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Platforms of individualized medicine: How Antisense Oligonucleotides, MicroRNAs, and Short Interfering RNAs can inform new treatments for rare diseases

Agarwal A[1]

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Abstract

Each individual rare disease affects at most 200,000 people, but they collectively affect 300 million people globally. They are difficult to treat because they present with specific, single or multiple, genetic mutations which challenge production feasibility and an adequate commerial return on investment. One possible solution is using RNA therapies such as antisense oligonucleotides (ASOs), short interfering RNAs (siRNAs), and microRNA (miRNA) as platforms to treat rare diseases because they can be created to target specific mRNAs and genetic mutations to correct the underproduction or overproduction of a desired or undesired protein respectively. This paper compares three categories of RNA therapeutics, ASOs, siRNA, and miRNAs, to determine their relative efficacies. It also includes two case studies researching hereditary transthyretin-mediated amyloidosis (hATTR) and heterozygous familial hypercholesterolemia. Each case study compares ASOs (Inotersen and Mipomersen) and siRNA therapies (Patisiran and Inclisiran). miRNA therapies only partially bind to RNA, making them less safe compared to ASOs and siRNAs, since they are capable of modulating non-target gene expression. Furthermore, ASOs are the only type that can up-regulate or down-regulate protein production as well as perform exon exclusion or inclusion to change the splicing pattern. These RNA therapies may be personalized, at least to subsets of mutational, transcriptional and/or translational dysfunction in rare diseases, while ensuring adequate commercial returns and production viability.

Keywords

Antisense oligonucleotides, Genetic therapy, Genetic diseases, Rare diseases, RNA interference, RNA knockdown, RNA splicing, miRNA therapy, siRNA therapy, Milasen, bioplatforms, Individualized therapy

[1]Corresponding Author: Aria Agarwal, Monta Vista High School, 21840 McClellan Rd, Cupertino, CA 95014, USA. aria.agarwal@gmail.com

Introduction

Rare diseases have small patient populations (by definition < 200,000 patients), but collectively, they affect 300 million people globally (1). Many rare diseases are caused by specific, single or multiple, genetic mutations that affect a small population, making them difficult to treat because any therapy treating a rare or ultra-rare disease may be commercially unviable to scale up to commercial production. One way this is being addressed is by using ribonucleic acid (RNA) bioplatforms such as antisense oligonucleotides (ASOs), short interfering RNA (siRNA), and microRNA (miRNA) to create individualized therapies for specific rare diseases. Using ASOs, siRNAs, and miRNAs as bioplatforms is useful because bioplatforms are cost-efficient and can be individualized, giving them potential to advance the future of rare disease treatment. Commercial companies have been developing ASOs since the 1970s, and there have been significant advancements in the field of RNA therapeutics since then (2). In 2019 researchers used ASOs as a platform to develop Milasen, the first drug ever created for a single patient to target her specific genetic mutation and correct it (3). This demonstrated the potential to develop individualized treatments using ASOs as a bioplatform, suggesting a promising future for individualized RNA therapeutics in the treatment of rare diseases.

ASOs are single-stranded RNA molecules and are generally implemented in two different ways, knockdown and splicing, to manipulate protein production. Knockdown ASOs prevent gene expression by binding to the target mRNA strand and inhibiting transcription. Splice-correcting ASOs control gene expression by modulating RNA splicing to either silence or express certain parts of the gene (4). This paper will compare the efficacy of ASOs to two other RNA-based therapies; short interfering RNA (siRNA) and microRNA (miRNA). siRNA functions similarly to the ASOs degradation mechanism, *viz*. by binding to a target mRNA and inhibiting transcription to prevent protein production, and a single siRNA has the potential to target multiple mRNAs (5). On the other hand, miRNAs bind imperfectly to target mRNA, allowing a single miRNA to bind to hundreds of mRNA targets, in order to inhibit transcription and protein production which eventually leads to degradation (5).

This paper will compare the efficacy and biochemical mechanisms of these three categories of Active Pharmaceutical Ingredients (APIs') using FDA-approved ASO and siRNA therapies and clinical trials of miRNA therapies. The future potential of various RNA therapeutics in treating rare diseases will also be discussed.

Methods

This paper provides a comprehensive review comparing the use of antisense oligonucleotides to other RNA therapies including siRNA and miRNA for the treatment of rare diseases. Relevant papers were identified in the PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (https://scholar.google.com/) databases using the keywords, "antisense oligonucleotides", "RNA", "genetic therapy", "genetic diseases", "rare diseases", "RNA interference", "RNA knockdown", "RNA splicing" and "antisense therapy."

Results

ASOs

Antisense oligonucleotides (ASOs) are small single-stranded RNAs that regulate protein production through occupancy-only mechanisms which involve the regulation of RNA splicing, RNA cleavage and degradation, or steric blocking (6). This section provides a summary of six ASOs; Milasen, Inotersen, Nusinersen, Eteplirsen, Mipomersen, and Givosiran. It explores the biological mechanisms, efficacy, percentage of adverse events, time of development, degree of individualization, and patient population that are representative of ASO function.

Occupancy-Only method

This section covers four FDA-approved ASOs —Milasen, Nusinersen, Golodirsen, and Eteplirsen —that function through the occupancy-only method of regulating or correcting RNA splicing through exon exclusion or inclusion (6).

Many ASO therapies that use occupancy-only mechanisms to up-regulate or down-regulate protein production modify exon exclusion or inclusion to change the splicing pattern, often creating proteins of different lengths as compared to the original, but that still function normally (6). One example of a therapy that uses exon inclusion is Nusinersen, used to treat Spinal Muscular Atrophy (SMA) a disorder that causes early mortality in infants, to progressive weakness in the muscles of adolescents and adults which is caused by a mutation in the SMN1 gene on chromosome 5q13 that should produce the SMN protein (7). There is another gene SMN2 that would produce the SMN protein but does not, due to exon 7 being skipped. Nusinersen targets the SMN2 gene to include exon 7 by targeting the pre mRNA to include exon 7, creating a functional protein that increases SMN protein levels, and improves quality of life for many patients (8).

ASOs can also perform exon exclusion to make a shorter, yet still fully functional protein. One example of this kind of ASO is Golodirsen, which treats Duchenne Muscular Dystrophy (DMD), a degenerative neuromuscular disease caused by a mutation in the dystrophin mRNA that inhibits dystrophin production (9). Golodirsen is an ASO that targets the DMD pre- mRNA and binds to alter the splicing pattern, so it excludes exon 53, to produce a shorter, yet functional protein. Another ASO that treats the same disorder is Eteplirsen. Eteplirsen targets a point mutation in the DMD gene, which introduces an early stop codon and inhibits the production of dystrophin (10). This ASO binds to exon 51 and skips over the stop codon, which allows the mRNA to be translated into a functional protein (10).

Kim et. al. (3) described the creation of Milasen to combat a rare neurodegenerative disease, CLN7 Batten's disease, in a six-year-old girl, Mila. Researchers discovered an insertion of an SVA retrotransposon between exon 6 and 7, that modulated the splicing of exon 6 in MFSD8 intron 6, which resulted in the production of an early stop codon, which, in turn, halted protein production (3). They decided to use antisense oligonucleotides as a platform to create Milasen which was modeled off the FDA-approved drug Nusinersen. Milasen functions through occupancy-only mechanisms and targets the i6.SA cryptic splice acceptor site to correct the missplicing of exon 6, and enable protein production by creating a different splicing pattern to skip over the stop codon (3). Researchers found that

while it did not cure Mila of her condition, it reduced the frequency and duration of seizures, and improved the quality of life (3).

Binding and degradation mechanisms

Some ASOs function by binding to the complementary mRNA target and activating RNase H to degrade or cleave the mRNA, inhibiting protein production (11). One example of this type of therapy is Inotersen, which was created to combat hereditary transthyretin-mediated amyloidosis by inhibiting hepatic TTR protein production (12). Transthyretin-mediated amyloidosis is caused by a mutation in the TTR gene, which results in a misfolded protein, producing transthyretin amyloid fibrils that are deposited into tissues and organs such as the heart, kidneys, gastrointestinal tract, and peripheral nerves (13). Inotersen targets both mutant and wild type transthyretin mRNA by binding to the mRNA through complementary base pairing, and RNase H degrades the mRNA while the oligonucleotide prevents binding of the ribosome to the mRNA through steric blocking in order to inhibit protein production. This effectively reduced the protein production by both mutant and wild-type TTR genes, which reduced the formation of transthyretin amyloid fibrils and slowed disease progression (12).

The only other FDA-approved ASO therapy that operates by cleavage or degradation is Mipomersen, a therapy that treats familial hypercholesterolemia which results from mutations in apolipoprotein B, causing elevated levels of low-density lipoprotein cholesterol (LDL-C) (14). Mipomersen is an ASO that binds to the apolipoprotein-B-100 target mRNA and activates RNase H which cleaves the mRNA to inhibit the production of apolipoprotein B-100. Mipomersen reduced LDL cholesterol concentrations and improved the symptoms of hereditary familial hypercholesterolemia (14).

Small Interfering RNA

Small interfering RNAs (siRNA) are noncoding double stranded RNAs that work to inhibit protein production. Once administered, the siRNA is loaded onto the RNA-induced silencing complex (RISC), and the passenger strand is cleaved by the enzyme AGO2. The fully complementary antisense siRNA strand then binds to the target mRNA and the mRNA is then cleaved (15). Cleaving the mRNA inhibits protein production, and often slows disease progression. This section explores four different FDA-approved siRNA therapies: Paitsiran, Lumasiran, Givosiran, and Inclisiran.

One example of an siRNA therapy is Patisiran, used to treat hereditary transthyretin-mediated amyloidosis which is caused by a mutation in the TTR gene, that results in a misfolded protein. It produces transthyretin amyloid fibrils that are deposited into tissues such as the heart, kidneys, gastrointestinal tract, and peripheral nerves (16). Patisiran works by binding to the untranslated 3 prime region of TTR mRNA in the liver, and the fully complementary siRNA strand binds to the mRNA to inhibit hepatic TTR production and leads to degradation to reduce protein production. It reduced transthyretin levels and neuropathy and increased the quality of life (16).

Another FDA-approved siRNA therapy is Lumasiran, which is a double stranded siRNA that combats the rare genetic disease Primary Hyperoxaluria Type 1 (PH1). PH1 is caused by

a deficiency of alanine-glyoxylate aminotransferase (AGT), which results in the overproduction of hepatic oxalate and leads to kidney stones and failure, nephrocalcinosis, and systemic oxalosis (17). Lumasiran targets the mRNA that codes for glycolate oxidase by complementary bonding to the target mRNA to inhibit translation in order to reduce hepatic oxalate production (17).

The third FDA-approved siRNA therapy is Givosiran, which treats Acute Hepatic Porphyria (AHP), caused by a defect in the enzyme aminolevulinate synthase 1 (ALAS1) which results in an accumulation of delta-aminolevulinate and porphobilinogen, resulting in seizures, psychosis, abdominal and back pain, and polyneuropathy (18-19). Givosiran targets the ALAS1 gene by binding to the hepatic ALAS1 mRNA and causing degradation, which prevents protein production and leads to a reduction in ALA and PBG levels and increased patients' quality of life by decreasing protein levels (19).

The fourth FDA-approved siRNA therapy is Inclisiran, which is used to treat Heterozygous Familial Hypercholesterolemia caused by mutations in the LDL receptor genes resulting in elevated levels of low-density lipoprotein cholesterol that can lead to cardiovascular disease (20). Inclisiran targets the hepatic PCSK9 gene to inhibit protein production by binding to the gene and activating RNase H which lead to degradation, which, in turn, reduces LDL cholesterol levels (20).

MicroRNA

The third type of therapy is microRNA (miRNA) therapy. miRNA are short non-coding single stranded RNAs that bind to the target mRNA through partially complementary base pairing to prevent protein production and promote gene silencing through mRNA degradation or steric blocking which inhibits protein production (5). They do not require exact complementary pairs to bind to target mRNAs, hence they can bind to and regulate hundreds of target mRNAs. This section explores three miRNA therapies that were or are currently in clinical trials- Miraversen, MRX34 and RG-101.

There are no FDA approved miRNA therapies, and many are still in the early phases of testing. Miravirsen is a miRNA therapy that is in phase II of clinical testing, that combats Hepatitis C. It targets miR-122, an miRNA expressed in the liver and protects the HCV genome from innate immune responses (21). Miravirsen partially binds to the 5 prime region of miR-122 to inhibit it, resulting in a decrease of HCV RNA levels (21). The second therapy is MRX34, which is under phase 1 clinical trial testing to combat liver tumors (21). MRX34 is a liposomal formulation of miR-34a, which is a tumor suppressor which induces p53 transcription (21). MRX34 targets miR-34a to upregulate transcription to target liver tumors (21). RG-101 is another miRNA therapy that is in phase 1B of clinical trial testing, and combats Hepatitis C. It works similarly to Miraversen, in that it inhibits the miR-122 gene to reduce transcription, but the chemical makeup differs (21).

Short orally active molecules that supercede ASOs and siRNAs

The development of RNA based therapies is also paving the way for an emerging field of orally administered small molecule therapies that function similarly to ASO, siRNA and

miRNA therapies (27-29). This paper will explore five examples of siRNA small molecule therapies that are either FDA approved or are currently undergoing clinical testing. Instead of targeting pre-mRNA or mRNA in the way ASO, siRNA and miRNAs do, these small molecule therapies target gene promoters, transcription factors and/or ribonucleoproteins (22). There are currently small molecule substitutes for both ASOs and siRNAs, but not miRNA therapies. One example of an orally administered ASOs is Ataluren, which treats duchenne muscular dystrophy like Eteplirsen (22). However, instead of targeting the dystrophin pre-mRNA, like Etepliersen does, Ataluren interacts with the ribosome during protein synthesis to suppress the nonsense mutation that causes a premature stop in protein production (22). Another example is risdiplam, which targets spinal muscular atrophy like Nusinersen, but is a splicing factor modulator (22). Yet another example is the tricyclic macrocycle MK-0616 which is undergoing clinical testing to treat heterozygous familial hypercholesterolemia like Inclisaran but inhibits the interaction of PCSK9 with LDL receptors. Tafamidis is an orally administered drug which treats hereditary transthyretin-mediated amyloidosis by binding to TTR to stabilize the tetramer and slow its dissociation. This mechanism is unlike that for Inclisiran, which treats this disease by targeting the mRNA and activating RNase for degradation (22). The last example is Oxabact, an oral formulation of *Oxalobacter formigenes*, an oxalate metabolizing bacterium, which treats primary hyperoxaluria type 1. Oxabact promotes the removal of endogenously produced oxalate from the plasma, into the gut, where it is metabolized by the bacteria (22). This is unlike the mechanism of action of Lumasiran, which reduces hepatic oxalate production by inhibiting translation by binding to the target mRNA. Lastly, Imetelstat is a telomerase inhibitor that binds with nucleic acids to competitively inhibit telomerase enzyme activity rather than inhibiting protein translation by binding to mRNA like a typical antisense therapy would (22). RNA based therapies may give way to these orally administered small molecule therapies in the future as they continue to develop.

Discussion

This section includes two case studies that treat the same disorders with ASOs and siRNAs. The two examples used are Inotersen (ASO) and Patisiran (siRNA), which both treat transthyretin-mediated amyloidosis, and Mipomersen (ASO) and Inclisiran (siRNA), which both treat heterozygous familial hypercholesterolemia. However, it is only possible to indirectly compare one type of ASO to siRNAs because siRNAs do not have the ability to correct missplicing, so the only ASOs this paper was able to indirectly compare are the binding and degradation type. The first subsection explores the mechanistic differences between ASOs, siRNAs and miRNAs, and the second subsection discusses broad differences between therapies, including safety, degree of potential individualization, and patient populations.

Mechanistic differences between ASO, miRNA and siRNA

While ASOs, miRNAs, and siRNAs produce similar results, there are significant differences in the way they bind to target mRNAs. ASOs and siRNAs bind to their targets using exact complementary Watson-Crick base pairing, increasing target binding specificity (14).

miRNA, on the other hand, binds partially to the target mRNA, which allows it to target hundreds or thousands of genes, making it non-specific and potentially dangerous (5). In terms of delivery, ASOs and miRNA are single-stranded molecules while siRNAs are double stranded molecules and processed by RISC which then binds to RNA as part of the complete RISC (5, 26). Since siRNA molecules are double stranded, they can more easily activate innate immune responses and cause off-target effects and toxicity, making them more prone to causing adverse effects than ASOs in this regard (4). ASOs are also 12-30 nucleotides in length but are typically 16-20 base pairs long while siRNAs are usually 19-22 nucleotides to form a stable duplex in order to be recognized by RISC, but both molecules typically target one RNA (2, 23). On the other hand, miRNA are normally 20-25 base pairs long and are not exactly complementary to their target (5). Cellular delivery is considerably larger for ASOs compared to siRNAs because many cells have surface receptors that uptake ASOs, while delivery for siRNA requires cationic and neutral lipids because hydrated phosphates are not well recognized by cell surfaces (4). miRNA therapies are delivered, usually by packaging into viral and non-viral vectors . Non-viral delivery methods include inorganic material-based delivery, lipid-based nanocarriers, polymeric or dendrimer-based vectors, cell-derived membrane vesicles, and 3D scaffold delivery systems (31). Another difference is the need for chemical modifications in ASOs and miRNAs versus siRNAs. Both ASOs and miRNAs require chemical modification to avoid nuclease degradation because they are unstable (15, 23). Synthetic chemically modified ASOs can also have increased potency and affinity for target molecules by RNase H cleavage (23). On the other hand, siRNAs are stable enough to use as therapy without requiring chemical modifications, however they can be modified to increase potency (23).

Another difference is that many ASO therapies that use occupancy-only mechanisms to up-regulate or deregulate protein production perform exon exclusion or inclusion to change the splicing pattern, often creating proteins of different lengths as compared to the original; but those that still function normally (4). This gives ASOs the unique ability to not only manipulate gene expression, but to correct mutations to produce normally functioning proteins by targeting pre-mRNAs without degradation by RNase H, while siRNA and miRNAs are limited to manipulating gene expression to control protein production by targeting mRNA. When ASOs target pre-mRNA, the use of RNA cleavage is not required, making them more desirable (6).

Table 1. Mechanistic Comparisons of ASOs, siRNAs and miRNAs

Type of therapy	Mechanism of Action	Structure of Molecule	Chemical Modifications	Specificity of Target	Cellular Delivery	Gene Regulation	Degree of Potential Individualization
ASO (4, 6, 23)	-knockdown or complete binding and degradation using occupancy-only methods -involves RNA splicing, RNA cleavage and degradation, or steric blocking -splice-switching method does not require degradation by RNase H because it targets the pre-mRNA	-single stranded -12-30 base pairs	-requires chemical modification to avoid nuclease degradation and due to instability -potency and affinity for target sequence can be increased by RNase H cleavage for synthetic ASOs	-exact pairing allows for specificity of target	-many cells have surface receptors that uptake ASOs -delivery vehicles can include liposomes	-canup/down-regulate protein production - perform exon exclusion or inclusion to change the splicing pattern by targeting pre-mRNA -can also target miRNA to block function -steric blocking: binding to ribosome to prevent gene expression	-has been individualized for single patient
siRNA (15, 23)	-complete binding and degradation -siRNA is loaded onto RISC and passenger strand is cleaved by AGO2 -antisense strand binds to target mRNA and is cleaved	-double stranded -19-22 base pairs	-stable enough without requiring chemical modification - can be modified to increase potency	-exact pairing increases specificity of target -double stranded nature can activate innate immune responses and cause off-target effects	-requires cationic and neutral lipids for delivery - hydrated phosphates are not well recognized by cell surfaces	-reduce protein production through cleavage by targeting mRNA	-has been individualized for small group of patients
miRNA (5, 15, 22, 31)	-partial binding to target mRNA -inhibits protein production through degradation or steric blocking	-single stranded -20-25 base pairs	-Requires chemical modification to avoid degradation RNase H and due to instability	- can target thousands of genes -nonspecific, potentially dangerous	-viral and non-viral vectors -non-viral vector examples: inorganic material, lipid-based nanocarriers, polymeric vectors, cell-derived membrane vesicles, 3D scaffold delivery	-reduce protein production through cleavage and degradation by targeting mRNA	-has not been individualized

ASOs also have the advantage of being able to up-regulate protein production by modifying splicing patterns, while siRNA and miRNAs can only reduce protein production through cleavage and degradation (14). Not only can ASOs target pre-mRNA and mRNA, they can also bind to miRNAs to prevent gene expression. RNase H independent ASOs can also carry out steric blocking by binding to ribosomes to prevent gene expression rather than by utilizing RNA cleavage (23). Table 1 summarizes the key mechanistic comparisons between ASOs, siRNAs and miRNAs.

Two Exploratory Case Studies

Treatment of Transthyretin-Mediated Amyloidosis with ASOs and siRNAs

Both ASOs and siRNA achieve similar results through controlling and modifying gene expression by altering or blocking mRNA targets (23). A major difference between ASOs and siRNA is that ASOs are single stranded while siRNAs are double stranded complexes (23). However, there also are some additional differences in the way these therapies perform for specific diseases.

Inotersen is an antisense oglionucleotide that was created to combat hereditary transthyretin-mediated amyloidosis (hATTR) by inhibiting hepatic TTR production to reduce protein production (12). Another therapy that treats the same disorder is Patisiran, an siRNA that works by binding to the untranslated 3 prime region of TTR mRNA in the liver to inhibit hepatic TTR production. Both molecules cause the degradation of the TTR mRNA (16).

There are many differences between the ASOs and siRNAs that treat hATTR, but one similarity is that they both function in similar ways, i.e. by binding to the target mRNA and degrading it. However, some differences include the mode of delivery, the cost of therapy and the pharmacokinetics and pharmacodynamic profiles. Inotersen is administered by subcutaneous injection, while Patisiran is administered by IV infusion (12, 16). Another differentiating factor is the frequency of administration; while Inotersen requires weekly injections, Patisiran requires intravenous therapy infusions every three weeks (12, 16). This makes it easier for patients to use Patisiran because there is a longer duration between doses. Another factor is the annual cost of medication. Inotersen is priced at $420,000 per year for one patient, and Patisiran is slightly more expensive at $451,430 (23, 24).

The other factors are the pharmacokinetics (PK) and pharmacodynamic (PD) profiles of both therapies. Important PK parameters include Cmax, the peak concentration of the drug in the bloodstream, Tmax, the time it takes to reach that peak, and t ½, which is the half-life of the drug (33). The PD parameters discussed are Emax, which represents the reduction of TTR levels from the baseline. For Inotersen, PK parameters included a Cmax of 6.39 μg/mL, Tmax was 1.5 to 4 hours, and the t ½ was 2-4 weeks (34). For Patisiran, PK parameters included a Cmax of 5.51 μg/mL, Tmax was 1.3 hours, and the t ½ was 3.2 weeks (33). The Cmax for Inotersen was higher, which implied that the maximum concentration of the drug in the bloodstream was higher for Inotersen than Patisiran, but also took slightly longer to reach that concentration with Inotersen taking 1.5-4 hours while Patisiran taking 1.3 hours. The t ½ for Inotersen was

much longer than Patisiran (2-4 weeks versus 3.2 days), indicating it remained in the plasma for longer. The PD parameters for Inotresen and Patisiran were a 70% and 87.8% reduction in TTR levels from the baseline respectively (33, 34).

Table 2. A comparison of Inotersen and. Patisiran for the treatment of Transthyretin-Mediated Amyloidosis

Type of Therapy	**Mechanism of Action**	**Frequency and Route of Administration**	**Cost of Therapy**	**PK/PD profile (33, 34)**	**Potential for off-target effects**	**Mean Difference in mNIS+7 Score with Confidence Interval**	**Mean Difference in Norfolk QOL-DN Score Range Confidence Interval**
ASO-Inotersen (12, 31)	RNA Cleavage/ degradation by binding -RNase H	subcutaneous injections every week	$420,000 annually per patient	PK Parameters: Cmax: 6.39 µg/mL Tmax: 1.5 to 4 hours t1/2: 2-4 weeks PD Parameters: - Emax: 70% reduction in TTR levels from baseline	low	-12.3 (-26,0,-6.3)	-11.3 (-20.3, -2,8)
siRNA-Patisiran (16, 31)	-RNA Cleavage/ degradation by binding -RNase H	IV infusions every 3 weeks	-$451, 430 annually per patient	PK Parameters: Cmax: 5.51 µg/mL Tmax: 1.3 hours t1/2: 3.2 days PD Parameters: - Emax: 87.8% reduction in TTR levels from baseline	low		

The last two factors are the Neuropathy Impairment Score (mNIS+7) which ranges from 11.0 to 153.5, and Norfolk Quality of Life- Diabetic Neuropathy (QOL-DN) score which ranges from -4 to 136 (16). For each score, a smaller number indicates a better quality of life. Since these two therapies were tested using individual case studies, direct head-to-head comparisons are not possible. However, Gorevic et.al. indirectly compared the efficacy of Patisiran and Inotersen using the Bucher and MAIC methods (32). The MAIC analysis explored the comparison with reweighting between the two studies and found that the mean difference between the mNIS+7 score after 15 months for Patisiran and Inotersen was -12.3 with a 95% confidence interval of (-26.0 to -6.3) (32). The MAIC analysis of the mean difference between the Norfolk QOL-DN Score Range after 15 months for Patisiran and Inotersen was -11.3 with a 95% confidence interval of (-20.3 to -2.8) (32). This data shows that while both therapies broadly improve the mNIS7+ and Norfolk

QOL-DN Score Range, Patisiran may do so more effectively. Table 2 provides a summary of the comparison between the two molecules.

Treatment of Heterozygous Familial Hypercholesterolemia with ASOs and siRNAs

Mipomersen and Inclisiran are two drugs that treat heterozygous familial hypercholesterolemia. The disease results from mutations in apolipoprotein B, causing elevated levels of low-density lipoprotein cholesterol (LDL-C) (14). Mipomersen is an antisense oligonucleotide that operates by cleavage or degradation. It binds to the apolipoprotein-B-100 target mRNA and activates RNase H which cleaves the mRNA to inhibit the production of apolipoprotein B-100 (14). The other therapy that treats this same disorder is Inclisiran, an siRNA that targets the hepatic PCSK9 gene to inhibit protein production by binding to the gene and activating RNase H, in turn, causing degradation, which reduces LDL cholesterol levels (20).

Some similarities between Mipomersen and Inclisiran include the mode of delivery, and how they operate on a cellular level. Both therapies operate similarly, by binding to the target mRNA, and preventing translation by degradation which inhibits protein production and lowers LDL-C levels in the blood. They are also both administered by subcutaneous injection that can be administered by the patient at home, making them easily accessible for patients. However, Mipomerson requires weekly injections while Inclisiran requires injections 3 months after the first dose, then every 6 months following. This makes the siRNA treatment more convenient for patients (14, 20). Another difference between the two therapies is cost; Mipomersen averages $5,759 per week, and 30 days of therapy is $23,038 while Inclisiran is $3,250 per injection but $6,500 annually (14, 25).

For Mipomersen, PK parameters presented with a Cmax of 1.2-4.3 µg/mL, Tmax was 3 to 4 hours, and the t ½ was 23-31 days (14). For Inclisiran, PK parameters presented with a Cmax of 0.509 µg/mL, Tmax was 4 hours, and the t ½ was 9 hours (34). The Cmax and t ½ for Mipomersen were greater, which meant that the maximum concentration of the drug in the bloodstream was higher, but also took longer to reduce by half. However, both therapies seemed to take about the same amount of time to reach Cmax (14, 34). The PD parameters for Mipomerson and Patisiran were a 24% and 53% reduction in LDL Cholesterol levels from the baseline respectively (14, 34). Table 3 provides a summary of the comparison between the two molecules.

Table 3. A comparison of Mipomersen and Inclisiran for the treatment of Heterozygous Familial Hypercholesterolemia

Type of Therapy	Mechanism of Action	Administration Route	Frequency Of Administration	Cost of Therapy	PK-PD profile (14, 35)	Potential for off-target effects
Mipomersen (14)	-RNA Cleavage/ degradation by binding -RNase H	subcutaneous injections	weekly	-1 week of therapy is $5.759. 65 and 30 days of therapy is $23,038.60.2	PK Parameters: Cmax: 1.2 to 4.3 µg/mL Tmax: 3 to 4 hours t1/2: 23-31 days PD Parameters: -Emax: 24% reduction in TTR levels from baseline	low
Inclisiran (20)	-RNA Cleavage/ degradation by binding -RNase H	subcutaneous injections	3 months for first dose then 6 months	-$3,250 per injection, $6,500 annually	PK Parameters: Cmax: 0.509 µg/mL Tmax: 4 hours t1/2: 9 hours PD Parameters: -Emax: 53% reduction in TTR levels from baseline	low

Broad comparison of ASO, siRNA and miRNA

In terms of broad mechanistic differences between ASOs, siRNAs and miRNAS, miRNA partially binds, making it potentially less safe since non-target gene expression may be affected (5). ASOs are the only RNA therapy out of the three categories that are able to correct misplicing and restore protein function through exon inclusion or exclusion (6).

Most ASOs treat rare diseases with small patient populations and have already been used as a bioplatform to create individualized therapies. This demonstrates its potential as a bioplatform for personalized medicine. An example is Milasen, which used attributes from the existing FDA approved therapy, Nusinersen, and had the same chemical modifications and similar length of nucleotides (3). Milasen was developed and administered about one year after diagnosis, which demonstrates the efficiency of using ASOs for individualized therapies that may be cost efficient as well. siRNAs also treat rare diseases with relatively small patient populations but have not been used or modified to treat individual patients. There is debate amongst the scientific community about whether ASOs or siRNAs are easier to develop. ASOs are single stranded while siRNAs are double stranded, making ASOs potentially cheaper and easier to deliver (26). miRNA therapy on the other hand has a large target patient population and is anticipated to treat cancers or infections, such as HCV. There are no current FDA approved miRNA therapies, and it has never been used as a platform to

create personalized therapies on the same level as ASOs.

Table 4. Broad Comparisons of ASOs, siRNAs and miRNAs

Type of Therapy	Mechanism of Action	Safety	Degree of Potential Individualization	Patient Population
ASO	knockdown or complete binding and degradation	-specific targets relatively safe	-has been used as a bioplatform -has been individualized for single patient	Small, rare disease
siRNA	complete binding and degradation	-specific targets relatively safe	-has been used as a bioplatform -has been individualized for small group of patients	Small, rare disease
miRNA	partial binding and degradation	nonspecific targets, greater off target effects	-has not been individualized -large patient population	large patient population

Limitations

Only the binding and degradation type of ASOs' could be used to compare to siRNAs, because siRNAs do not have the capability to alter splicing, unlike the splicing ASOs such as Milasen, Nusinersen, Eteplirsen, and Golodirsen. Due to this limitation, this paper does not contain in-depth comparisons between splice switching ASOs and siRNAs, because they do not target the same mRNAs. These splice switching ASOs target pre mRNAs while siRNAs only target mRNA (6).

Conclusion

Antisense oligonucleotides provide an efficient bioplatform that allows scientists to build on previous ASO therapies and create new, individualized therapies to treat rare diseases. The mechanistic differences between the three categories affect their promise for the future of individualized RNA therapy. When compared with siRNAs and miRNAs, ASOs have the advantage of being able to up-regulate protein production by modifying splicing patterns, while siRNA and miRNAs can reduce protein production only by cleavage and degradation mechanisms (5, 6). ASOs and siRNAs possess binding complementarity to their targets whereas miRNA only partially binds, which makes the specificity of the former, greater, reducing potentially harmful off-target effects (5). ASOs are a promising bioplatform for the future of RNA therapeutics, with its ability to modulate mRNA expression. They also support a future of personalized medicine since they can be individualized with relatively less time and cost.

There will be a need to revisit these conclusions in the future since many miRNA drugs are still in clinical development. There will likely be new comparisons that can be made between the three categories in the future. In the future, specific experimentation could be done to directly compare ASOs, siRNAs and miRNAs that all target the same mutation, which would eliminate any confounding variables. The development of

RNA based therapies is also paving the way for an emerging field of orally administered small molecule therapies that function similarly to ASO, siRNA and miRNA therapies (27-29). Some examples include ASOs that can be orally administered using enabling excipients such as sodium caprate, tripeptide PCSK9 inhibitors, and splicing factor modulators (27-29). RNA based therapies may give way to these orally administered small molecule therapies; however further exploring these ideas is beyond the scope of this paper.

ASOs provide a promising platform for the future as their ability to be highly individualized could make them more accessible in the future, because the cost of production may be reduced due to ASOs being used as a bioplatform. With modifications made only to the sequence of nucleotides, scale up and productions lines, equipment and processes may be expected to be minimally different upstream and significantly similar downstream, enabling rapid switch-out, short lead, down and turn-around times and the ability to manufacture significantly smaller batch volumes. Perhaps individualized ASO therapy will one day be commercialized instead of being developed and administered in academic or specialized manufacturing settings, hence furthering its accessibility to the general public. ASOs, siRNAs and miRNAs are advancing the field of RNA therapeutics, and someday may enable individualized treatments for rare diseases.

Abbreviations

AGT- Alanine-Glyoxylate Aminotransferase
ALAS1- Aminolevulinate Synthase 1
ASO- Antisense oligonucleotide
DMD- Duchenne Muscular Dystrophy
hATTR- Hereditary Transthyretin-Mediated Amyloidosis
LDL-C- Low-Density Lipoprotein Cholesterol
mNIS+7- Neuropathy Impairment Score
miRNA- Micro Ribonucleic Acid
PH1- Primary Hyperoxaluria Type 1
PD- Pharmacodynamic
PK- Pharmacokinetics
RISC- RNA-induced silencing complex
QOL-DN- Norfolk Quality of Life- Diabetic Neuropathy
siRNA- short interfering ribonucleic acid
SMA- Spinal Muscular Atrophy

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