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The application of antisense oligonucleotides in disease treatment

Lokkwan Chan[1, 5], Yuanxi Dai[2, 6], Yijia Su[3, 4, 7]

[1]Fudan International School, Shanghai, China
[2]Abbey College Cambridge, Homerton Gardens, Cambridge, United Kingdom
[3]Shanghai International Studies University, Shanghai, China
[4]Corresponding author

[5]1910851105@mail.sit.edu.cn
[6]1911511109@mail.sit.edu.cn
[7]0211104011@shisu.edu.cn

Abstract. Scientific research in recent years has made every endeavor to combat against various untreatable diseases in neurodegenerative or genetic diseases under the guidance of constantly updated underlying gene-associated pathological mechanisms. However, substantial therapeutic challenges still remain and many original causes of diseases are under great debate. Problems of drugs for neurological disorders involve high resistance to drug molecules by the blood-brain barrier (BBB) and only work efficiently in certain populations of patients, which is also a side effect of genetic malady. The introduction of Antisense oligonucleotides (ASOs) brings a new era for these diseases and meets the requirement. The US Food and Drug Administration (FDA) has proven ten oligonucleotide drugs until 2020. This article reviews some of their clinical trials with mechanisms, up-to-date attempts in inventing new ASO therapy, continuous safety and efficacy in preclinical and clinical trials of proven ASO, and great potential usage for RNA virus therapy.

Keywords: Antisense Oligonucleotides, Neurodegenerative Disease, Genetic Diseases.

1. Introduction

Antisense oligonucleotides (ASOs) belong to Oligonucleotides. They are nucleic acid polymers with single-stranded synthetic structure of various chemistries. ASO is generally classified into 2 distinct categories: RNase H competent and steric block[1]. Both of these factors possess the capability to impede the process of translation by causing the degradation of RNA or obstructing connections between RNA molecules and/or RNA-protein complexes [1]. As shown in the picture above, ASO treatments utilise the mechanism of ASO and target the specific gene whose mutation leads to the development of the disease. By silencing the gene's expression, ASO treatment can effectively stop the development of the disease. The Steric blockers that lack RNase H competence primarily target transcripts with high affinity. They can thereby intervene in the splicing process to select specific exons, which results in the splice correction [1]. Moreover, the RNase H-competent blockers degrade RNA by binding with targeted RNA and undermining its sequence [1].

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Zamecnik and Stephenson (1978) made the initial endeavour to employ ASO as a cellular management tool for the purpose of impeding viral propagation and cell transformation. During the past 45 years, ASO therapy has been engaged in managing several neurological disorders and hereditary diseases. The utilisation of ASO in the context of clinical therapy has yielded encouraging outcomes. As of January 2020, the FDA has officially approved 10 oligonucleotide medicines for use as therapeutic medications on the market[1]. It has proved the efficiency of ASO therapy. After the COVID-19 pandemic invaded the world, scientists considered applying ASO to RNA virus treatment.

Nevertheless, there are still challenges and obstacles that need to be conquered. ASO has difficulties being transferred to specific organs other than the liver [1]. The estimated course of therapy is 2 to 4 treatments per year, and the gene mutation needs to be avoided or dealt with [1]. Nevertheless, in general, ASO's application in clinical use is with great promise.

2. The potential use of ASO in neurological DisorderS

ASO brings an insight into various neurodegenerative and neuromuscular disorders, such as spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD), which have already gotten approval for ASO therapy by the FDA[2]. This review paper will summarise updated ASO design, some in vivo experiments and clinical trials of SMA, Alzheimer's Disease (AD) and Huntington's disease (HD). One prominent obstacle encountered in numerous neurological illnesses is the limited ability of ASO to traverse the intact blood-brain barrier (BBB) with higher efficiency owing to its charge and size, hence impeding its capacity to prevent intracellular breakdown. It is imperative to take into account the administration of ASO to the central nervous system (CNS) [2]. Receptor-mediated endocytosis could be used. Specifically, A biotinylated ASO was captured with a conjugate monoclonal antibody for streptavidin or using nanoparticles. Another mechanism is cell-penetrating peptide (CPP), ASOs encapsulated in exosomes, or the adeno-associated virus vector possessing high delivery ability and nonpathogenic property [2].

2.1. Spinal muscular atrophy (SMA)

SMA is classified as a recessive neurodegenerative condition in somatic chromosome due to the occurrence of homozygous or occasionally heterozygous deletions or mutations in the survival motor neuron 1 (SMN1) gene, causing a reduction in alpha-motor neurons (MNs) amount inside the spinal cord[3]. According to the SMA Foundation, it is estimated that SMA has an effect on 10,000 to 25,000 people in the US. Type 1 SMA has the most severe symptoms after birth, such as breathing problems and shorter life expectancy. Type 2 and 3 patients may have difficulty sitting up and walking respectively.

SMN1 is the gene located on chromosome 5q, which contains motor neurons. SMN2 is a similar gene to SMN1, with five different nucleotides. Cytosine is transitioned to Thymine in exon 7 of SMN2, which makes the splicing site in SMN2 much less likely to transcribe highly operational SMN protein (only 10% of production) by skipping exon7, compared to SMN1, which constantly undergoes correct splicing [2]. However, the loss of ability for SMN2 to produce functional protein due to the genetic problem leads to SMN2 being the only site for MN synthesis among the SMA patient. The more copy of the gene SMN2, the less severe the disease, so the treatment of SMA targets at making exon 7 in SMN2 more inclusive during the splicing process [3].

2'-MOE PS antisense medicine Nusinersen (MOE ASO), received approval from the FDA in the year 2016. The design of this molecule is intended to specifically target to the pre-mRNA sequence of intron 7 in SMN2, hence inhibiting the activity of undesirable splicing factors. More exon 7 are included, and thus SMN protein level increases [4].

In a phase-1 clinical research, the safety and tolerability of intrathecal Nusinersen were assessed through an open-label study design. The trial encompassed a cohort of 28 individuals of 2-14 years old diagnosed with either SMA type 2 or type 3. The participants were divided into cohorts receiving four increasing single-dose levels of Nusinersen (1, 3, 6 mg for 6 children; 9 mg for 10 patients) [5]. The drug level of Plasma and CSF was also compatible with preclinical results, and prolonged drug

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efficacy with 4-6 months half-life was proven by extended pharmacokinetics [5]. This trial led to further progress for ASO therapy to treat SMA [ClinicalTrials.gov identifiers: NCT01494701 and NCT01780246].

An open-label phase-2 study examined the tolerability, pharmacokinetics, and clinical efficacy of Nusinersen on infants aged 3 weeks to 7 months with SMA type 1 and 6 mg and 12 mg doses were injected into 20 participants [6]. 77 serious adverse events among 16 patients and 4 death were observed, but it was thought to be drug-unrelated. Several motor milestones with compound motor action potential, survival cases compared to baseline showed high efficacy of the drug, especially for 12 mg dose [6]. The most notable finding in this trial was that the drug enhanced SMN2 transcription with higher inclusion of exon7 and thus SMN protein synthesis level by autopsy analysis [6]. In addition, multiple cell types in the brain and motor neurons in the spinal cord were revealed to absorb the drug efficiently [ClinicalTrials.gov NCT01839656 NCT02193074 NCT02386553].

A double-blind phase 3 clinical trial of Nusinersen on infants with SMA also supported the previous two trials' results showing that the drug had noticeable improvements in muscle function and event-free survival rate by having more motor-milestone response compared to the control group [7]. This study especially suggested that to derive the maximum benefit of the therapy, patients should be under treatment at an early age [7].

Based on a recent meta-analysis study on the effect of Nusinersen, the adverse events associated with the drug were only 0.57%, and the potential drug-related side profile was 7.76% [8]. However, as mentioned above, being unable to get across the BBB is the main problem of Nusinersen, and intrathecal injection is required [8]. It is estimated that the procedure led to several adverse effects, such as lower back pain and vomiting, particularly in children. 14.16% of Post-Lumbar Puncture Syndrome (PLPS) was thought to be because of the injection method rather than nusinersen. Another problem with the drug is that it has not been approved in some countries with poor or middle income [8]. The cost is also unaffordable in general (US$125,000 per injection).

Currently, multiple clinical reports of Nusinersen showed successful results on infants. More clinical studies targeting the efficacy of the drug on SMA elder children or adults are needed. Another meta-analysis based on 384 5q-SMA onset adult patients treated with Nusinersen showed that amelioration was observed in achieving MCID in HFMSE (43.3%) and RULM(38.9%) in the lasting follow-up assessments [9].

2.2. Alzheimer's Disease (AD)

AD causes approximately 60% to 80% of cases of senile dementia. It is a progressive neurodegenerative disorder that is currently unable to be cured. The hallmarks of the disease are plaque deposition related to abnormal accumulation of Amyloid beta-peptide(Aβ) and Neurofibrillary Tangles formed by Tau protein, which may occur decades before symptoms. There are multiple clinical features, such as reading problems, poor judgment, or short-term memory loss. More severe AD patients even suffer from basic function loss of heart rate, which leads to death. It is estimated that the population of patients over 65 in America will rise to 13.8 million by 2050. Currently, several drugs are approved by the FDA, but most of them cannot solve the underlying biology of the disease. Among them, aducanumab and lecanemab treat AD by Aβ removement and slowing down cognitive degradation progress. However, the drug is only for patients with early diagnosis, such as mild dementia, and adverse effects by intravenous infusion are also a problem.

The amyloid hypothesis was widely studied during the last 30 years and is believed by some scientists to be a promising target for AD treatment. The cleavage of Amyloid Precursor Protein(APP) by beta-secretase1(BACE1) and gamma-secretase produces Aβ[10]. The hypothesis states that overproduction of Aβ in some individuals starts the disease, which stimulates Plaque deposits, chronic inflammatory reactions, abnormal accumulation of toxic hyperphosphorylated Tau protein and other cognitive downstream events [10].

ASOs could target at mRNA of APP to downregulate Aβ or enzymes [11]. A study designed a new ASO to alter BACE1 transcription and prevent it from production. Among kinds of 2'-OMethyl-

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modified ASOs, ASO2 targeting exon 2 on the BACE1 gene is the most effective at downregulating transcription (by 90%) [12]. The Phosphorothioate backbone variant could be further combined. The number of BACE1 dropped by 45%[12]. Studies in vivo are required to test the safety and efficacy in the future [12].

Splice-switching antisense oligonucleotides (SSOs) were also designed to target at Aβ42 level reduction. It is a single-stranded and shorter ASO [13]. Instead of activating RNase H ASO, this SSO work by skipping the splicing of exon 17 on the APP gene, which accounts for toxic Aβ42 production, and then splicing mRNA lacking exon 17 as an alternation that disables γ-secretase cleavage. The drug was tested in vivo. Expected results of amount decrease and suppression in long periods of APP were shown [13].

Another pathomechanism approved in recent research show that Tau protein pathology may also be the major driving factor of AD[11]. A microtubule-related protein called tau (MAPT) gene is coded for tau [11]. Patients with Aβ lead to protein phosphorylation by kinases such as CDK5 or ERK1. After separation from microtubules of Tau, Tau aggregates into insoluble' Paired Helical Filaments' and then Neurofibrillary Tangles(NFTs) [11]. This toxic effect progress into synaptic dysfunction and neuronal loss. The ASO therapy modifies the post-translation of Tau protein, such as hyperphosphorylation and attachment with Tau to microtubules [11].

One ASO drug on the clinical trial is IONIS-MAPTRx (ISIS 814907/BIIB080), downregulating NFT by reducing MAPT mRNA. MAPTRx binds to the MAPT pre-mRNA intron9 and involves ribonuclease H1 to degrade mRNA. The Tau protein could not be translated. A phase 1b clinical trial with a double-blind, randomised, multiple-ascending dose situation is tested for the drug's tolerability and safety, and pharmacokinetics in cerebrospinal fluid (CSF). 3:1 of MAPTRx and placebo were given to 46 patients. Mild or moderate adverse effects were observed in 94% MAPTRx participants and 75% placebo group instead of severe side effects. The level of the tau is decreased in the CSF, and the 60, 115 mg ASO group at 24 weeks post last dose shows >50% average decrease from baseline. It is the pioneer in clinical trial of ASO therapy for Alzheimer's in humans, and more clinical tests are required for MAPTRx [14].

Another ongoing phase 2 study tested BIIB080 of ASO. It is a randomised, double-blind and parallel group to assess the safety and efficacy of the drug on elder people aged 50 to 80 with the onset of mild AD or AD-related Dementia. The participant group are larger (735 participants)[ClinicalTrials.gov Identifier: NCT05399888]

However, much conflicting evidence raises doubt on the role of Aβ, and a recent study suggested that stochastic and genetic factors of penetrance reduction play a role in AD [10].

AD is found to be related to neuroinflammation and oxidative stress [11]. miRNA-ASO targets at signalling pathways are promising because more than 70% of miRNAs are included in the brain with the ability of crucial mechanism regulation of the disease [11]. ASO also could achieve multi-targeting, higher specificity, more splicing options, and transcription at the same time[11]. However, ASO is still novel to AD treatment and needs more preclinical as well as clinical trials to support its use in the future. The study lacks an animal model to mimic the human situation [11].

2.3. Huntington's disease (HD)

As a fatal genetic condition featured by progressive neurodegeneration and abnormal movement patterns, HD (also known as Huntington's chorea) is inherited and autosomal-dominant-gene-determined. The aforementioned condition is an uncommon hereditary genetic disorder due to the huntingtin (HTT) gene mutation. The initial manifestations manifest as a combination of motor, cognitive, and mental impairments, often observed between the ages of 30 and 40.

Currently, HD is still considered incurable. Lowering the HTT gene is a promising treatment strategy for HD, which can be achieved by ASOs targeting transcripts and degrading the targets [15]. Thus, several ASO drugs were invented and put into clinical trials. However, according to the suboptimal results, the drug application process for human therapy had been halted.

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Roche's ASO HD drug Tominerson was developed and showed successful Phase I/II study results. Despite showing adverse effects on a large scale of subjects, most are mild and found non-related to the drug injection. In the transitional phase following the Phase I/II study and preceding the Open-Label Extension (OLE) Study, the injection administration was temporarily suspended to evaluate the safety and tolerability of the drug at varying space between injections. The subjects were divided into a 4-week group and an 8-week group. Based on the PK/PD model, it was established that dosage at 8-week intervals over a period of 15 months resulted in exceeding the preclinical effectiveness threshold in both the cortex and the caudate [15]. The finding built a foundation for the subsequent experiment.

Phase III trial received an unsatisfactory result. In March 2021, it was determined that additional administration of the dosage was not feasible due to the presence of unfavorable safety indicators in the risk/benefit assessment. In the cohort receiving treatment for a duration of 8 weeks, a total of 15% of the patients, including 90% of the entire study population, reported experiencing adverse effects and exhibited severe symptoms [15]. The data represented 8.4% of the total patient population in the 16-week group, which accounted for 86.2% of all patients [15]. The performance of the group treated by Tominersen was worse than the placebo-controlled group [16]. Hence, the trial was halted. Two allele-selective ASOs designed by Wave Life Sciences are: WVE-120101 and WVE-120102 [15]. During the trial, approximately 53.8% of subjects reported to have severe adverse effects. Additionally, mHTT or total HTT decrease was not observed [16]. Thus, the development was also terminated.

To address the issue at hand, researchers employ Cyclodextrin-Based nanoparticles (CDs) to attain a more pronounced reduction of mHTT protein and the allele SNP1 which causes HD [16]. CDs have higher versatility for chemical modification; hence the nanoparticles can help enhance the specific targeting ability of ASO. There are three different CDs: CD1, CD2 and CD3. Compared to the control group, ASO therapy with CD3 has the most remarkable effect. The mHTT decreased by 90% during the trial [16]. The strategy still needs further development and research, but it is considered a promising method to help ASO therapy achieve better efficacy.

3. Genetic Diseases Treated with ASO Treatments

3.1. Introduction

Many genetic diseases are caused by abnormal mRNA production due to gene mutation. These abnormal mRNA lead to the production of dysfunctional proteins that disrupt various physiological processes, contributing to disease development. In recent years, therapies that target specific mRNA have been developed; the first ASOs were invented in this context. The ASOs' unique sequences are complementary to their target gene sequences, which can specifically degrade and inhibit the target's translation, preventing non-functional proteins from forming. Clinical trials involving ASOs are conducted on patients with genetic diseases, and results demonstrate that ASOs are successful in use for therapies.

3.2. Familia Hypercholesterolemia (FH)

FH is a genetic disorder with autosomal codominant inheritance that causes a rise in low-density lipoprotein cholesterol (LDL-C) levels in the bloodstream [17]. Familial hypercholesterolemia (FH) can be identified in individuals through the inheritance of a mutant form of one of three genes: the LDL receptor gene (LDLR), the kexin type 9 gene (PCSK9), or the apolipoprotein gene (APOB). The aforementioned genes are accountable for roughly 70-95% of familial hypercholesterolemia (FH) diagnoses. In the absence of medical intervention, individuals who remain untreated face a significantly elevated susceptibility to cardiovascular events and premature development of atherosclerosis [17]. Consequently, therapy is required.

Mipomersen, known by its brand names Kynamro and Genzyme, is classified as a second-generation ASO therapy specifically targeting the coding area of apoB-100 mRNA within the liver. The biochemical activities give rise to a substrate that is prepared for intrahepatic endonucleases. Therefore, the suppression of apoB-100 synthesis causes a fall in LDL-C quantity. In 2013, the FDA

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granted approval for the use of nonstatin medicine for the specific objective of decreasing LDL-C levels in Homozygous Familial Hypercholesterolemia (HoFH) patients.

Various randomised, double-blind phase III trials were performed on patients with FH. One of the research conducted in March 2013 assessed mipomersen's effectiveness in patients diagnosed with Heterozygous Familial Hypercholesterolemia (HeFH) and coronary heart disorders. Following a duration of 28 weeks, there was a notable significant reduction of 28% in the levels of LDL-C [18]. Similarly, apoB-100 levels were decreased by 26.5%, and the cholesterol level by 19.4% among 124 HeFH patients [18]. A subsequent phase III clinical research was undertaken involving individuals with homozygous familial hypercholesterolemia (HoFH) yielded encouraging findings. The trial revealed a notable reduction in LDL-C levels by 24.7% compared to the placebo group's decline of 3.3% over 26 weeks [18]. Although research has demonstrated that mipomersen can successfully decrease the apoB-100 and LDL-C levels in FH patients, the treatment is limited for treating HoFH. Furthermore, the therapy costs are expensive and may lead to adverse effects [18, 19].

3.3. Hereditary Transthyretin Amyloidosis (hATTR)

hATTR is classified as a systemic genetic illness arising from encoding transthyretin (TTR) mutations. The gene variations most frequently observed in association with this condition are Val30Met and Val122Ile. The occurrence of these mutations results in the misfolding of transthyretin, which subsequently leads to the buildup of abnormal amyloid protein deposits throughout the whole human body [20]. The gradual buildup of aberrant amyloid proteins has the potential to cause dysfunction and mortality in multiple organs and tissues, such as the peripheral nerves and heart [21]. Liver transplantations have emerged as the prevailing therapeutic approach for hATTR in contemporary times, owing to the liver's central role in the formation of TTR. Individuals with the Val30Met gene mutation exhibit a high likelihood of experiencing successful recovery and prolonged survival, with illness stabilisation rates ranging from approximately 80% to 90% [22]. Nevertheless, individuals who possess gene variants other than Val30Met exhibit a mere 60% rate of illness stabilization [22]. Moreover, liver transplantations are subject to numerous limits, such as limited availability of donor organs, compatibility based on blood type, and various other determining variables. Hence, there is a need for improved therapeutic interventions.

Inotersen (IONIS-TTRRX) is an antisense oligonucleotide inhibitor that belongs to the second generation of 2'–O–methoxyethyl–modified inhibitors. Its purpose is to suppress the synthesis of the wild-type and mutant TTR protein [21]. Inotersen comprises a sequence of 20 nucleotide base pairs that specifically bind to the complementary base pairs found in the mRNA of human TTR. The process of hybridization induces the activation of RNase H1, which subsequently facilitates the cleavage and destruction of the mRNA, as stated in reference [22]. Therefore, suppressing both mutant and wild-type TTR protein synthesis leads to the stoppage of hATTR advancement. The therapeutic approach for hATTR is a relatively new advancement with significant promise. In the year 2018, the FDA and Health Canada provided their authorisation for utilising Inotersen, also known by its brand name Tagsedi, for the therapeutic management of stage 1 and 2 of polyneuropathy hATTR in adult individuals [22].

A phase III clinical trial was performed to examine the effectiveness of Inotersen in addressing subjects diagnosed with stages 1 and 2 hATTR. The investigation was conducted in accordance with a placebo-controlled, double-blind, and randomised experimental design. The study's results demonstrate a significant enhancement in the Inotersen cohort vs. the placebo cohort. The Modified Neuropathy Impairment Score+7 (mNIS+7) demonstrated a substantial reduction of 19.7 points, while the Norfolk Quality of Life-Diabetic Neuropathy (QoL-DN) questionnaire exhibited a drop of 11.7 values at week 66 [21]. The group that was administered Inotersen had an average rise of 5.8 points in initial mNIS+7 and 1.0 points in baseline QoL-DN. On the other hand, it is noteworthy that the placebo control group exhibited a notable rise in baseline mNIS+7 scores by 25.5 and baseline QoL-DN scores by 12.7, suggesting a favourable reaction to the administered intervention [21].

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Although the clinical study has provided evidence of the positive impact of Inotersen on both the quality of life and the progression of disease in individuals diagnosed with hATTR, it is important to acknowledge that side events continue to be observed. Glomerulonephritis (3%) and thrombocytopenia (3%) were identified as the most prevalent and frequently occurring side effects. It is important to acknowledge that a single patient expired as a result of one of the adverse events [22]. Furthermore, it should be noted that the utilisation of Inotersen is presently restricted to individuals who do not have end-stage disease. Additionally, there is a deficiency in statistical power to evaluate the efficacy of Inotersen treatment on cardiomyopathy28 accurately.

3.4. Familial Chylomicronemia Syndrome (FCS)

FCS is a rare metabolic disease that follows an autosomal recessive inheritance pattern characterised by chylomicronemia and severe hypertriglyceridemia, causing elevating triglyceride (TG) levels [23]. Patients are diagnosed with FCS when mutations occur in the presence of two recessive or biallelic in any of the five canonical FCS genes: LPL, APOC2, APOA5, LMF1, and GPIHBP1 [24]. According to studies, 95% of FCS cases are caused by the mutant LPL gene, which is responsible for gene-coding lipoprotein lipase (LPL) [22]. LPL is a crucial enzyme for the breakdown of TG in circulating lipoproteins, including chylomicrons or very-low-density lipoprotein (VLDL) [22]. Due to the mutant genes of LPL, the proteins produced are dysfunctional, resulting in the complete inhabitation of chylomicron hydrolysis [24]. Consequently, patients' downstream levels of lipoprotein species will be deficient, such as low-density lipoprotein (LDL), VLDL, and high-density lipoprotein (HDL) [24]. However, there are no successful treatments for FCS apart from using specific medications, restricting dietary fats, and abstaining from alcohol consumption [22]. Thus, effective therapy is needed.

A therapeutic strategy employed in the treatment of FCS involves the specific targeting of the APOC3 gene, responsible for encoding the APOC3 lipoprotein. The APOC3 lipoprotein has been seen to inhibit the function of lipoprotein lipase (LPL), leading to elevated levels of plasma triglycerides (TG). This occurs due to a decrease in the liver's uptake of lipoproteins that are rich in triglycerides. Moreover, it facilitates the liberation of triglycerides in the hepatic organ[23]. Volanesorsen, known by its brand name Waylivra, is a therapeutic agent that utilises ASO technology to selectively target the 3' end of apoCIII mRNA. The process of mRNA molecule degradation is initiated by the binding event, which then leads to the cleavage of the molecule by RNase H[23]. The degradation of apoCIII mRNA leads to heightened LDL activity, hence stimulating the elimination of TG and leading to a decrease in TG levels in the plasma [23]. The European Commission granted clearance to Volanesorsen (marketed as Waylivra) in May 2019 to treat adult individuals diagnosed with FCS. However, more postmarketing studies are required before final approval may be granted [23].

The ASO treatment was used in phase III clinical trials for treating FCS, hypertriglyceridemia, and familial partial lipodystrophy. Results show that the group treated with Volanesorsen experienced a significant reduction in plasma TG levels by 77%, while the placebo group experienced an increment in TG levels by 18%. Furthermore, by week 52, the Volanesorsen group sustained a decreased level of TG at around -50.1%. In addition, individuals treated with The individual who had experienced a minimum of two instances of pancreatitis in the previous five years documented a complete absence of attacks subsequent to the administration of Volanesorsen. Another phase III clinical trial was conducted to treat hypertriglyceridemia using Volanesorsen. The results were similar to the previously mentioned study, with the Volanesorsen group experiencing a 71.8% reduction in TG levels, whereas the placebo group had a 0.8% reduction [23]. Both studies suggest the effectiveness of Volansorsen in lowering TG levels. However, it should be noted that these benefits are counterbalanced by the occurrence of drug-induced thrombocytopenia in clinical trials. Moreover, injection site reactions are observed in approximately 30-40% of patients, and upper respiratory tract infection in around 10-15% [24].

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4. The potential use of Antisense oligonucleotides in RNA virus treatment

After SARS-CoV-2 became the leading cause of the worldwide pandemic, researchers explored the possibility of using ASO to treat COVID-19. The therapy has proved to be efficient in mice. It is potential for ASO to become a therapeutic for COVID-19 and even other RNA-virus-related diseases. LNA ASO can undermine the structure of the RNA sequence and prevent genetic material replication. There are two ways: forming a steric blocker and activating the enzyme RNase H to degrade RNA. LNA ASO destroys the RNA sequence and stops the translation process as a steric blocker and an enzyme activist. The preclinical trial has been performed on mice and shows a promising result. Mice were injected with 400 μg daily for four doses. The results showed that the viral titer has remarkably decreased by over 80 folds. Additionally, the preclinical trials using LNA ASO led to a dramatic decline (80-90 folds) of the Nucleocapsid (N) and Spike (S) expression detected by RT-qPCR [25].

Thus, it is demonstrated that ASO is antiviral-efficient against SARS-CoV-2. The therapeutic effectiveness on the human body and its application to other RNA-related diseases are to be expected. Nevertheless, the result still needs to be further guaranteed. ASO has the ability to undermine the structure of the RNA sequence. Therefore, it will be an excellent method to cope with the constant mutation of the RNA virus. The production of ASO is well established and has already covered a large scale [25]. Thus, the cost might be partially unaffordable, but due to those advanced techniques, achieving worldwide currency is still a challenge.

5. Conclusion

In the last several decades, ASO created hope for treating incurable diseases. In summary, we make a review addressing the current progress by summarizing clinical trials of several approved drugs in use and the most recent study of drug design with ongoing trials in neurological disorders, genetic diseases, and potential application in RNA virus remedy: SMA with approved nusinersen, AD with new design and tests in MAPTRx and BIIB080, HD with unsatisfactory results and promising usage of CD, FH with mipomersen, hATTR with Inotersen, FCS with Volansorsen and positive outcome in LNA ASO for SARS-COVID19. The main edge of this novel therapy over the conventional one with molecular antibody therapy is achieving sequence-specific binding which targets at coding or noncoding RNA. ASO also has the ability of multiple targeting and simultaneous transcription with high specificity and giving much splicing choice[11]. The ephemeral reaction of ASO brings convenience to clinical formulations and adjusting injection doses. ASO brings the probability of triggering a mild immune response to deliver the drug to the human brain with higher efficiency in neurological disease remedy, though it still could not directly get across BBB. However, adverse effect is a problem of ASO and it almost happens from mild to severe in all the approved drug. The delivery method of ASO to the brain such as intrathecal injection is also thought to contribute to adverse effect. Future clinical trials are required for the involvement of safety aspects related to toxic property in preclinical studies, by enhancing conjugation of peptide, antibody and nanoparticles of lipid. In addition, the preclinical or clinal tests for different patient populations and stages in certain diseases are highly required, such as SMA in adult and ending stage in hATTR. The technology in analyzing statistics on cardiomyopathy and animal models should also be improved to investigate the disease. Moreover, policies should be adjusted to lower the cost of ASO.

Author contribution

All the authors contributed equally, and their names were listed in alphabetical order.

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