Editing Approaches to Treat Alpha-1 Antitrypsin Deficiency

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TOPIC IMPORTANCE: Alpha-1 antitrypsin (AAT) deficiency is a genetic disorder most commonly due to a single G to A point mutation (E342K), leading to debilitating lung and/or liver disorders and is associated with increased mortality. The E342K point mutation causes a conformational change of the AAT protein resulting in its retention in liver hepatocytes. This reduces AAT secretion into the serum resulting in higher protease activities due to the lack of inhibition from AAT, causing damage to healthy lung tissue. The current standard of care for lung manifestations involves weekly IV augmentation therapy and is considered suboptimal for these patients. Furthermore, there is currently no approved treatment for liver manifestations. The unmet medical need for patients with AAT deficiency remains high, and new treatment options are needed to treat the underlying disease etiology.

REVIEW FINDINGS: Advances in genomic medicines may enable treatment by editing the DNA or RNA sequence to produce wild-type AAT instead of the mutated AAT caused by the E342K mutation. One approach can be achieved by directing endogenous adenosine deaminases that act on RNA to the E342K RNA site, where they catalyze adenosine to inosine conversion through a process known as RNA editing. The A-I RNA change will be read as a G during protein translation, resulting in an altered amino acid and restoration of wild-type AAT secretion and function.

SUMMARY: In this review, we will discuss the pathophysiology of AAT deficiency and emerging treatment options with particular focus on RNA editing as a disease-modifying treatment for both liver and lung disease. CHEST 2024; ■(■):■-■

KEY WORDS: AATD; ADAR; DNA editing; RNA editing; RNA therapeutics

Alpha-1 antitrypsin (AAT) is a ∼52-kDa glycoprotein serine-protease inhibitor consisting of 394 amino acids encoded by the *SERPINA1* gene in liver hepatocytes. At steady state, levels of AAT reach 20 to 53 μM in plasma, 10 to 40 μM in interstitial fluid, and 2 to 5 μM in the epithelial lining fluid.[1] Although AAT inhibits several serine proteases, one of its primary functions is to neutralize the activity of neutrophil elastase. Without controlled inhibition, increased neutrophil elastase activity can damage lung tissues by degrading elastin and other structural proteins, leading to damage to the

ABBREVIATIONS: AAT = alpha-1 antitrypsin; AATD = alpha-1-antitrypsin deficiency; ADAR = adenosine deaminase acting on RNA; GalNAc = N-acetylgalactosamine; LNP = lipid nanoparticle; PS = phosphorothioate

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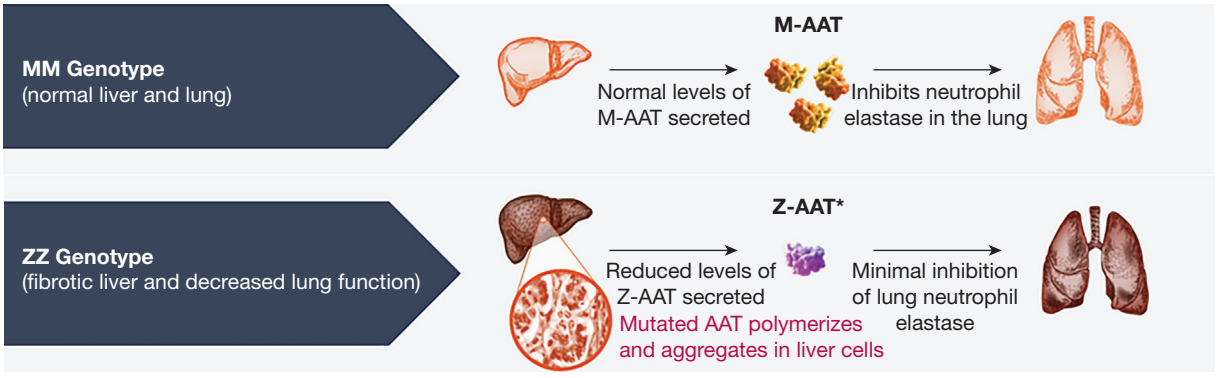

Figure 1 – *AAT section in MM and ZZ genotype. *Z-AAT significantly less active compared to M-AAT. AAT = alpha-1 antitrypsin.*

walls of alveoli and bronchi, ultimately resulting in emphysema.[2] In addition, AAT also exhibits antiinflammatory, immunomodulatory, and tissue protective effects[3] and is released into the circulation as an acute phase reactant in response to inflammation, infection, and tissue injury.[4] During these conditions, AAT levels in the bloodstream can increase fourfold to sixfold compared with steady-state levels.[4]

Alpha-1-antitrypsin deficiency (AATD) is a genetic disorder that leads to low plasma levels of AAT and is a significant risk factor for the development of lung diseases (Fig 1). Mutations in *SERPINA1* can result in misfolding, affecting its export out of hepatocytes, its ability to bind to other proteins, and overall function in vital organs.[5] The normal allele of AAT is designated as Pi*M and is found in approximately 90% of the population. The most common AATD-causing alleles are Pi*S (Glu264Val) and Pi*Z (Glu[E]342Lys[K] also known as E366K based on the full length protein). Individuals who are carriers of 2 Z alleles (Pi*ZZ) have only 10% to 20% of the normal levels of circulating AAT. The severity and manifestation of AATD depends on the genotype and resultant serum AAT level with Z being one of the more significant risk factors for developing liver and lung disease. Blanco et al[6] estimated the prevalence of Pi*ZZ in non-Hispanic White individuals in the United States to be 1 in 4,126 people, consistent with data from Ashenhurst et al.[7]

Lung manifestations of AATD due to insufficient inhibition of neutrophil elastase include COPD and early emphysema. The progression of these manifestations depends on genotype, degree of deficiency in circulating AAT, and environmental risk factors (eg, tobacco use). Based on initial genetic studies, a plasma AAT level of 11 μM (57 mg/dL) has historically been considered the threshold concentration at which the lung appears to be able to protect itself from excess proteinase activity.[8,9] However, studies and data analyses have suggested that this level may not be fully protective and patients with active disease may benefit from additional AAT.[10]

Furthermore, patients with AATD due to the Z mutation are also at risk of developing liver disease, which often progresses to fibrosis and cirrhosis (Fig 1). In the liver, the Z allele leads to enhanced protein degradation and/or aggregation of polymerized mutated AAT in hepatocytes, causing proteotoxic liver stress and damage. The degree of accumulation of Z-AAT polymers in the liver is correlated with hepatocyte injury and progression of fibrosis.[11,12] Risk factors for more severe liver disease in adults include age > 50 years, male sex, obesity, metabolic syndrome, and diabetes mellitus.[13]

Literature Search

Management of Lung Disease

The primary treatment approach for patients with AAT < 11 μM and evidence of lung disease is AAT augmentation therapy. This therapy involves administering exogenous AAT protein derived from the plasma of healthy individuals through IV infusion once per week. The aim of AAT augmentation therapy is to increase functionally normal AAT in the blood and lungs to a level where lung damage is less likely to occur.[14,15] Studies of augmentation therapy demonstrate reduced loss of lung density, slowing the decline in lung function in patients with moderate obstructive lung disease.[16,17] Retrospective analyses suggest a reduction in mortality risk and a prolongation of the time to lung transplant.[18-20] Withdrawal of AAT augmentation therapy from

deficient patients results in a substantial increase in pulmonary exacerbations and hospitalizations.[21] AAT augmentation therapy regimens can be inconvenient for patients due to the need for lifetime weekly infusions.[22] Although several AAT augmentation therapy products have been approved, they are not reimbursed in every country. As a result, many patients have no access to treatment.[23]

New therapeutic options for AAT replacement therapy are in development and undergoing clinical trials. Kamada's inhaled AAT would allow for delivering AAT directly to the lungs and hence possibly improve the quality of life of patients with AATD.[24] Although a clinical trial in patients with severe AATD suggested there was no associated reduction in exacerbation frequency,[25] a new phase 3 study in moderately affected patients is underway to determine the efficacy and safety of AAT inhalation therapy (NCT04204252). In addition, a new IV therapy involving a recombinant AAT-IgG4 Fc fusion protein to enhance AAT half-life is currently under clinical investigation. Results from a phase 1 study suggest that normal AAT serum levels (> 20 μM) are maintained when dosed every 3 weeks, with the potential for longer intervals.[26] Further clinical studies are being planned for the fusion protein along with understanding the impact of activity for the recombinant protein beyond neutrophil elastase. Finally, Grifols is investigating a subcutaneous formulation (Alpha-1 15%) and comparing the formulation to the IV formulation.[27]

Management of Liver Disease

Currently, there is no approved pharmacologic treatment for AATD-related liver disease and liver transplantation is the only way of resolving advanced liver cirrhosis. Existing evidence demonstrates survival and rapid normalization of serum AAT concentrations in both adults and children after transplantation.[28] Of note, AAT augmentation therapy is not recommended because clinical experience suggests that AATD-related liver disease is unaffected by this therapy.[29]

Several new therapeutic approaches for the treatment of AATD-related liver disease are under investigation, which aim to reduce the intrahepatic Z-AAT burden by (1) correcting or stabilizing the folding of the mutated Z-AAT to allow its secretion; and/or (2) decreasing Z-AAT polymers by either stimulation of their degradation or silencing their production. The latter would also reduce circulating Z protein and could potentially exacerbate lung risk based on observations from humans that are null for the *SERPINA1* gene.[30]

One possibility to stabilize the mutated Z-AAT is to use chaperones which are proteins that keep AAT in its native conformation, facilitating proper folding and enhancing secretion.[31] The corrector VX-864 was being assessed in a phase 2 trial in individuals with the PiZZ genotype but the small increase in serum (~2.2-2.3 μM) in AAT concentrations observed so far is deemed insufficient to confer any clinical benefit beyond, possibly, liver disease.[32]

Small interfering RNAs are double-stranded RNAs that can be tuned to recognize specific target RNA sequences (eg, the mutated *SERPINA1* messenger RNA) and reduce the RNA. An open-label phase 2 study with fazirsiran showed a reduction in intrahepatic and serum levels of Z-AAT, a decrease in polymerized intrahepatic Z-AAT polymers, and a reduction in liver enzymes associated with ongoing hepatocyte injury.[33] The decrease in serum Z-AAT concentrations after fazirsiran therapy was not accompanied by exacerbation of pulmonary disease despite reduced circulating Z protein, even among those with preexisting emphysema. However, long-term controlled studies with a larger sample size will be needed to evaluate pulmonary function.

Literature Search

A search via PubMed for editing therapies in AATD was performed. Articles that described and were specific for RNA editing or DNA editing were used for inclusion in this review. Background for any technology was sourced back to the original publication.

Evidence Review

Treatment of Liver and/or Lung Disease via Editing

Gene editing offers the prospect of not only reducing the synthesis of mutant Z-AAT but also simultaneously increasing the synthesis of wild-type M-AAT, thus addressing both the gain of function liver disease and the loss of function lung disease in this population. It also allows for the maintenance of endogenous physiologic regulation of corrected *SERPINA1* expression and wild-type M-AAT synthesis as an acute phase reactant to inflammation and tissue injury.

DNA Editing to Treat AATD

DNA editing involves a permanent genetic change to introduce the normal DNA or correct a mutated DNA

sequence in vivo, with the potential for life-long treatment after a single therapeutic dose. These approaches typically involve the CRISPR-Cas9 proteins, coopted from the bacterial immune system, that direct DNA editing machinery to a target genome locus through a guide RNA. For the Z mutation, this has been shown preclinically through the following approaches: (1) knock in of the normal *SERPINA1* sequence to direct expression of functional AAT,[34-36] (2) knock out the mutant *SERPINA1* sequence to reduce levels of Z-AAT in the liver,[35,37] or (3) base editing to correct the E342K mutation.[38]

Conventional CRISPR-Cas9 systems mediate editing by inducing double-stranded DNA breaks at target loci, which are then repaired via nonhomologous end joining or homology-directed repair.[39] Nonhomologous end joining can introduce insertions or deletions leading to frameshift mutations and premature stop codons to knock out the gene. Homology-directed repair, on the other hand, can be leveraged by concurrently introducing a DNA repair template to attain the desired edit; however, this repair mechanism shows variability in efficiency across cell types and is primarily active in dividing cells, potentially limiting efficacy in adult-onset diseases. Base editing approaches still use Cas9 and guide RNA to direct the enzyme to a target sequence, but do not involve double-stranded breaks and instead direct the chemical alteration of a nucleobase.[40,41] A cytidine deaminase is conjugated to CRISPR-Cas9 to catalyze G→A or C→T conversions, whereas adenosine deaminases can convert A→G or T→C.

Delivery of CRISPR-Cas9 components to the liver has been achieved through adeno-associated virus vectors and lipid nanoparticles (LNPs), with the former approach limited by vector size to multiple components, and potential toxicity and immunogenicity issues. Furthermore, the CRISPR-Cas9 system itself could activate an immune response due to its bacterial origins. The durability of these delivery methods has not been extensively evaluated; however, the potential for long-term effects after a single dose could reduce patient burden and side effects that may accompany frequent dosing. CRISPR-based gene insertion into the albumin locus, an approach used by Intellia Therapeutics, Inc and other companies for lung-related phenotypes of AATD, enables continued and stable expression of *SERPINA1* sufficient to maintain AAT protein levels > 1 year in nonhuman primates and potentially indefinitely. Base editing, on the other hand, has unique advantages because its mechanism avoids double-stranded breaks in the DNA. Because correction does not depend on the DNA repair machinery, editing efficiency may be improved because nondividing cells could also be targeted. Furthermore, because the precise E342K mutation can be edited back to the wild-type sequence, this approach has the potential to address both lung and liver manifestations of AATD by reducing liver aggregations and increasing functional AAT in circulation.

Another major concern with CRISPR-Cas9 technology is the potential for off-target genome edits, which are permanent mutations outside the target region that could inadvertently disrupt protein function.[42] In a PiZ mouse model, adeno-associated virus delivery of CRISPR-Cas9 components including a homology-directed repair template led to editing and secretion of M-AAT. However, there was 23% insertion or deletion rate around the guide RNA target region, including mutations predicted to generate potentially toxic truncated AAT protein.[34] Base editing using LNP delivery to correct the E342K mutation in the PiZ mouse model successfully achieved sustained editing in the liver, rescuing serum AAT liver histology. However, this base editing approach resulted in > 18% bystander editing in patient fibroblasts and up to 5% in the livers of treated NOD scid gamma-PIZ mice.[38] Similar bystander editing was observed with base editing of AATD-derived induced pluripotent stem cells.[43]

RNA Editing to Treat AATD

RNA editing approaches are also designed to correct genomic mutations, but at the transcript level (Fig 2). The transient nature of RNA editing reduces the possibility of permanent mutations to the patients' genome. Adenosine deaminases acting on RNA (ADARs) are also endogenous to most cell types, including hepatocytes which have been shown previously to be amenable to oligonucleotide delivery. Redirecting endogenous ADAR editing activity using synthetic oligonucleotides to position 342 on *SERPINA1* has shown promise in cell-based assay systems.[44] Advantages over DNA editing include reduction in off-target events, avoidance of an exogenous immunogenic protein, avoidance of the permanence of changes induced by DNA editing, and activity in both dividing and nondividing cells.

RNA editing is especially attractive when considering treatment for a broad class of disease severity observed in patients with AATD due to the Z mutation. Furthermore, in PiMZ carriers for AATD, a smaller or less frequent dose may be suitable to reduce the

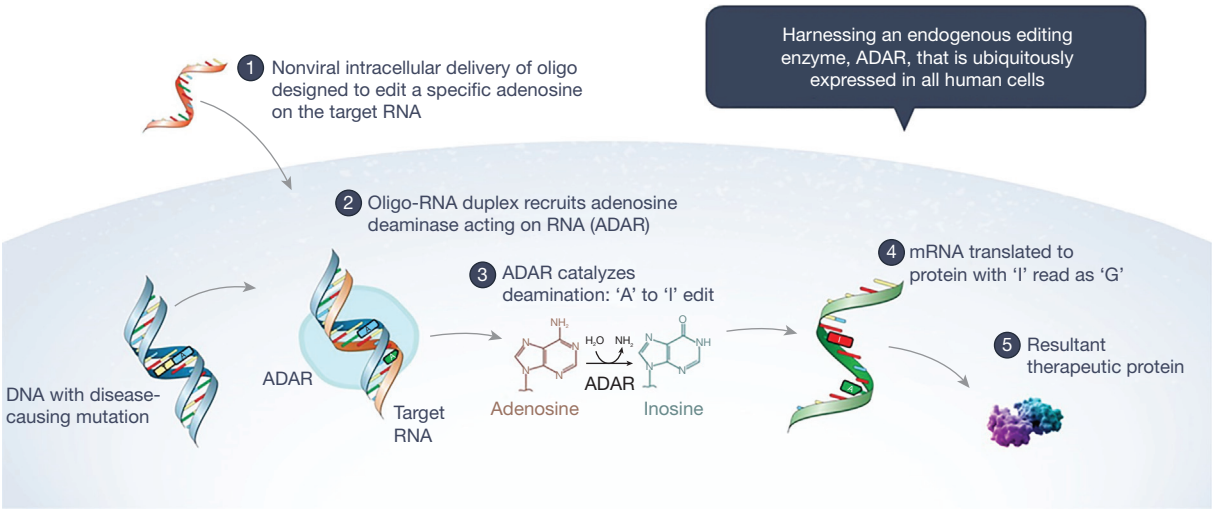

Figure 2 – *Oligo-targeted RNA editing. ADAR = adenosine deaminase acting on RNA; mRNA = messenger RNA.*

synthesis and polymerization of Z-AAT and the sensitization of the hepatocyte to second-hit injury from environmental, viral, or other insults. Finally, RNA editing allows titratable dosing to ensure the synthesis of sufficient quantities of M-AAT and ensure the reduction of polymeric Z-AAT in the liver. The furthest in development for patients with AATD with the Z mutation are as follows: (a) Wave Life Sciences' WVE-006 in clinical development which is an RNA editing candidate delivered via N-acetylgalactosamine (GalNAc),[45] and (2) Korro Bio's KRRO-110 which is an RNA editing oligonucleotide delivered via LNP.[46] Recently, Wave Life Sciences found administration of WVE-006 in two PiZZ participants, 6.9 μM circulating M-AAT, and a 10.8 μM total AAT concentrations on day 15 after a single dose, compared to undetectable M-AAT and total AAT levels at baseline.[45]

Oligonucleotide Delivery to Liver Hepatocytes

Despite the myriad of challenges that limit the delivery of oligonucleotides, design strategies that improve oligo stability, tissue targeting, cellular uptake, and endosome escape have all contributed to the successful implementation of three clinically validated delivery strategies for liver hepatocytes. These approaches include gymnotic delivery, LNP encapsulation, and GalNAc conjugate-based delivery. The success of these approaches is reflected in the approval of 15 liver-targeting oligo drugs by the US Food and Drug Administration.[47] In the next section, we will provide a brief overview of these three delivery technologies including insights into their strengths and limitations.

Gymnotic Delivery: Chemical modification of oligonucleotides can improve their stability in vivo. Alterations to the phosphate backbone, sugars, and bases of oligonucleotides enhance their stability and has allowed for the systemic dosing of gapmer antisense oligonucleotides (ASOs).[48] Improving oligo stability by replacing the phosphodiester backbone with phosphorothioate (PS) linkages has the added benefit of increasing binding to circulating proteins. Unfortunately, high doses of PS-ASOs are needed to achieve therapeutically relevant concentrations in liver hepatocytes due to their delivery to a multitude of other tissues. The increased level of PS-ASOs in circulation carries with it an associated class-specific toxicity called thrombocytopenia and can also lead to acute kidney injury via damage to the proximal tubules.[49,50]

LNP Delivery: Encapsulation of oligonucleotides into LNPs is an approach to prevent oligo degradation and avoid potential toxic side effects of systemically delivered PS-containing oligonucleotides.[51] After IV injection, LNPs interact with the circulating apolipoprotein E, which then directs the LNPs to liver hepatocytes through apolipoprotein E's interaction with low density lipoprotein receptors on the hepatocyte cell surface. This interaction triggers endocytosis of the entire LNP and its oligo cargo.[52,53] Once internalized, LNPs are localized in early endosomes with LNP cargo released into the cytosol via protonation of the ionizable lipids on the LNPs due to the low pH found in the maturing endosomal compartments.[54] This process is much more efficient than the standard endosome escape rate for naked oligonucleotides and allows for a significant

mRNA (E342K)

5'-UGCAUAAGGCUGUGCUGACCAUCGAC**AAG**AAAGGGACUGAAGCUGCUGGGGCC

Figure 3 – *Section of PiZ mutated SERPINA1 mRNA 26 nucleotides upstream and downstream of the editing site (underlined). mRNA = messenger RNA.*

lowering of the total dose, and quick release of oligo cargo to allow for a faster onset of action.

The discovery of biodegradable ionizable lipids has enabled an improved safety profile for LNPs, and impacted their stability and potency. The first approved siRNA-LNP medicine, patisiran, was approved in 2018.[55,56] Since then, the therapeutic potential of LNPs has been expanded far beyond liver and small interfering RNAs with targeting of extrahepatic organs, and the delivery of much larger cargos including sgRNAs for CRISPR-Cas9 approaches and mRNA cargoes for vaccines and protein replacement therapies. The development of LNP-delivered mRNA vaccines for SARS-CoV-2 has proven to be safe and effective in billions of people.[57-59] Overall, LNP-based systems can be a delivery vehicle for the development of safe and effective therapies for a wide range of liver and extrahepatic diseases.

GalNAc Conjugate Delivery: An oligo conjugate approach offers an improvement over gymnotic delivery due to its tissue specificity and lowered toxicity. GalNAc is a carbohydrate ligand that binds with high affinity to ASGR, a highly expressed receptor on the hepatocyte cell surface.[60] Conjugation with GalNAc has been shown to increase drug efficacy by 10-fold in vivo in preclinical species, which enables the lowering of the total dose and an improvement in the therapeutic index. Indeed, shifting PS-ASOs from gymnotic to conjugated delivery dramatically changed the distribution of PS-ASOs from liver sinusoidal endothelial cells to hepatocytes, and significantly improved potency.[61] Another benefit of GalNAc-mediated delivery is the extended duration of action seen originating from a slow release of oligo from an intracellular depot, thought to be late endosomes and lysosomes.[62]

Using Endogenous ADAR Activity for RNA Editing

ADAR is an RNA-editing enzyme that catalyzes the posttranscriptional conversion of adenosine to inosine in RNA molecules. This process can lead to the alteration of the coding potential of RNA and affects gene expression, RNA stability, and splicing. There are three known isoforms of ADAR: ADAR1, ADAR2, and ADAR3, which differ in their expression patterns and substrate specificities. In using established oligonucleotide chemistry for targeting ADAR, there is the ability to leverage major oligonucleotide advances in the last 4 decades to enhance the drug-like properties, enabling their development as therapeutics.[63-65]

ADAR Recruitment Domain

There have been previous reports of successful A to I editing of RNA by recruitment to endogenous ADARs using synthetic oligonucleotides, and this work has also been reviewed elsewhere.[66-68] With this approach, the oligonucleotide is delivered to cells that contain a therapeutically relevant mRNA to which it binds through Watson-Crick interactions. Endogenously expressed ADAR is engaged, the target adenosine on the mRNA strand is positioned in the active site of the enzyme, and A to I conversion is catalyzed. A variety of oligonucleotides with different structural features have been used to elicit A to I editing, most of which are inspired by natural substrates that are known to be deaminated with very high efficiency.[69,70] Two notable advances in the field are the recruiting endogenous ADAR to specific transcripts for oligonucleotide mediated RNA editing (RESTORE)[71] and leveraging endogenous ADAR for programmable editing of RNA (LEAPER) approaches.[72] Studies have been performed using nuclear magnetic resonance spectroscopy to interrogate the structure of ADAR2 double-stranded binding motifs (dsRBM1 and dsRBM2) in complex with GluR-2 R/G RNA.[73,74] The data obtained suggest a mechanism for recognition of shape and primary sequence of the double-stranded RNA (dsRNA) that could inform structure-based design of oligonucleotide drugs.

Allele	Amino acid 342	Codon	Protein
Z	Lysine	**A**AG	**Z-AAT**
M	Glutamic Acid	**G**AG	**M-AAT**

Figure 4 – *Allele to AAT protein classification.*

Deamination Domain of ADAR

The deamination domain of ADAR1/2 prefers binding of certain natural sequence motifs,[75] particularly those where the edited adenosine is flanked by a uridine (5' nearest neighbor), and such sequences are frequently used as models in ADAR editing studies.[76] The PiZ mutation (E342K) in *SERPINA1* mRNA (Fig 3) contains an adenosine that comprises a mutated AAG codon encoding lysine instead of glutamic acid. When positioned in the active site of ADAR, this adenosine is flanked by a cytidine (5' nearest neighbor) and an adenosine (3' nearest neighbor) resulting in a 5'-CAA-3' motif that is predicted to not be a site that is naturally preferred for editing, as determined using the inosine predict algorithm.[75] Therefore, to enable development of a drug with good efficacy, an oligonucleotide targeted against the E342K site may require extensive optimization to overcome an antipreference for editing. Structural studies of ADAR2 in complex with RNA have hinted at potential strategies that could be applied for optimization.

Translation of the mRNA to Protein

Following the specific RNA edit on a coding region by ADAR, the translational machinery reads the edited A to I as a guanosine[77] to correct the detrimental lysine to the amino acid glutamic acid at position 342 (Fig 4). The resultant protein secreted would be the active M-AAT which will be able to protect the lung from excess protease activity while at the same time reducing the pathogenic aggregation of the Z-AAT in the liver.

Summary

New therapeutic approaches for the treatment of AATD have potential to improve on the current standard of care by addressing the underlying disease mutation.[63] Editing approaches to correct the E342K mutation have the ability to normalize wild-type protein secretion and reduce mutant protein synthesis. Unlike existing therapies, these approaches address both the deleterious accumulation of Z aggregates in the liver and restoring the protective lung functions of AAT.

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