expression of oncogenes or restore the function of tumor suppressor genes.[107] Modulating RNA editing pathways using ADAR-based therapeutics can also enhance the immune system's ability to recognize and eliminate cancer cells, particularly in "cold tumors" that are typically unresponsive to immunotherapy.[108] This approach offers a novel avenue for cancer therapy, potentially improving patient outcomes and expanding the efficacy of existing treatments.

Modulating protein interactions via RNA editing

Protein interactions are fundamental to virtually all biological processes, and their dysregulation often underlies diseases such as neurodegenerative disorders, cancers, and immune dysfunctions.[109] Precisely editing specific adenosine residues in mRNA transcripts allows for amino acid substitutions that alter protein binding interfaces without affecting overall protein structure or expression levels. This approach, where we can fine-tune protein functions and interactions in a transient, reversible manner, could disrupt pathological protein complexes to mitigate disease progression, or enhance beneficial interactions improving cellular responses to stress or injury. Modulating protein interactions at the RNA level provides a degree of control that is often unattainable with traditional small-molecule inhibitors or monoclonal antibodies, which may suffer from limited specificity or difficulty in targeting intracellular or traditionally "undruggable" proteins. Advancements in gRNA design, high-resolution structural data, and computational modeling are crucial for identifying critical adenosine residues whose editing yields desired modifications without inducing unintended binding or editing effects. As research progresses, modulating protein-protein interactions via RNA editing could become a powerful tool in the therapeutic arsenal, offering personalized and dynamic treatment options for complex diseases.

Conclusion

ADAR-mediated RNA editing has swiftly transitioned from a fundamental biological phenomenon to a promising therapeutic approach for addressing a wide range of genetic disorders. By enabling precise and reversible modifications of RNA transcripts through an endogenous mechanism, this technology offers unique advantages, minimizing permanent off-target effects and enhancing dynamic regulation of gene expression. Crucially, advances in understanding ADAR enzyme functions, coupled with innovations in guide RNA design and delivery methods, have propelled the field toward clinical applications. Therapies entering human trials underscore the tangible potential of this approach to improve patient outcomes. While challenges remain in achieving optimal specificity and efficient delivery, ongoing research and the concerted efforts of the scientific community are steadily overcoming these obstacles. The convergence of scientific insight and technological innovation positions ADAR-mediated RNA editing to become a cornerstone of precision medicine, offering new hope for treating diseases that have long lacked effective therapeutic options.

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Conflicts of interest

P.M. is a scientific co-founder of Shape Therapeutics, Boundless Biosciences, Navega Therapeutics, Pi Bio, and Engine Biosciences. The terms of these arrangements have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies.

Author contributions

Joseph Rainaldi, Prashant Mali, Sami Nourreddine (Conceptualization, Writing). All authors approved the final version of the manuscript.

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