RNA BIOLOGY
2021, VOL. 18, NO. S1, 41–50
https://doi.org/10.1080/15476286.2021.1983288

REVIEW

OPEN ACCESS Check for updates

Site-directed RNA editing: recent advances and open challenges

Hamid Mansouri Khosravi and Michael F. Jantsch

Center of Anatomy & Cell Biology Division of Cell & Developmental Biology Medical, Unviersity of Vienna Schwarzspanierstrasse Vienna, Austria

ABSTRACT
RNA editing by cytosine and adenosine deaminases changes the identity of the edited bases. While cytosines are converted to uracils, adenines are converted to inosines. If coding regions of mRNAs are affected, the coding potential of the RNA can be changed, depending on the codon affected. The recoding potential of nucleotide deaminases has recently gained attention for their ability to correct genetic mutations by either reverting the mutation itself or by manipulating processing steps such as RNA splicing. In contrast to CRISPR-based DNA-editing approaches, RNA editing events are transient in nature, therefore reducing the risk of long-lasting inadvertent side-effects. Moreover, some RNA-based therapeutics are already FDA approved and their use in targeting multiple cells or organs to restore genetic function has already been shown. In this review, we provide an overview on the current status and technical differences of site-directed RNA-editing approaches. We also discuss advantages and challenges of individual approaches.

ARTICLE HISTORY
Received 12 June 2021
Revised 25 August 2021
Accepted 16 September 2021

Introduction

Up to 60% of all genetic disorders are caused by point mutations [1,2]. These can affect transcript stability, translation, or RNA-splicing. Moreover, in coding regions point mutation can induce amino-acid substitutions and introduce or eliminate stop codons. Most point mutations lead to loss of function mutations but also gain of function mutations can be observed. As point mutations can be found in all classes of proteins, the consequences can be numerous and range from tumour formation, over neuromuscular diseases to metabolic disorders, to name a few. Today, more than 6,000 diseases of genetic origin are known, with numbers constantly rising [3].

Technologies for the treatment of genetic disorders are expanding with many ongoing clinical trials, some already completed or even approved [4]. Some remedies are available to treat symptoms, however, only 10% of genetic disorders are treatable by conventional methods [5]. A promising and widely tested approach for the treatment of genetic disorders is gene therapy or gene editing, with 1800 ongoing or completed trials worldwide [4].

Gene editing was pioneered in the 1990s [6] and aims at manipulating or replacing defective genetic material. Technologies involved engineered zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and engineered meganucleases [7]. The most recent and versatile tool are CRISPR-Cas9 RNA-programmed nucleases and their numerous derivatives [8]. While genome editing has great potential, there are essential concerns precluding its widespread clinical use. First and foremost, genomic manipulation introduces permanent alterations to the genome both at targeted sites but also at off-target sites [9]. Further, genome editing remains largely impractical for post-mitotic cells that lack reliable DNA-break-repair mechanisms [10,11]. Finally, systemic delivery of large protein-encoding constructs or proteins themselves that are required to cut DNA poses a problem in itself that still needs to be overcome.

Recently, a new approach that can bypass some of the above obstacles has emerged. Here, instead of inducing permanent modifications in DNA, RNA is transiently targeted, which can be used for therapeutic and research purposes [12,13]. Tunability and the transient nature of changes introduced to RNA turn this strategy to a safer and potentially more applicable tool for genome editing [14–16]. In this review, we provide an overview of recent developments and approaches towards site-directed RNA editing.

Site-Directed RNA Editing (SDRE)

Directed targeting of RNAs is intensively explored since the discovery of siRNAs and miRNAs, where many therapeutic approaches were taken to exploit endogenous siRNA and miRNA machineries to repress gene expression [17,18]. Similarly, antisense-based approaches have been developed to regulate mRNA levels or to correct splicing defects [19].

Attempts to recode RNAs utilizing RNA-editing are relatively novel. RNA-editing describes the alteration of an RNA sequence by introducing or removing nucleotides from an RNA or by changing the character of a nucleobase by deamination. For therapeutic purposes, the two types of deamination reactions, converting cytidine to uridine and adenosine to

CONTACT Michael F. Jantsch Michael.Jantsch@muv.ac.at Center of Anatomy & Cell Biology Division of Cell & Developmental Biology Medical, University of Vienna Schwarzspanierstrasse 17 A-1090 Vienna, Austria

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

inosine are considered most useful. As inosines are decoded as guanosines during translation, both C to U and A to I changes can directly alter coons and recode RNAs.

A-to-I editing was first described in the late 1980s [20]. A-to-I editing is mediated by adenosine deaminases acting on RNA (ADARs). These enzymes bind double-stranded or structured regions in RNAs via their double-stranded RNA-binding domains [21]. An adenosine that is typically located within the double-stranded region is then recognized by the catalytic deaminase domain and converted to inosine. Substrate-binding and deamination occur with relatively low specificity, leading to abundant and widespread editing *in vivo* [22]. Still, some sites are edited with very high specificity.

In mammals, two catalytically active ADARs are found, ADAR1 and ADAR2. ADAR1 harbours three double-stranded RNA binding domains (dsRBDs), while ADAR2 contains only two dsRBDs [23]. ADAR2 is primarily expressed in the nervous system, the vasculature and the intestine [24]. Due to its nuclear localization signal, the protein is localized to the nucleus. Most naturally occurring editing events that lead to mRNA recoding are mediated by this enzyme. ADAR1, in contrast, is expressed from two promoters. An interferon-inducible promotor leads to the expression of a 150 kDa protein that is mainly localized to the cytoplasm due to the inclusion of a first exon that contains a nuclear export signal [25]. Cytoplasmic ADAR1 p150 is involved in antiviral defence but also modifies endogenous repeat-derived RNAs. A shorter ADAR1 isoform is constitutively expressed and gives rise to a 110kDa protein that is mainly localized to the nucleus due to a nuclear localization signal overlapping its third double-stranded RNA-binding domain [26]. The therapeutic potential of ADARs was already explored in the middle of the 1990s by Ribozyme Pharmaceuticals for thier use in correcting a premature stop codon in dystrophin and thus treatment of muscular dystrophy [27].

Strangely, the idea of therapeutic RNA editing vanished quickly. However, the concept was revived almost two decades later by independent groups [28,29]. Meanwhile, several companies are testing RNA-editing systems to develop potential treatments for different maladies ranging from acute pain to severe genetic disorders such as muscular dystrophy. The potential of RNA editing has already been tested in model systems for the RNA-repair of several genetic disorders related to Duchenne muscular dystrophy, cystic fibrosis, Hurler's syndrome, Rett-syndrome, or ornithine transcarbamylase deficiency [13,29–33].

C to U editing is naturally mediated by Apobec enzymes [34]. Indeed, the catalytic domain of Apobec1 has been artificially targeted to a reporter RNA to alter its coding. Similarly, Apobec3-derived catalytic domains have been used to edit DNA. Most interestingly, site-directed C to U editing was also achieved with an in vitro evolved ADAR variant that accepts cytosines rather than adenosines as a substrate for deamination [35].

Below, we list existing tools and strategies that have been put forward as solutions for site-directed RNA editing. These are also illustrated in Figure 1.

λN-fusions

One of the first strategies to establish site-directed RNA editing involved λN-BoxB-ADAR fusion constructs. This technology pioneered by the Rosenthal group builds on the well-known interaction between the Lambda N protein (λN) and the BoxB RNA hairpin [29]. To allow site-specific targeting, the deaminase domain of ADAR2 is fused to the RNA binding domain of the λN protein. The λN protein naturally binds to short stem-loop RNA structures called BoxBs. Cotransfection of a guide RNA (gRNA) that harbours a BoxB sequence and that is antisense to the region to be edited mediates the targeting of the λN-ADAR fusion to the target sequence for modification [29]. Like all guide RNAs, the length determines the specificity but also delineates the region where the double-strand specific catalytic deaminase domain will act. The main advantage of the λN-BoxB method is the small size of the editing complex. This enables packaging of the editing complex and several copies of the gRNA into a single virus [31].

Like most SDRE approaches, λN-BoxB also leads to off-target editing in the mRNA/gRNA duplex [36]. Moreover, transcriptome-wide off-target editing can be observed which may be explained either by overexpression of the editase [36] or the long duplex formation of gRNA with mRNA [37]. However, off-target editing levels can be reduced to a lower rate by introducing an NLS to the enzyme and by a reducing the guide RNA concentration [13]. Interestingly, the λN-system has been used successfully in cultured neurons but also in whole mouse brains to correct mutations in MeCP2, the cause of Rett-syndrome [38]. Thus, despite the artificial character of the construct and the need for viral vehicles for delivery, its use for therapeutic purposes seems realistic.

SNAP-ADAR

The SNAP-ADAR technology established by Stafforst and colleagues is a well tunable SDRE strategy that has been used in cells but also embryos [12,28,39]. The system fuses the deaminase domain of ADAR to the SNAP enzyme and uses a modified nuclease-resistant guide RNA (gRNA) conjugated to O6-benzyl-guanine (BG) [28,40]. The SNAP-tag moiety is engineered to form a covalent linkage with O6-benzyl-guanine (BG) as a substrate. Subsequently, the SNAP-ADAR complex linked to BG gRNA will be guided to the target for modification. Remarkably, SNAP-ADAR, can reach an editing efficiency of 90% when a hyperactive mutant version of ADAR2 is being used. This is the highest efficiency reported today [13].

The SNAP-tag was also fused to the catalytic C-terminus of murine APOBEC1 thereby allowing C-to-U editing in an RNA-guided manner. Applying the SNAP-APOBEC1 fusion, site-specific C-to-U editing with an efficiency of 20% was detected in a GAPDH transcript with no detectable A-to-I RNA editing [41].

Besides the BG modification of the gRNA most recently, a HALO tag was introduced to allow orthogonal targeting of RNAs by engineered APOBEC enzymes. The HALO tag requires guide RNAs to be labelled with a 1-chloroalkane moiety [42]. The chemistry involved allows also double

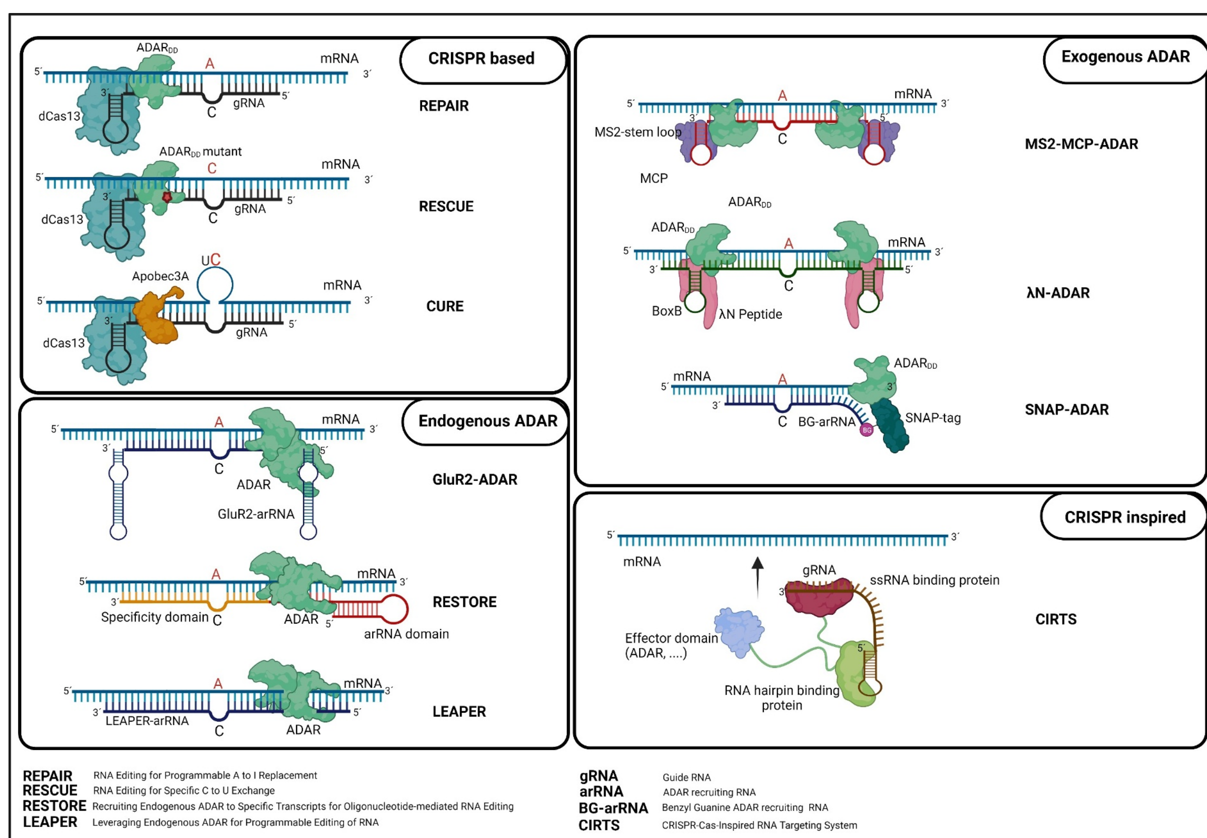

Figure 1. Overview on current strategies for site-directed RNA editing

Top left: CRISPR-based editing approaches take advantage of the programmable targeting of an (inactive) dCAS13 moiety fused to a catalytically active deaminase domain from ADAR (REPAIR). Alternatively a mutated (DD) version of the ADAR catalytic domain can be used to change substrate specificity from A to C (RESCUE). A fusion to Apobec3G introduces specificity for UC dinucleotides that must be located in a bulged region (CURE). In all cases the dCas13 fusion is recruited by specific CRISPR guide RNAs. **Top right**: ADAR catalytic domains can also be recruited by fusing them to RNA-binding domains that bind to specific sequences or by fusing them to a SNAP-tag using click chemistry. Guide RNAs that specifically target the region to be edited also contain a region that recruits the ADAR fusion protein, MCP, λN-peptide, or a SNAP tag. **Bottom left**: Endogenous ADARs can be recruited via guide RNAs that either partly mimic an endogenous target (GluR2-arRNA, RESTORE) or that generate double-stranded RNAs of different lengths that recruit ADARs (LEAPER). Background editing can be reduced by appropriate chemical modifications to the guide RNAs or by optimizing base composition to avoid background editing. **Bottom right**: CIRTS, a CRISPR inspired system uses a combination of RNA binding domains fused to an ADAR catalytic domain in conjunction with a guide RNA to recruit the artificial and ectopically expressed protein fusion to regions of interest in the transcriptome. The modularity of this system allows recruitment of different catalytic domains and targeting of multiple RNAs.

linkage of a BG and 1-chloroalkane moiety therefore allowing simultaneous recruitment of two fusion proteins (e.g. ADAR1 & ADAR2, or ADAR2 & APOBEC1 fusions). The efficiency of this system reaches ~70% editing for A to I editing and ~50% for C to U. C to U editing was therefore higher than with the RESCUE approach (see below) [35].

Off-target editing can be observed in this system that can be separated into guide-RNA dependent and guide-RNA independent background editing. guide RNA-dependent off-target editing can be reduced by optimizing guide RNAs with respect to sequence but also to chemical modifications, further adjustments can be introduced to optimize stability, specificity, and targeting efficiency [43,44]. A key advantage of the SNAP technology is the use of short gRNAs (ca. 20 nt), which can be efficiently transfected into a broad range of cells using technologies that have been developed for antagomirs and siRNAs [45,46]. Since the ADAR fusion is of human origin, only a low chance of immunogenicity exists.

Still, BG -modified gRNA are not genetically encodable and may have toxic side effects. Additionally, in scenarios where repeated treatments are required, SNAP-ADAR may not be the best strategy as too many components would need to be delivered in a tissue or cell-specific manner [12,13]. As for all ADAR-derived systems, also the editing efficiency of SNAP-ADAR is influenced by next-neighbour preferences [13].

MS2-MCP-ADAR

Another way to recruit an engineered ADAR is the MS2-MCP-ADAR approach which fuses the deaminase domain of ADAR to the bacteriophage MS2 coat protein (MCP). Normal or hyperactive ADAR domains can be used (MCP-ADAR, or MCP-$ADAR_{DD}$). The MCP binds the MS2 stem-loop RNA. The antisense gRNA that determines the double-stranded region in which the target adenosine is located is then fused to the MS2 stem-loop. The MS2 loop region then recruits the MCP-ADAR or $ADAR_{DD}$ fusion protein to the editing site [47,48].

High editing efficiency is a clear advantage of this system. However, there is notable off-target editing that needs to be considered [47,48]. Clearly, a disadvantage of the system is the promiscuous binding of MCP to short structured regions in RNAs [49]. Further, the system requires many components that need to be introduced to cells. The latter problem has recently been overcome by generating multi-purpose vectors that allow the simultaneous expression of guides and optimized ADAR-fusions from a single plasmid [50].

MS2-mediated RNA editing was also used for C to U editing by generating a MS2 APOBEC1 fusion [51]. The system was used to restore gene expression of blue fluorescent protein (BFP) by correction of a termination T to C mutation with 21% efficiency and low off-target editing [51]. Improvement of this system might be achieved by altering the MS2 stem loop and using gRNAs of different lengths.

CRISPR-Cas13 approaches for SDRE

Recently, CRISPR-based genome editing approaches have gotten broad attention, primarily due to their programmable nature and wide applicability. The discovery of the type VI CRISPR nuclease Cas13, which binds to single-stranded RNA directed by a programmable CRISPR RNA (crRNA) guide, has expanded the use of CRISP-Cas systems from DNA targeting to RNA targeting [52,53–56]. The RNAse activity of Cas13 can be deactivated by mutating two critical catalytic residues, thus allowing repurposing for the recruitment of other domains [52,53,56,57]. As Cas13 does not require a protospacer adjacent motif (PAM) sequence, the targeting space of Cas13 fusions is almost unlimited [52,56].

REPAIR

REPAIR (RNA Editing for Programmable A to I Replacement) was the first Cas13-based RNA editing system to be reported. In this system, $ADAR2_{DD}$ is fused to a Cas13b orthologue derived from *Prevotella sp*. to create a programmable RNA editor. The gRNA used in this system has a 5′ homology region of 30 to 84 nucleotides that hybridizes with the substrate, carrying an A-C mismatch to the target adenosine. A spacer sequence then separates a direct repeat region at the 3′ end that is used to recruit dCas13b to the sequence of interest [58]. Using this system, editing levels of ~30% could be routinely achieved on different substrates. The considerable off-target effects found for REPAIRv1 could be reduced by site-directed mutagenesis of the catalytic domain ($ADAR2_{DD}$-E488Q/T375G). A reduction of the size of the Cas13 moiety facilitated the genetic delivery of the system. The advanced platform termed REPAIRv2 achieves significantly lower off-target editing while maintaining high-efficiency on-target editing of 27% and 13% on endogenous *Kras* and *Ppib* transcripts [58]. Besides the lower off-target effects of the improved system, the lack of motif preferences surrounding the target adenosine allows repair of any adenosine in the transcriptome using REPAIRv2.

RESCUE

The first platform established for C-to-U RNA editing was termed RESCUE (RNA Editing for Specific C to U Exchange) [59]. It is based on the introduction of mutations in $ADAR_{DD,}$ that allow the deamination of cytosines. Consequently, the mutant enzyme allows both A-to-I and C-to-U modifications, thereby doubling the number of mutations that can be corrected. The engineered enzyme was fused to a deactivated Cas13 orthologue analogous to REPAIR.

Besides allowing both A-to-I and C-to-U editing, the RESCUE system can be minimized for AAV packaging and delivery [60]. However, the double functionality of RESCUE can also be problematic. The expanded deamination spectrum increases the risk for unintended transcriptomic modifications. To tackle this problem, RESCUE-S was developed by rational mutagenesis of S375A of $ADAR_{DD}$. This mutation results in an approximately 1.8-fold reduction in C-U off-targets and an almost 12-fold reduction in A-I off-targets while maintaining specificity [13]. Still, while potentially versatile in its use, a broadened target spectrum may also be problematic when it comes to therapeutic uses of this system.

CURE (C to U editing)

A recently developed C to U editing tool is termed CURE (C to U RNA Editor) [61]. CURE fuses the APOBEC3A (A3A) cytidine deaminase to dCas13. Unlike RESCUE, CURE only deaminates cytosines, turning this system to the first cytidine-specific C-to-U RNA editor. As A3A has a high specificity for UC dinucleotides, off-target editing at other dinucleotides is very low. Further, as A3A edits Cs in specific loop regions unconventional guide RNAs are designed to induce loops at the target sites. Thus, CURE has a high specificity towards C to U editing and shows significantly fewer target mutations than RESCUE. The specificity of CURE was further optimized by tightly controlling the intracellular localization by the addition of a nuclear localization signal which further reduced off target editing [61]. CURE-X uses CasRx instead of Cas13. CasRx harbours internal flexible loops into which A3A was inserted therefore further decreasing off-target editing. Taken together, CURE can reach up to 60% editing at endogenous targets. Depending on the localization and type of fusion used in CURE, off-target edits vary dramatically with the highest off-target edits observed in cytoplasmically localized versions, the lowest with nuclear or CURE-X fusions. Thus, at specific sites CURE is superior over RESCUE. However, the preference for UC dinucleotides reduces the number of targetable C residues.

CIRTS

Recently, a synthetic RNA targeting strategy called CRISPR-Cas-Inspired RNA Targeting System (CIRTS) was developed. This system is entirely built from parts of human proteins [62]. This programmable RNA-targeting platform consists of: (1) a gRNA with a complementary sequence to the target RNA of interest. The gRNA also harbours a specific RNA structure that interacts with the engineered hairpin binding

protein; (2) an RNA hairpin-binding protein as the core of this system that serves as a selective, high-affinity protein binder on the engineered gRNA; (3) a ssRNA binding protein that binds to the gRNA, which stabilizes and protects the target interaction; and (4) an effector protein that acts on the targeted sequence [60,62].

Compared to other artificial editing systems, CIRTS consists of smaller components, which can ease the delivery by systems like adeno-associated virus (AAV) [13,62]. CIRTS is a versatile programmable platform that can accommodate multiple protein domains. These domains can act as 'writers', 'readers', or 'erasers' to target effectively any transcript of interest and can therefore be used for the delivery or removal of many RNA modifications. Moreover, besides RNA-editing proteins for the conversion of A-to-I or C-to-U, CIRTS has been used to deliver effector proteins such as translational activators or RNA degrading enzymes, all in a gRNA-dependent manner [60]. The human-derived nature of CIRTS components vanishes the concerns for immunogenicity for in vivo application of this strategy [60]. Thus, CIRTS may be of value for therapeutic interventions and research purposes [60].

However, there are also challenges to be tackled for efficient use of CIRTS. For instance, an RNA hairpin-binding protein used to bind to RNAs has an endogenous RNA hairpin binding partner that could influence stem-loop RNA trafficking. Also, the cationic peptide ß-defensin-3, another component of the system, might still interact with its intracellular binding partners and stimulate unwanted biological responses [62].

Endogenous ADAR Approaches

As an alternative to recruiting ectopically expressed ADAR- or Apobec fusion proteins to editing sites, the recruitment of endogenous ADARs requires fewer components and reduces the potential for immune responses. However, the recruitment of endogenous ADARs also strongly relies on the abundance and substrate-specificity of ADAR family members. Importantly, recruiting endogenous ADARs relies on the efficient creation of RNA structures that are recognized by ADARs. This is typically achieved by introducing exogenous RNAs that basepair with endogenous substrate RNAs to generate these structures.

GluR2-ADAR

The idea to recruit endogenous ADARs by mimicking endogenous substrates was independently proposed by two labs [32,63]. Both labs exploit the well-understood structure of the Gria2 (GluRB) RG stem-loop. By building a sequence that looks like the Gria2 stem-loop but that extends on either the 5′- or 3′-end to hybridize with the target RNA, a favourable binding site for ADARs is brought in proximity to the substrate which at the same time is forced to form a double-strand. The gRNA, therefore, contains two sections; 1) the ADAR recruiting region (ARR) that interacts and recruits hADAR2, 2) the antisense region (ASR), which is complementary to the target RNA and determines the target site.

In case hyperactive or other ADAR versions are required, these can also be packaged into an AAV together with the GluR2-adRNA sequences. However, the primary benefit lies in the ability to recruit endogenous ADARs [64]. In fact, additional ectopic expression of ADAR2, has led to high toxicity, possibly due to off-target editing [1,13,65]. A recent report demonstrates that shortening the guide RNA can reduce the off-targeting rate [64].

RESTORE

An improved system termed RESTORE (Recruiting Endogenous ADAR to Specific Transcripts for Oligonucleotide-mediated RNA Editing) uses short (20–40 nucleotide) chemically modified antisense oligonucleotides. The modified gRNA is recruiting endogenous ADAR to the target location and is composed of two domains; 1) an invariant R/G ADAR recruiting domain to guide the endogenous ADAR to the gRNA-mRNA hybrid, and 2) the programmable specificity domain that dictates target mRNA binding [15,60].

The high potential of RESTORE for therapeutic application was shown by correcting mutations in disease-relevant transcripts. In fact, the ease and versatility of delivery of the ASO may simplify the clinical application through applying several delivery strategies for oligonucleotides considerably. Delivery could be achieved via liposomal nanoparticles or by linkage to moieties that facilitate cellular uptake such as *N*-acetylgalactosamine (GalNAc) which target hepatocytes [60,66]. Several chemical modifications were applied and tested for the RESTORE guide RNAs to improve efficiency and specificity. These included 2′O-CH3, phosphorothioate linkage, and locked nucleic acids. While the former modifications are known to improve the stability of RNAs when introduced at their ends, it was surprising to see that also internal 2′O-CH3 additions helped to improve editing efficiency.

LEAPER

LEAPER (Leveraging Endogenous ADAR for Programmable Editing of RNA) utilizes long antisense RNAs (71–191 nucleotides) to generate double-stranded regions to attract ADARs [67]. While LEAPER does not intend to mimic a particular substrate, the long regions resemble typical repeat-derived ADAR1 substrates that stimulate binding and editing by endogenous ADAR1. LEAPER shows an efficiency of up to 50% on endogenous targets and limited off-target editing [67]. Like in other approaches, the gRNA contains a single A-C mismatch at the target site to direct editing [67]. Interestingly, partial randomization of guide RNAs also allowed selection of optimized nucleotide compositions. Moreover, LEAPER was used to simultaneously target editing at different sites allowing multiplex editing through the use of several gRNAs [60]. The lack of specific chemical modifications in the RNA allows delivery by viral vectors, by transcriptionally active plasmids, or as a synthetic oligonucleotide into different cell types, including multiple human primary cell types [60].

Optimizing RNA design

Common to most mentioned approaches is the need for an efficient guide RNA design. Interestingly, the length of complementarity, structure formed, and mode of enzyme recruitment varies between the systems discussed. This resembles the situation found *in vivo*, where the structures of endogenous editing substrates vary widely and clearly depend on the type of enzyme recruited [68,69]. Thus, guide RNA design will most likely need to be optimized for each substrate individually.

Common problems of all RNA-based therapeutics are their stability, antigenicity, specificity and deliverability. For instance, phosphorothioate linkage and 2′O-CH3 modifications have been widely used to increase stability and reduce antigenicity. While these modifications are mainly introduced at the ends of RNAs, 2′O-CH3 was also shown to boost editing when introduced centrally. Target adenosines are more efficiently deaminated when found opposite a C in an non-basepaired conformation [68]. Interestingly, the Beal lab could show that a combination of a guide RNA that is abasic opposite the target A and an engineered ADAR2 that puts a phenylalanine at position 488 the target specificity can be dramatically increased while decreasing off-target editing, as the phenylalanine prevents the base flipping required for editing [70]. Similarly, introduction of a deoxycytidine opposing the target A can boost editing with a hyperactive variant of ADAR2 [71]. Still, both approaches require the use of an engineered ADAR enzyme that needs to be introduced to cells. Most interestingly, introduction of a Benner's base Z opposing the A also leads to a boost in editing in combination with endogenous ADAR, thus precluding the need for an engineered ADAR [71].

Off-target editing poses another problem. Here, introduction of non-standard nucleotides such as 2′-methoxy or 2′-fluoro have been shown to reduce off-target editing significantly [43]. Lastly, specific delivery of nucleic acids poses a problem. Current methods range from lipid nanoparticle delivery over coupling to specific moieties such as cholesterol [72], GalNac [66], circRG peptides [73], or specific peptides [74]. Still, as new routes of cellular uptake are being tested, RNA-delivery technologies can be expected to improve.

Therapeutic use of SDRE

All approaches discussed above may be used for SDRE. Still, advantages and disadvantages exist for all systems. For example, the big size of Cas proteins and their bacterial origin makes their delivery challenging and will also activate an immune response when used in organisms [75]. The problem of immune stimulation will also hold true for other protein fusions including MS2 or λN fusions. Interestingly, λN fusions have been successfully used to correct MeCP2 deficiency in the brain. The success of this approach most likely also lies in the different types of immune responses observed in the central nervous system [76].

ADAR recruiting platforms are less complex and with new chemical modifications of guide RNAs being explored, off-target editing will hopefully decrease while maintaining or even improving on-target editing. However, a direct comparison of currently available tools is difficult as no side by side benchmarking has been performed.

Here we list successful attempts to recode known mutations in RNAs:

REPAIRv1 was applied to correct mutations in AVPR2 (W293X) causing X-linked nephrogenic diabetes insipidus and in FANCC (W506X) causing Fanconi anaemia, respectively. In HeK293 cells expressing the mutant cDNAs 35% correction was achieved for AVPR2 and 23% for FANCC. Another 33 ectopically expressed mRNAs could be repaired with up to 28% editing efficiency [58]. REPAIRv2 was used to correct mutations in ectopically expressed ABCA4 and USH2A which account for 25% of all US cases of retinal degeneration [13]. USH2A could be edited with 43% efficiency in HEK293T cells [13]. Nonetheless, in vivo data is still missing on the applicability and efficiency of this system.

A genetically encodable guide RNA to re-target ADAR2 was used to correct a mutation in a PINK1-reporter RNA (linked to Parkinson's disease) with 65% efficiency [32]. Endogenous substrates were edited to 38% using this system [32].

Direct targeting of human mutations in corresponding cell lines was achieved using RESTORE where a PiZZ mutation leading to α1-antitrypsin deficiency or a critical tyrosine 701 in STAT1 were targeted. An editing rate of up to 40% was reached for STAT1 and PiZZ mutations with quite low off-target rates. In this system, reasonable editing rate was also achieved in the absence of IFN-α, indicating independence from the induced expression of ADAR1 [15].

LEAPER was used to target a premature stop codon in a TP53 reporter using three different ADAR-recruiting (ar) RNAs [33]. All three TP53-W53X-targeting arRNAs corrected the mutation with an editing yield of 25% to 35% with the variable off-target phenomenon.

Correction of G-to-A mutations was also tested in two mouse models. An AAV8-ADAR2-GluR2adRNA construct was used to target a stop codon in the mdx model of Duchenne muscular dystrophy [30]. Here, two adjacent adenosines needed to be edited to change a stop codon (TAA) to tryptophan (TGG). Intramuscular injection of an AAV-GluR2-adRNA-ADAR2 (both with and without the hyperactive E488Q mutation) resulted in 0.8% correction and gave rise to partial dystrophin protein restoration [30]. In the second model, a G > A splice variant was targeted in the *spfash* mouse model for ornithine transcarbamylase (OTC) deficiency. Systemic injection of the construct led to 4.6–8.2% correction of the pre-mRNA and depletion of the incorrectly spliced fraction of mRNA. Moreover, a 2.5–5% restoration of ornithine transcarbamylase protein was detected [13,30].

A BoxB-λN-$ADAR_{DD}$ fusion was used to repair a *Mecp2* mutation in vivo. For delivery, an AAV expressing a guide RNA and a hyperactive ADAR2 fusion was injected in the hippocampus of mice. Excitingly, up to 50% editing rates and restoration of protein function was observed [38]. On the

downside, up 2900 and 909 off target editing sites were observed in the presence and absence of guide RNAs, repectively. Most off-target sites were edited to less than 30%. Still, these data suggest that optimized guide RNA design may further help to reduce background editing for this system [38].

Lastly, SDRE by RESCUE was tested across nine genes containing 24 synthetic disease-relevant mutation targets. Here, editing rates up to 42% were achieved in HeLa cells [59].

Advantages of SDRE

Compared to genomic editing, SDRE bares several advantages:

(Safety) SDRE clearly reduces the risk of permanent off-target mutations that are intrinsic to DNA editing [13]. The transient nature of RNA reduces the potential risks associate with the observed off-target editing events [77].

(Tunability) SDRE is tunable, both, with respect to guide RNA and enzyme concentrations that govern editing rates [1,13].

(Delivery) The relatively small nature of the components involved allows easier delivery of SDRE components than those required for genomic editing. This is especially true when endogenous ADARs are recruited by a single antisense guide RNA.

(Antigenicity) Since ADARs and Apobecs are mammalian enzymes, their proteins are not foreign to the immune system. In contrast, Cas9 originated from microorganisms, and can introduce an immune response in the human body. In fact, antibodies to CRISPR-Cas proteins are already circulating on a wide fraction of people [78–80].

(Admission) Today, already six RNA based therapeutics are FDA-approved. Thus, the approval of RNA editing treatments could be considerably accelerated.

(Ease) Genomic DNA is sequestered in the nucleus and tightly bound by histones. In contrast, RNAs can be targeted in the nucleus and the cytoplasm [29]. Nonetheless, RNAs may also be less accessible during translation, as shown in recent studies [14].

(Applicability) Therapeutic CRISPR-Cas9-based genome editing could be used primarily for the manipulation of stem cells or early embryos. In contrast, SDRE can be used to treat conditions in postmitotic cells [81].

Challenges

Besides the mentioned advantages, there are still some challenges that need to be resolved before SDRE is ready for clinical applications.

(Stability and durability) The low stability of RNA and transient nature of the treatment needs to be improved. Thanks to intensive research on the therapeutic use of small RNAs, numerous chemical modifications that improve stability are known today [14,40,82,83]. Most recently, embedding of guide RNAs in circular RNAs has been shown to increase their stability by weeks [84]. This may indeed dramatically improve the usability of SDRE.

(Immunogenicity) Unmodified RNAs trigger the innate immune system. This problem has already been recognized in the past and can be overcome by introducing appropriate nucleic acid modifications that evade recognition by RNA sensors [85–87].

(Versatility) Probably the biggest obstacle is the fact that RNA editing enzymes are limited to specific alterations, typically A to I and C to U deaminations. Moreover, catalytic domains show substrate specificities. Some of these restrictions can be overcome by introducing specific amino acid exchanges to the catalytic domains [59,88,89]. However, this is only an option when the active protein is ectopically expressed. When endogenous proteins are harnessed, this option is not available [90].

(Delivery) Recruiting the appropriate machineries to tissues and RNAs of interest is complex. The efficiencies of delivery vary largely, depending on the tissue of interest. Direct injection into cells or surrounding fluids is quite efficient [13,91]. Similarly, targeting specific cells with appropriately modified nucleic acids can be achieved [92,93]. However, not all cell types and tissues can be targeted today. Also, intracellular release of transfected nucleic acids poses another challenge to efficiency of SDRE [94].

Inside the cell, target RNAs need to be accessible. It was already shown that translation interferes with SDRE [40]. It can therefore be assumed that RNA-binding proteins may prevent access to target sites thereby limiting editing efficiency.

(Specificity) Off-target editing has not yet been assessed for all systems in a transcriptome-wide manner [95]. Although the transient nature of off-target effects will vanish with time it is still important to prevent undesired changes in the transcriptome [13]. However, as outlined above, altering guide RNA-sequences and their chemical modifications can help to decrease off-target editing [14,40,46,70,71].

Outlook

While site-directed RNA-editing is a relatively new field, a rapidly growing community has achieved impressive technological improvements. Importantly, ADAR- and Apobec-derived enzymes will only be able to correct a fraction of existing genetic conditions. However, with more than 170 chemical modifications known to occur in different types of RNAs [96] it is likely that also other enzymes will be harnessed to repair, optimize and modify genetic information at the RNA level.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Work in the lab of MFJ is funded by the Austrian Science Foundation (FWF) Grant nrs F8007 and P30505. This paper was based upon work from COST Action CA16120 EPITRAN, supported by COST (European Cooperation in Science and Technology); austrian science fund [F8007]; austrian science fund [P30505];

ORCID

Michael F. Jantsch http://orcid.org/0000-0003-1747-0853

References

[1] Chen G, Katrekar D, Mali P. RNA-Guided Adenosine Deaminases: advances and Challenges for Therapeutic RNA Editing. Biochemistry. 2019;58(15):1947–1957.
[2] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018;19(12):770–788.
[3] Orphanet: About rare diseases. 2020. [cited 2021 Jul] Available from: www.orpha.net.
[4] Ginn SL, Alexander IE, Edelstein ML, et al. Gene therapy clinical trials worldwide to 2012 – an update. J Gene Med. 2013;15(2):65–77.
[5] Treatment for genetic disorders. 2020. [cited 2021 Aug] Available from: https://www.rx-genes.com/.
[6] Woolf TM. Therapeutic repair of mutated nucleic acid sequences. Nat Biotechnol. 1998;16(4):341–344.
[7] Method of the Year. Nature. Methods. 2011;2012(9):1.
[8] Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–821.
[9] Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol. 2018;36(8):765–771.
[10] Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. Nat Med. 2015;21(2):1947–1957.
[11] Ran FA, Hsu PD, Wright J, et al. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013;8(11):2281–2308.
[12] Vogel P, Stafforst T. Critical review on engineering deaminases for site-directed RNA editing. Curr Opin Biotechnol. 2019;55:74–80.
[13] Fry LE, Peddle CF, Barnard AR, et al. RNA editing as a therapeutic approach for retinal gene therapy requiring long coding sequences. Int J Mol Sci. 2020;21(3):777.
[14] Vogel P, Moschref M, Li Q, et al. Efficient and precise editing of endogenous transcripts with SNAP-tagged ADARs. Nat Methods. 2018;15(7):535–538.
[15] Merkle T, Merz S, Reautschnig P, et al. Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides. Nat Biotechnol. 2019;37(2):133–138.
[16] Gulei D, Raduly L, Berindan-Neagoe I, et al. CRISPR-based RNA editing: diagnostic applications and therapeutic options. Expert Rev Mol Diagn. 2019;19(2):83–88.
[17] Chakraborty C, Sharma AR, Sharma G, et al. Therapeutic miRNA and siRNA: moving from Bench to Clinic as Next Generation Medicine. Mol Ther Nucleic Acids. 2017;8:132–143.
[18] Fu Z, Zhang X, Zhou X, et al. In vivo self-assembled small RNAs as a new generation of RNAi therapeutics. Cell Res. 2021;31(6):631–648.
[19] Scharner J, Aznarez I. Clinical applications of single-stranded oligonucleotides: current landscape of approved and in-development therapeutics. Mol Ther. 2021;29(2):540–554.
[20] Bass BL, Weintraub H. An unwinding activity that covalently modifies its double-stranded RNA substrate. Cell. 1988;55(6):1089–1098.
[21] Nishikura K. Functions and regulation of RNA editing by ADAR deaminases. Annu Rev Biochem. 2010;79(1):321–349.
[22] Bazak L, Haviv A, Barak M, et al. A-to-I RNA editing occurs at over a hundred million genomic sites, located in a majority of human genes. Genome Res. 2014;24(3):365–376.
[23] Bajad P, Jantsch MF, Keegan L, et al. A to I editing in disease is not fake news. RNA Biol. 2017;14(9):1223–1231.
[24] Jain M, Mann TD, Stulic M, et al. RNA editing of Filamin A pre-mRNA regulates vascular contraction and diastolic blood pressure. EMBO J. 2018;37(19):e94813.
[25] George CX, Wagner MV, Samuel CE. Expression of interferon-inducible RNA adenosine deaminase ADAR1 during pathogen infection and mouse embryo development involves tissue-selective promoter utilization and alternative splicing. J Biol Chem. 2005;280(15):15020–15028.
[26] Fritz J, Strehblow A, Taschner A, et al. RNA-regulated interaction of transportin-1 and exportin-5 with the double-stranded RNA-binding domain regulates nucleocytoplasmic shuttling of ADAR1. Mol Cell Biol. 2009;29(6):1487–1497.
[27] Woolf TM, Chase JM, Stinchcomb DT. Toward the therapeutic editing of mutated RNA sequences. Proc Nat Acad Sci. 1995;92(18):8298.
[28] Stafforst T, Schneider MF. An RNA–deaminase conjugate selectively repairs point mutations. Angew Chem. 2012;51(44):11166–11169.
[29] Montiel-Gonzalez MF, Vallecillo-Viejo I, Yudowski GA, et al. Correction of mutations within the cystic fibrosis transmembrane conductance regulator by site-directed RNA editing. Proc Natl Acad Sci U S A. 2013;110(45):18285–18290.
[30] Katrekar D, Chen G, Meluzzi D, et al. In vivo RNA editing of point mutations via RNA-guided adenosine deaminases. Nat Methods. 2019;16(3):239–242.
[31] Sinnamon JR, Kim SY, Corson GM, et al. Site-directed RNA repair of endogenous Mecp2 RNA in neurons. Proc Natl Acad Sci U S A. 2017;114(44):E9395–E402.
[32] Wettengel J, Reautschnig P, Geisler S, et al. Harnessing human ADAR2 for RNA repair - Recoding a PINK1 mutation rescues mitophagy. Nucleic Acids Res. 2017;45:2797–2808.
[33] Qu L, Yi Z, Zhu S, et al. Leveraging Endogenous ADAR for Programmable Editing on RNA. Nat Biotechnol. 2019 Nov;37(11):1380.
[34] Anant S, MacGinnitie AJ, Davidson NO. apobec-1, the catalytic subunit of the mammalian apolipoprotein B mRNA editing enzyme, is a novel RNA-binding protein. J Biol Chem. 1995;270(24):14762–14767.
[35] Abudayyeh OO, Gootenberg JS, Franklin B, et al. A cytosine deaminase for programmable single-base RNA editing. Science. 2019;365(6451):382–386.
[36] Vallecillo-Viejo IC, Liscovitch-Brauer N, Montiel-Gonzalez MF, et al. Abundant off-target edits from site-directed RNA editing can be reduced by nuclear localization of the editing enzyme. RNA Biol. 2018;15(1):104–114.
[37] Jo DH, Koo T, Cho CS, et al. Long-term effects of in vivo genome editing in the mouse retina using campylobacter jejuni cas9 expressed via adeno-associated virus. Mol Ther. 2019;27(1):130–136.
[38] Sinnamon JR, Kim SY, Fisk JR, et al. In vivo repair of a protein underlying a neurological disorder by programmable RNA editing. Cell Rep . 2020;32(2):107878.
[39] Hanswillemenke A, Kuzdere T, Vogel P, et al. Site-directed rna editing in vivo can be triggered by the light-driven assembly of an artificial riboprotein. J Am Chem Soc. 2015;137(50):15875–15881.
[40] Vogel P, Schneider MF, Wettengel J, et al. Improving site-directed rna editing in vitro and in cell culture by chemical modification of the guiderna. Angew Chem. 2014;53(24):6267–6271.
[41] Stroppel AS, Latifi N, Hanswillemenke A, et al. Harnessing self-labeling enzymes for selective and concurrent A-to-I and C-to-U RNA base editing. Nucleic Acids Res. 2021;49(16):e95–e95.
[42] Los GV, Encell LP, McDougall MG, et al. HaloTag: a novel protein labeling technology for cell imaging and protein analysis. ACS Chem Biol. 2008;3(6):373–382.
[43] Vogel P, Moschref M, Li Q, et al. Efficient and precise editing of endogenous transcripts with SNAP-tagged ADARs. Nat Methods. 2018;15(7):535–538.
[44] Reautschnig P, Vogel P, Stafforst T. The notorious R.N.A. in the spotlight - drug or target for the treatment of disease. 2017 May 4;14(5):651-668.
[45] Kim D-H, Behlke MA, Rose SD, et al. Synthetic dsRNA dicer substrates enhance RNAi potency and efficacy. Nat Biotechnol. 2005;23(2):222–226.

[46] Krützfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature. 2005;438(7068):685–689.
[47] Bhakta S, Azad MTA, Tsukahara T. Genetic code restoration by artificial RNA editing of Ochre stop codon with ADAR1 deaminase. Protein Eng Des Sel. 2018;31(12):471–478.
[48] Azad MTA, Bhakta S, Tsukahara T. Site-directed RNA editing by adenosine deaminase acting on RNA for correction of the genetic code in gene therapy. Gene Ther. 2017;24(12):779–786.
[49] Helgstrand C, Grahn E, Moss T, et al. Investigating the structural basis of purine specificity in the structures of MS2 coat protein RNA translational operator hairpins. Nucleic Acids Res. 2002;30(12):2678–2685.
[50] Tohama T, Sakari M, Tsukahara T. Development of a single construct system for site-directed RNA editing using MS2-ADAR. Int J Mol Sci. 2020;21(14):4943.
[51] Bhakta S, Sakari M, Tsukahara T. RNA editing of BFP, a point mutant of GFP, using artificial APOBEC1 deaminase to restore the genetic code. Sci Rep. 2020;10(1):17304.
[52] Abudayyeh OO, Gootenberg JS, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science. 2016;353(6299):aaf5573–aaf.
[53] East-Seletsky A, O'Connell MR, Knight SC, et al. Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. Nature. 2016;538(7624):270–273.
[54] Konermann S, Lotfy P, Brideau NJ, et al. Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors. Cell. 2018;173(3):665–76.e14.
[55] Shmakov S, Smargon A, Scott D, et al. Diversity and evolution of class 2 CRISPR-Cas systems. Nat Rev Microbiol. 2017;15(3):169–182.
[56] Abudayyeh OO, Gootenberg JS, Essletzbichler P, et al. RNA targeting with CRISPR-Cas13. Nature. 2017;550(7675):280–284.
[57] Smargon AA, Cox DBT, Pyzocha NK, et al. Cas13b Is a Type VI-B CRISPR-Associated RNA-Guided RNase Differentially Regulated by Accessory Proteins Csx27 and Csx28. Mol Cell. 2017;65(618–30.e7):618–630.e7.
[58] Cox DBT, Gootenberg JS, Abudayyeh OO, et al. RNA editing with CRISPR-Cas13. Science. 2017;358(6366):1019.
[59] Abudayyeh OO, Gootenberg JS, Franklin B, et al. A cytosine deaminase for programmable single-base RNA editing. Science. 2019;365(6451):382.
[60] Aquino-Jarquin G. Novel engineered programmable systems for ADAR-mediated RNA editing. Mol Ther Nucleic Acids. 2020;19:1065–1072.
[61] Huang X, Lv J, Li Y, et al. Programmable C-to-U RNA editing using the human APOBEC 3A deaminase. EMBO J. 2020;39(22):e104741.
[62] Rauch S, He E, Srienc M, et al. Programmable RNA-guided RNA effector proteins built from human parts. Cell. 2019;178(1):122–34.e12.
[63] Fukuda M, Umeno H, Nose K, et al. Construction of a guide-RNA for site-directed RNA mutagenesis utilising intracellular A-to-I RNA editing. Sci Rep. 2017;7(1):41478.
[64] Nose K, Hidaka K, Yano T, et al. Short-chain guide RNA for site-directed A-to-I RNA editing. Nucleic Acid Ther. 2020;31(1):58–67.
[65] Chen L, Li Y, Lin CH, et al. Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma. Nat Med. 2013;19(2):209–216.
[66] Nair JK, Willoughby JL, Chan A, et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust rnai-mediated gene silencing. J Am Chem Soc. 2014;136(49):16958–16961.
[67] Qu L, Yi Z, Zhu S, et al. Programmable RNA editing by recruiting endogenous ADAR using engineered RNAs. Nat Biotechnol. 2019;37(9):1059–1069.
[68] Eggington JM, Greene T, Bass BL. Predicting sites of ADAR editing in double-stranded RNA. Nat Commun. 2011;2(1):319.
[69] Uzonyi A, Nir R, Shliefer O, et al. Deciphering the principles of the RNA editing code via large-scale systematic probing. Mol Cell. 2021;81(11):2374–2387.e3.
[70] Monteleone LR, Matthews MM, Palumbo CM, et al. A bump-hole approach for directed RNA editing. Cell Chem Biol. 2019;26(2):269–77 e5.
[71] Doherty EE, Wilcox XE, van Sint Fiet L, et al. Rational Design of RNA Editing Guide Strands: cytidine Analogs at the Orphan Position. J Am Chem Soc. 2021;143(18):6865–6876.
[72] Benizri S, Gissot A, Martin A, et al. Bioconjugated Oligonucleotides: recent Developments and Therapeutic Applications. Bioconjug Chem. 2019;30(2):366–383.
[73] Alam MR, Dixit V, Kang H, et al. Intracellular delivery of an anionic antisense oligonucleotide via receptor-mediated endocytosis. Nucleic Acids Res. 2008;36(8):2764–2776.
[74] Henke E, Perk J, Vider J, et al. Peptide-conjugated antisense oligonucleotides for targeted inhibition of a transcriptional regulator in vivo. Nat Biotechnol. 2008;26(1):91–100.
[75] Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. Nat Commun. 2018;9(1):3497.
[76] Galea I, Bechmann I, Perry VH. What is immune privilege (not)? Trends Immunol. 2007;28(1):12–18.
[77] Step aside CRISPR, RNA editing is taking off. Nature-News Feature Nature, 2020.
[78] Wagner DL, Amini L, Wendering DJ, et al. High prevalence of Streptococcus pyogenes Cas9-reactive T cells within the adult human population. Nat Med. 2019;25(2):242–248.
[79] Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat Med. 2019;25(2):249–254.
[80] Simhadri VL, McGill J, McMahon S, et al. Prevalence of Pre-existing Antibodies to CRISPR-Associated Nuclease Cas9 in the USA Population. Mol Ther Methods Clin Dev. 2018;10:105–112.
[81] Montiel-Gonzalez MF, Diaz Quiroz JF, Rosenthal JJC. Current strategies for site-directed RNA editing using ADARs. Methods. 2019;156:16–24.
[82] Yu A-M, Jian C, Yu AH, et al. RNA therapy: are we using the right molecules? Pharmacol Ther. 2019;196:91–104.
[83] Doherty EE, Wilcox XE, van Sint Fiet L, et al. Rational Design of RNA Editing Guide Strands: cytidine Analogs at the Orphan Position. J Am Chem Soc. 2021;143(18):6865–6876.
[84] Katrekar D, Yen J, Xiang YC, et al. Robust RNA Editing via Recruitment of Endogenous ADARs Using Circular Guide RNAs. Mol Ther. 2021;29:126.
[85] Freund I, Eigenbrod T, Helm M, et al. RNA Modifications Modulate Activation of Innate Toll-Like Receptors. Genes (Basel). 2019;10(2):92.
[86] Gehrig S, Eberle M-E, Botschen F, et al. Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. J Exp Med. 2012;209(2):225–233.
[87] Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. Chem Biol. 2012;19(1):60–71.
[88] Eggington JM, Greene T, Bass BL. Predicting sites of ADAR editing in double-stranded RNA. Nat Commun. 2011;2(1):319.
[89] Matthews MM, Thomas JM, Zheng Y, et al. Structures of human ADAR2 bound to dsRNA reveal base-flipping mechanism and basis for site selectivity. Nat Struct Mol Biol. 2016;23(5):426–433.
[90] Betts L, Xiang S, Short SA, et al. Cytidine Deaminase. The 2·3 Å Crystal Structure of an Enzyme: transition-state Analog Complex. J Mol Biol. 1994;235(2):635–656.
[91] Hanswillemenke A, Kuzdere T, Vogel P, et al. Site-directed RNA editing in vivo can be triggered by the light-driven assembly of an artificial riboprotein. J Am Chem Soc. 2015;137(50):15875–15881.
[92] Debacker AJ, Voutila J, Catley M, et al. Delivery of Oligonucleotides to the Liver with GalNAc: from Research to Registered Therapeutic Drug. Mol Ther. 2020;28(8):1759–1771.
[93] Hangeland JJ, Flesher JE, Deamond SF, et al. Tissue Distribution and Metabolism of the [32 P]-Labeled Oligodeoxynucleoside

Methylphosphonate-Neoglycopeptide Conjugate, [YEE(ah-GalNAc) 3]-SMCC-AET-pU m p T 7, in the Mouse. Antisense and Nucleic Acid Drug Development. 1997;7(3):141–149

[94] Juliano RL. Intracellular Trafficking and Endosomal Release of Oligonucleotides: what We Know and What We Don't. Nucleic Acid Ther. 2018;28(3):166–177.

[95] Montiel-González MF, Vallecillo-Viejo IC, Rosenthal JJC. An efficient system for selectively altering genetic information within mRNAs. Nucleic Acids Res. 2016;44:e157–e.

[96] Boccaletto P, Machnicka MA, Purta E, et al. MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res. 2018;46(D1):D303–D7.