Review Article

siRNA: A comprehensive review of marketed products till August 2022

Abstract

Small interference RNA (siRNA) is a double-stranded RNA of 21~25 nucleotides. siRNA functions using a natural phenomenon known as RNA interference (RNAi), a gene silencing mechanism. Hypothetically, siRNA can target and regulate the expression of any disease-related gene in a sequence-specific manner. In 1993, this mechanism was noticed in a nematode *Caenorhabditis elegans,*later discovered in humans. After two decades, in 2018, the first siRNA therapeutics (Patisiran) were developed successfully and got approval from USFDA. Followed by three more siRNA drugs (Givosiran, Lumasiran, and Inclisiran) approved in consecutive years to treat rare, inherited genetic disorders. Recently approved one is Vutisiran with a similar indication of patisiran.Limitation of conventional therapies, this new& standard pharmacotherapy opens a new era of changing the treatment options of human diseases.Six siRNA candidates are in phase III clinical trials and are hoped to enter the pharmaceutical market soon. Challenges faced during the development of these novel therapies were off-target effects, target-specific delivery, cellular uptake, recognition by the innate immune system, limited efficacy, and others. However, chemical modification of the siRNA nucleotides in sugar, base, and phosphate moiety makes it successful in overcoming obstacles. In addition, a non-viral delivery carrier also helped in many aspects during formulation. This study is a narrative review and will summarize pharmacokinetic, pharmacodynamic, design approaches, and other attributes faced during the development of marketed siRNA products.

Keywords: siRNA, RNAi, patisiran, givosiran, lumasiran, inclisiran, LNP, GalNac.

Introduction:

The foremost function of RNAsregarded as transcription and protein biosynthesis. Mostly but not all RNAs being copied from DNA template through an enzymatic process,thereforeit can be considered as anintermediary between DNA and protein(1)(2). Over time, a considerable number of studies revealed that RNAs have a more distinctive role than translating genetic information from DNA to proteins. RNA without being involved in protein translation can perform cellular functions both in unicellular and multicellular organisms(3). All DNA templated RNAs are considered transcripts, but less than 3% of these genetic transcripts can encode protein (4)(2). This lead to introduce a term non-coding RNAs (ncRNAs). These are classes of RNA transcripts, they do not do transcription of proteins but have role in regulating transcription & translating the protein coding genes(5). ncRNAs can be classified according to their function, size, structure, conservation and found as having both long and short sequence of nucleotides (nt)(6). Long non-coding RNAs (lncRNAs) are the largest group comprising all ncRNAs having nucleotide sequence of > ~200(7). Short sequenced ncRNAs referred as small non-coding RNAs (sncRNAs) consist of nucleotides less than 200. sncRNAs are classified as microRNA (miRNA) with 21-23 nt, small interfering RNAs (siRNAs) having 21-25 nt and piwi-interacting RNAs (piRNAs) with 21-26 nt(8).

First small non-coding RNA, the *lin-4* RNA detected in nematode (*Caenorhabditis elegans*) in 1993was involved in controlling the larval development.This *lin-4* and later the second riboregulatory*let-7* became the starting member of microRNA (miRNA) class(9)(10). Until now, miRNAs are observed in animals, plants, unicellular algae, and even viruses and mis-regulation of specific ones will benefit theailment of human diseases(11).The piRNAs were first discovered in germline cells and observed as influencing somatic cell renewal process.piRNAs binds with the piwi proteins whereas siRNAs and miRNAs bind with AGO proteins of the piwi/argonaute(Ago) protein family(12)(13).This Ago protein and small RNAs complexes can target mRNAs for transcriptional gene silencing(14).

A pathway named RNA interference (RNAi) involved in the processing of siRNA(15). RNAi is a natural defense mechanism to protect genome that degrade exogenous genes such as viruses (16, 17).This mechanism was first observed in plants and later found in protozoa, flies, nematodes, insects, parasites, mouse&human cell lines too(16).RNAi use small double stranded RNA (including siRNA and miRNA) and do degradation of targeted mRNA in homology dependent manner. Longer double stranded RNAs are the precursor for miRNA (endogenously) and siRNA (endo and exogenously), cleavage done by RNase III endonuclease Dicer. However,piRNAs processed from long single stranded RNAs in a dicer independent manner(18)(19).

RNAi mechanisms is useful in targeting and suppressing different pathological proteins expressions which is difficult to regulate with traditional pharmaceutical technologies. Thus, RNAi therapeutics and use of siRNA became a new drug class and hope for modern medicineinvolving gene regulation (20).Furthermore, this technique raises a new option for such diseases having a known genome background but lacking in availability of useful conventional drugs(21). In 2001, first RNAi pathway was applied for gene silencing by using synthetic siRNA(22). In 2004, first clinical trial of siRNA therapy has been done by Acuity Pharmaceuticals among patients with age related macular disorders (23).siRNA can be delivered systemically to treat diseases including neurodegenerative disorders, metabolic disorders, many

types of cancers, viral infectious diseases including AIDS, cardiovascular disease i.e. hypercholesterolemia, gastrointestinal disorders(21)(23)(24)(25).

This review will summarize current knowledge on marketed siRNA products in terms of their designing strategies, delivery approaches, pharmacodynamics & pharmacokinetics profile, and emerging challenges. Published articles are searched using specific keywords in Pubmed, Google scholar.

Mechanism of naturally occurring siRNA

RNAi, a posttranscriptional gene silencing pathway also referred to as RNA-based gene silencing (RBGS), is advantageous over antisense or antibody-based therapies for gene silencing(26). The first step of this phenomenon initiated with cleavage of longer dsRNA (30 to 100 nucleotides) into siRNA by dicer and characterized by two non-paired nucleotides (overhang) at 3′ end of each strand(27).On the next step, siRNA recognized by RNA induced silencing complex (RISC) which is a ribonucleoprotein effector complex and bind with it.(28)(29).Unwinding of siRNA is required to bind RISC and it is facilitated by ATP dependent helicase enzyme(30).Activated RISC can combine with one strand of siRNA and present it as the template or guide strand for complementary mRNA.Another strand which is known as passenger strand is efficiently removed(31)(32). The strand having thermodynamically less stable 5′ end is favorably selected as guide strand and binds to the PAZ (PIWI/Argonaute/Zwille) domain of AGO2 (preferential for human) protein of RISC. PAZ domain is significant for attaching with the 3′ endof the guide strand through its hydrophobic pocket and the MID (middle) domain has a 5′ phosphate end binding site(33)(34).For degradation, a phosphodiester bond of mRNA is cleaved which has complementary base pair at 10 & 11 from 5′ end of siRNA.Cellular exonuclease removed the resulted fragments of mRNA and after completion of the process targeted mRNA dissociate from siRNA. RISC get free and can take part in next cleavage(35). siRNA is very specific in targeting disease causing mutant allele and leaving behind the normal mRNA even if they are mismatched by only one base pair (36).

Design of siRNA for targeting human cells

To attain the maximum effect of siRNA-based therapeutics, to improve safety, to resist degradation by endogenous endonucleases and exonucleases and also to increase selectivity for RISC, chemical modifications should be applied(36)(37, 38).Earlier development started with unmodified strands of siRNA, but that failed in many aspects including limited efficacy and off-target effect induced toxicity. Chemical modification can be done on the nucleotide base, ribose moiety and phosphate backbone. One settlement is substitution of the 2′-OH of ribose with a 2′-O-methyl (2′-OMe) ,2′-methoxyethyl (2′-MOE) or 2′-deoxy-2′-fluoro (2′F) group. To suppress the innate immune response, substitution on certain nucleotides can be done with locked nucleic acid (LNA), unlocked nucleic acid (UNA) or glycol nucleic acid (GNA). Phosphonate can be modifiedby introducing phosphorothioate (PS) linkage, achieved by replacing one nonbridging oxygen of a phosphodiester with sulfur. This improves the plasma protein binding and longer circulation of unmodified counterparts, resistance to nuclease degradation although some shows reducing affinity for target. Replacement of uridine and cytidine residuesof base such pseudouridine, 2-thiouridine, N6-methyladenosine, 5-methylcytidine shows escaping from immune recognition and resistant to nuclease(37, 38)

However chemical modification is useful,but the size, hydrophilicity and charge of dsRNA have to consider overcoming the major challenges for systemic circulation, tissue penetration, cellular uptake and endosomal degradation(37, 38).For systemic deliverypolymers, lipids, peptides, antibodies, aptamers, exosomes, RBC, inorganic nanoparticles can be used as carriers. For selecting appropriate carriers along with efficacy&safety, manufacturing issues need to be considered too.To achieve optimum efficacy of siRNA through systemic delivery, obstacleslike off-target effects, innate immune response, lysosomal degradationmay occur (39). Extrinsic and intrinsic factors including pH, overexpressed level of enzymes, magnetic fields, UV irradiationmay be advantageous in designing siRNA nanoparticles(40).Final challenge is to choose the site of administration, it will selectively affect the bioavailability and volume of distribution.During development, till date, systemic delivery through intravenous (IV) infusion and subcutaneous (SC) injection, locally via inhalation (to the lungs), site-specific injection (in the eye) or topical administration has been applied.Though systemic deliverycan pass the first pass metabolism of liver, administration is troublesome for patients and due to intrinsic pharmacokinetic properties via systemic delivery it is difficult to reach all targeted tissue(37, 38)

FDA approved siRNA therapies

The US FDA approved the first RNAi therapy Patisiranto treat polyneuropathy caused by hereditary transthyretin amyloidosis (hATTR) on August 2018 under the brand name ONPATTRO™ and marketized by Alnylam Pharmaceuticals.Hereditary transthyretin amyloidosis (hATTR) is a rare, multisystem, inherited, fatal disease and cause progressive disability. Transthyretin (TTR) is a plasma protein known as amyloid, having 127 amino acids in four identical subunits bound together as a pair of dimers and largelyproduced by liver cells. They have a role as transport protein and transport vitamin A (retinol) along with retinol binding protein (RBP) and plasma thyroxine. In hereditary transthyretin mediated amyloidosis, the mutated and misfolded form of TTR protein gets accumulated in many extracellular spaces of heart, peripheral nerves, eyes, kidney, GIT. The clinical presentation of hATTR varies according to the TTR's mutation type and deposition site. Mostly mutated TTR accumulation found on peripheral nervous system (known as peripheral or amyloid neuropathy) and cardiovascular system (known as cardiopathy). Mutated *TTR-gene* code for TTR proteins having reduced stability whichfirst dissociate into two dimers and then in monomer which undergo conformational changes.TTR-gene mutation causes increased formation of misfolded amyloidogenic C-terminal fragments and full-length monomer which can deposit readily. Patisiran target the specific sequence of *TTR-mRNA*, degrade it and decrease the expression of both mutant and wild-type variants of TTR(41)(42)(43)(44)(45).More than 130 mutated transthyretins founded till now and predominant mutations found in 30th position in between val-met (valine-to-methionine)(46)(44). Another mutation, The Val122Ile was found primarily in African American individuals with cardiopathy as clinical manifestations(47).

Patisiran is a double stranded synthetic siRNA. Two partially complementary each strand of it contains 21 nucleotides. It isformulated as lipid nanoparticle (LNP) (encapsulated in lipid nanoparticles) and comprised of siRNA(ALN-18328) as the drug itself along with four lipid excipients. These formulated liposomescan deliverpatisiran specifically to hepatocytes and prevent endogenous enzymes degradation in vivo. Formulation of lipid nanoparticle is accomplished with a hydrophobic core of lipid encapsulating the siRNA by forming micelle and outer coating of hydrophilic polyethylene glycols to stabilize the core(48)(49)(45).Among these

four lipids, 2 of them were introduced for first time,DLin-MC3-DMA [(6Z, 9Z, 28Z, 31Z)-heptatriaconta-6, 9, 28, 31-tetraen-19-yl-4-(dimethylamino) butanoate] a cationic lipid and PEG_{2000}-C-DMG (α-(3′-{[1,2-di(myristyloxy)proponoxy] carbonylamino} propyl)-ω-methoxy, polyoxyethylene; whereas remaining two have previous history of using in approved drugs namely DSPC [1,2 distearoyl-sn-glycero-3-phosphocholine]a phospholipid and cholesterol(46). These four are mixed in an acidic and pH-dependent manner(44). Each lipid particles perform diverse role including cationic lipid helps in particle formation, encapsulation of siRNA, cellular uptake, fusion with endosomal membrane and release of siRNA in cells cytoplasm from endosome. Along with stabilization in circulation after administration, PEG helps elongate circulation time and adequate uptake by liver where TTR is predominantly synthesized. Phospholipid DSPC and cholesterol additionally helps in maintaining physicochemical stability of LNP(48)(46, 49).

During formulation of patisiran, siRNA is slightly changed to avoid the off-target effect with improved stability. Sugar (ribose) residue of 11 nucleotides modified with a 2′-*O*-methoxy group and 4 nucleotides with 2′-deoxy thymidine residues(48)(50). Affinity of a siRNA for its targeted mRNA completely depends on the ribonucleotides' sequence. For setting upWatson-crick base pairing, guide strand of patisiran need to be complementary with mutated TTR-mRNA. The sequence of antisense strand of patisiran is 5′-AUGGAAUACUCUUGGUUAC-3′ with previously mentioned modification. It will bind to the 3′ untranslated UTR region of complementary sequence 5′-GUAACCAAGAGUAUUCCAU-3′ of mutated and wild type *TTR* mRNA (instead of targeting any mutated region)(48)(51)(38).

After intravenous administration, in the systemic circulation, the outer lipid layer of modified polyethylene glycol (PEG2000-C-DMG)gets dissociated and replaced by serum protein apolipoprotein E (ApoE). ApoE interacts with the cholesterol of the lipid complexand ApoE covered drug recognized by hepatocytes surface receptors apolipoprotein E.Thus, drug is delivered to the hepatocytes andsequentially send the drug to endosomes. In the acidic environment of endosome, DLin-MC3-DMA developed a positive charge due to its pH dependent ionization characteristics. In the endosome, osmosis started and continued until disruption and dissociation of lipid vesicle. Finally, with the help of electrostatic &hydrophobic interaction of dissociated lipid particles and endosomal membrane, siRNA released into the cytoplasm of liver cells to control the *TTR-gene* expression. Then siRNA assemble with theArgonaute slicer protein (Ago2) in RISC, unwinding into sense and antisense strand and antisense strand bind to the homologous*TTR-mRNA*. Thereby cleavage of mRNA andinhibition ofboth mutant and normal TTR protein synthesis. Consequently, serum & tissue deposition of amyloid reduced and with pause in disease progression(38)(44)(48)(49).

Phase I clinical trial of patisiran was done on healthy individuals, dose range was 0.01-0.5mg/kg and produced serum reduction of TTR in a dose dependent manner.Phase II clinical study among patients of amyloidosis with polyneuropathy yielded a maximum TTR reduction of 82.9% to 86.7% at the highest dose of 0.3 mg/kg. In phase III trial, patients with hATTR amyloidosis with polyneuropathy (APOLLO), TTR level was reduced by 81% (median) with the same dose of phase II. Till date, patrisiran has found relatively safe with mild-moderate side effects and recommended dosage is 0.3 mg/kg once every 3 weeks for patients weighing less than 100 kg and 30 mg once every 3 weeks for patients weighing ≥ 100 kg. Dosesmust be administered as a single intravenous infusion over approximately 80 minutes. (45, 49, 52).Metabolism occurred by ribonuclease enzymes to nucleotides, with less than 1% excreted unchanged in the urine and

having a half-life of 3.2 days (53)(45).Almost amount of administered dose eliminated by metabolism and only 1% eliminated through urine as unchanged(54). Before patrisiran administration, patients need to be premedicated with H_1 and H_2 blockers, acetaminophen, and corticosteroids. As TTR transport retinols, daily vitamin A supplements are recommended(47).

Givosiran(GIVLAARI®) (ALN-AS1), from Alnylam Pharmaceuticals, the second approved siRNA therapeutics got its approval on 20 Nov 2019 in the USA to treat hepatic porphyria (AHP) among adults. In EU, it achieved positive signin January 2020 for the treatment of hepatic porphyria (AHP) but also among patients ≥ 12 years(55). AHP is a metabolic disorder and mutation in the genes encoding for enzymes needed in the haem biosynthetic pathway (in hepatocytes)are manifested. It is a rare, inherited, life-threating disease having four types-Acute intermittent porphyria (AIP) (third enzyme), hereditary coproporphyria(sixth), variegate porphyria (VP) (seventh)and δ-aminolevulinic acid dehydratase (ALAD)-deficient porphyria. *HMBS*, *CPOX*, *PPOX* and *ALAD* are the mutated genes responsible for above mentioned types, respectively. Women of reproductive age are most vulnerable to AHP. As enzymes of haem biosynthetic pathway lost its function, haem availability will be reduced in contrast of its increased demands. The triggered the activation of heam biosynthetic pathway and up-regulation of hepatic δ-aminolevulinic acid synthase 1 (ALAS1), the first and rate-limiting enzyme for normal haem synthesis. For instances, pathway is not accomplished with haem synthesis but ended up with accumulation of neurotoxic haem precursor and intermediate metabolites δ-aminolevulinic acid (δ-ALA) ,porphobilinogen (PBG) and porphyrins. (56-58).Haem biosynthesis starts with succinyl coA& glycine which converts into δ-ALA and ALA synthase. On the next step, δ-ALA converts to porphobilinogen, further steps halted due to lack of downstream proper functioning enzymes. This accumulated metabolites give rise to severe abdominal pain, vomiting, tachycardia, hypertension, respiratory paralysis, hepatocellular carcinoma, chronic renal failure, and chronic neuropathy(59).

Givosirando the downregulation of hepatic ALAS1, by targeting hepatic *ALAS1* gene and stop its translation to ALAS1 enzyme.This will consequently reduce the accumulation of toxic intermediatory by-products. To deliver givosiran, it is conjugated with a trivalent N-acetylgalactosamine(GalNac) ligand. GalNac helps uptake siRNA by hepatocytes because of exclusive expression of a transmembrane receptor asialoglycoprotein receptors (ASGPR) on liver cells. ASGPR have a high affinity and specificity for GalNac. It will also help to devoid the off-target effect with higher efficacy. Without this GalNac ligand, the siRNA will be unable to show any efficacy in liver.It further undergoes extensive chemical modification to protect the GalNac from nuclease degradation. 2’ position of nucleotides either modified with 2’-deoxy-2’-fluoro or a 2’-O-methyl group in the ribose sugar moieties. To achieve the in-vivo efficacy,a few phosphodiester bonds are replaced with phosphorothioate (PS). These terminal phosphrothionatelinkages of most Givosiranare well tolerated thus increases sustainability during in-vivo efficacy. Though not clearly understood, the hypothesis is thatupon entering the liver by binding with ASGPR enzyme, endocytosis triggered and GalNac ligand degraded in endosomes. Upon internalization to hepatocytes, RNA cleaved by cellular enzymes into ~20 nt base pairing and loaded into RISC, separated into two strands.Then follow the general mechanism of cleaving $ALaS_1$mRNA by RISC-antisense strand binding and stop the synthesis&translation of $ALaS_1$ protein. Thereby accumulation of neurotoxic ALA and PBG on systemic circulation (57-59)(60).Givosiran is administered as subcutaneous injection at a dose of 2.5mg/kg/ monthly and showed 90% protein binding(57). If we see the pharmacokinetic profile of Givosiran, it has~1-

week $t_{1/2}$ life in the liver and 4-10 hours in plasma; metabolized by nuclease and formed active metaboliteAS(N-1)3′ with 35-75%plasma exposure. Upon metabolism, givosiran lose one nucleotide from the 3′ end of antisense strand and the resulted metabolite AS(N-1)3′ which is equipotent to givosiran. No significant drug-drug interactions has been observed(61)(16)(57). 5-14% excreted unchanged in urine whereas 4-13% is recovered as its active metabolite AS(N-1)3′ givosiran(59)

As a continuous success of siRNA therapeutics, as a third component **Lumasiran**(Oxlumo™) (ALA-GO1)received its global approvalon 19 November 2020 in EU to treat an ultra-rare autosomal recessive disorderprimary hyperoxaluria type 1 (PH_1) for all age group whereas in the USA it got approval for adult & pediatric patients. Alnylam Pharmaceuticals also develop it.$PH_1$is the most severe type among PH disorders and clinically manifested by deficiency of alanine-glycolate aminotransferase (AGT) , a liver-specific peroxisomal enzyme due to genetic defect in AGT encoding gene *AGXT*.This enzymecatalyzesglyoxylate metabolismand converts it to glycine in peroxisome by transamination. In the deficiency of AGT, glyoxylateaccumulates, excess glyoxylates transported to cytoplasm and oxidized to oxalate(glyoxylate is the direct precursor of oxalate)by lactate dehydrogenase type A (liver-specific) and increased plasma concentration of oxalate. Excess oxalates transport to kidney to excrete through urine and there they bound with calcium, form insoluble calcium oxalate crystals. This ultimately leads to deposition of calcium oxalate crystals in the kidneys and urinary tract and gradually cause kidney disease (nephrocalcinosis, recurrent kidney stones)& ultimately kidney failure. In addition, multi-organ damage from systemic oxalosismay occur due to deposition of calcium oxalate crystals in other tissues, including bone, blood vessels, heart, eye, and skin(62-64).

Lumasiran, the synthetic dsRNA,was designed to silence the gene expression of *HAO1* and prevent its translation to glycolate oxidase (GO). GO enzyme is responsible for converting glycolate to glyoxylate in hepatic peroxisomes, soglyoxylate availability will be reduced, ultimately reducing the conversion to oxalate(65). One strand of lumasiran is composed of 21-base and another of 23-base. Modification done on 10 nucleotides with 2′-F substitution and the other 34 nucleotides substituted with 2′-OMe (66). It also has phosphonothioate linkages at the terminals.Designing approach of lumasiran is similar of givosiran. Like givosiran, triantennary N-acetylgalactosamine (GalNAc) -a carbohydrate was used to deliver lumarsiran and GalNac conjugated with the sense strand of siRNA. Gal-Nacresidue interacts with the highly expressed asialoglycoprotein receptors-1 in hepatocytes. Asialoglycoprotein receptorsensured the specific delivery of siRNA to liver and facilitates the hepatic uptake by receptor-mediated endocytosis.Once enter the acidic environment of endosome, siRNA released from the GalNaccomplex in the cytoplasm, followed by loaded on RISC complex and do silencing *HAO1* gene. This silencing has been done irrespective of mutationbecause GO is upstream of the defective AGT enzyme. High glyclolate levels in the liver could be toxic, cause acidosis but found it as well-tolerated elevations which indicates lumasiran as safe strategy to treat PH_1(62, 64, 65)(67)(68).

Lumasiran is recommended to administration subcutaneously in the USA and EU with a dosage regimen of three loading doses (once monthly) and followed by maintenance dosing. Patients weighing less than 10 kg, loading dosage is 6 mg/kg once monthly whilethe maintenance dosage is 3 mg/kg once monthly. Patients weighing less than 20 kg, loading dosage is 6 mg/kg once monthly whereas the maintenance dosage is 6 mg/kg once monthly. Patients weighing less than $\geq$20 kg, kg, loading dosage is 3 mg/kg once monthly whereas the maintenance dosage is 3

mg/kg once monthly. No dose adjustment is needed if the GFR rate is 30 to < 90 mL/min/1.73 m2. Due to lacking data availability safety monitoring is required for patients under age 1 year and having GFR less than 30 mL/min/1.73 m^2.Lumasiran absorbed rapidly and reach the maximum plasma concentration within 4 hours of administration.77–85% of lumasiran is bound to plasma protein and volume of distribution is 4.9 L. Metabolized by endo- and exonucleases to shorter length oligonucleotides. All clinical trials data have stated dose dependent increase of plasma and urinary glycolate concentration and 24-hour urinary oxalate excretion found >0.7 mmol. Also characterized by rapid elimination from plasma which indicate rapid uptake by liver. Excreted via urine and accounting for 7%–26% of drug clearance as lumasiran, rest of the administered dose excreted as inactive metabolites(62, 64, 65, 68).Lumisiran have a longer half-life with a the mean terminal plasma half-life of 5.2 h (68).

The last approved siRNA therapeutics till date is **Inclisiran**(Leqvio®) (ALN-60212), developed by Novartis pharmaceuticals under a license from Alnylampharmaceuticals.It got first approval on December 2020 in the EUand on December 2021 by FDAto treat hypercholesterolemia(main reason of atherosclerosis) and mixed dyslipidemia along with diet.Statin was the mainstay drug to treat hypercholesterolemia by reducing the causable factor LDL (Low density lipoprotein). Thensometreatment gapshave been observed including variety in individual response, nonadherence, discontinuation the therapy over the time (within a year), high incidence of cardiovascular among patients with persistent level of LDL. Above reasonsandfor patients who are intolerant and contraindicated to statins, inclisiran opened a new window for them.Statins are the HMG-CoA reductase inhibitors in cholesterol synthesis pathway and reduce the atherogenic cholesterol levels in plasma. In 2003, serine protease proprotein convertase subtilisin–kexin type 9(PCSK9) was discovered, a circulating protein that can accelerate the degradation of the LDL receptor. So, it reduced the binding of LDL to the receptor and consequently regulated the plasma LDL cholesterol level (LDL-C). Gain of function mutations in this *PCSK9* may cause severe hypercholesterolemia. Initially, these circulating *PCSK9* was targeted by human monoclonal antibodies and reduced its binding to LDL-receptor, thus clear LDL from plasma.Later, development of inclisiran that target hepatic production of PCSK9 being an alternative therapy to reduce LDL-C level(69-71).

Sense strand sequence is 5′-Cms-Ums-Am-Gm-Am-Cm-Cf-Um-Gf-Um-dT-Um-Um-Gm-Cm-Um-Um-Um-Um-Gm-Um-L96-3′ whereas antisense strand is composed with a sequence of 3′-Ams-Ams-Gm-Am-Um-Cf-Um-Gf-Gm-Af-Cm-Af-Am-Af-Am-Cf-Gm-Af-Af-Af-Ams-Cfs-Am-5′. As a characteristic of siRNA, at 3′ terminus of the antisense strand overhanging of 2 nucleotides (adenosine) observed(72). Inclisiranhelpful with blocking the translation of PCSK9 both intra & extracellularly, long biological half-life produces sustained effect. Inclisiran, the synthetic double stranded siRNA targeted the PCSK9 mRNA. For ensuring target-specific delivery, GalNAc (a triantennary carbohydrate) used as a carrier which was conjugated with the sense strand of inclisiran. This triantennary carbohydrate is complementary of ASGPR receptor of hepatocytes which ensures specific hepatic uptake. To increase the stability, modification done on siRNA strand with 2′-O-methylnucleotides (m on sequence) or 2′-O-fluoronucleotides(f on sequence) with phosphorothioate (s on sequence) (PS).After uptake by hepatocytes, antisense strand integrated with RISC and followed by degradation of PCSK9 mRNA (target the 3′ UTR) and thus preventing PCSK9 protein translation(69, 70, 73, 74)

Recommended to administer as subcutaneous injection with a dose of 284 mg (each mL contains inclisiran sodium equivalent to 189 mg inclisiran). After initial dosing further boosting on the

90th day and then on 6 months. Inclisiranshows dose dependent PK profile over the dose range of 24–756 mg on single administration. After administration of recommended dose, shows the peak plasma concentration after ~4 hours and depleted within ~48 hours from plasma. No trace of accumulation has been found after multiple dosing, indicating rapid localized hepatocyte uptake. In vitro plasma protein binding was 87% with a volume of distribution was ≈ 500 L. Like other siRNAs described above, metabolized by nucleases into inactive shorter nucleotides. Biological half-life is ~9 hours and renal clearance rate was 16%. No drug-drug interaction has been found with concomitant administration with statins(69, 70, 73).

Recently, a new RNAi therapeutics **Vutisiran** get approval from USFDA in June 2022. This second-generation siRNA is developed by Alnylam Pharmaceuticals to treat hATTR like patrisiran(75). To treat this polyneuropathy, vutisiran target the TTR-mRNA. Vutisiran helps in reducing TTR-protein level in serum by initiating degradation of TTR-mRNA in the liver. It is a long acting subcutaneously administered drug once in three months. Its actually administered in patients with patisiran during phase III clinical trial and demonstrated reduction of serum TTR-protein in a dose-dependent manner(76, 77).As it is a newly approved drugs, detailed information about formulations is yet to publish.

siRNA therapies on clinical trials

On continuation of earlier success, siRNA therapeutics are continuing its development as novel therapies. Some promising siRNAs are now on clinical trials and will be marketized in upcoming years. Nedosiran, developed by Dicerna pharmaceuticals, can potentially treat primary hyperoxaluria, continuing its phase III clinical trial. Fitusiran, a result of collaborative work between Alnylam and Sanofi Genzyme. Its promising to treat hemophilia A and B in Phase 2 and 3 trials. Teprasiran was first systemically administered siRNA drug to treat acute kidney injury and received an orphan drug status by FDA. Cosdosiran, under development by Quark Pharmaceuticals treat nonarteritic anterior ischemic optic neuropathy (NAION) and primary angle glaucoma. It has developed as local delivery instead of systemic. It is also achieved the orphan drug status. Tivanisiran, is being developed by Sylentis, undergoing phase III clinical trial, treats ocular pain and dry eye disease (66, 78).

Table 1: Approved and phase III clinical trial siRNAs

Approved siRNAs	siRNAs on clinical trial
• Patisiran	• Nedosiran
• Givosiran	• Fitusiran
• Lamisiran	• Teprasiran
• Inclisiran	• Cosdosiran
	• Tivanisiran

Table 2: Overview of pharmacokinetics and pharmacodynamics information of marketed siRNA therapeutics

	Patisiran	**Givosiran**	**Lumasiran**	**Inclisiran**
Other names	ONPATTRO™	GIVLAARI®	Oxlumo™	Leqvio®
siRNA	ALN-18328	ALN-AS1	ALN-GO1	ALN-60212
Developer	Alnylam Pharmaceuticals	Alnylam Pharmaceuticals	Alnylam Pharmaceuticals	Novartis Pharmaceuticals
Indication	Polyneuropathy caused by hereditary transthyretin amyloidosis (hATTR) (USA) stage 1 or 2 polyneuropathy (EU) (45).	Acute hepatic porphyria (AHP)	Primary hyperoxaluria type 1 (PH_1)	Hypercholesterolaemia
Approval status	Approved August 2018 by US FDA and EMA (45)	20 Nov 2019 in the USA In EU, in January 2020	November 2020 in EU and USA	December 2020 in the EU, December 2021 by FDA (79)
Target	*TTR* mRNA	*ALAS1* mRNA	*HAO1mRNA*encoding for GO	*PCSK9 mRNA*
Mechanism of action	Reduce the synthesis of both mutant and normal TTR protein	Hepatic δ-aminolevulinic acid synthase 1 inhibitors	Inhibit glycolate oxidase (GO) translation	Target hepatic production of PCSK9 reduces degradation of LDL receptor and increases LDL-C level.
Delivery carrier	Lipid nanoparticle prepared by using four lipid excipients.	Triantennary N-acetylgalactosamine (GalNAc) -a carbohydrate	TriantennaryN-acetylgalactosamine (GalNAc) -a carbohydrate	GalNAc (a triantennary carbohydrate)
Modification in siRNA strand	Ribose residue of 11 nucleotides modified with a 2′-*O*-methoxy group and 4 nucleotides with 2′-deoxy thymidine residues	2′ position of nucleotides either modified with 2′ -deoxy-2′ -fluoro or a 2′ -O-methyl group Phosphodiester bonds are replaced with	10 nucleotides with 2′-F substitution and the other 34 nucleotides substituted with 2′-OMe. It also has phosphorothioate linkage.	Substitution with 2′-O-methylnucleotides or 2′-O-fluoronucleotides and replace phosphodiester linkage with phosphorothioate.

phosphorothioate.

Recommended dose	0.3 mg for body weight <100 kg 30 mg for body weight ≥ 100 kg	2.5 mg/kg	Loading dose- 6 mg/kg/once monthly maintenance dose- 3 mg/kg/once monthly – body wt.<10kg Loading dose- 6 mg/kg/once monthly maintenance dose- 6 mg/kg/once monthly – body wt.<20kg Loading dose- 3 mg/kg/once monthly maintenance dose- 3 mg/kg/once monthly – body wt. ≥ 20kg	284 mg as a single injection
Administration	Every 3 weeks	Once monthly	Once monthly	On 3rd month and 6th month after first administration followed by every 6th month dosing
Delivery Route	IV (Infusion)	Subcutaneous injection	Subcutaneous injection	Subcutaneous injection
Half-life	Terminal elimination half-life 3.2 days	~1-week in the liver and 4-10 hours in plasma	Mean terminal plasma half-life is 5.2 h	Biological half-life is ~9 hours
Metabolism	Cleaved by ribonuclease enzymes	Metabolized by nuclease and formed active metabolite AS(N-1)3	Metabolized by endo- and exonucleases to shorter length oligonucleotides	Metabolized by nucleases into inactive shorter nucleotides
Excretion	Mostly through metabolism, 1% unchanged in urine.	5-14 % as givosiran, 4-13% as AS(N-1)3′ givosiran	7-16% excreted via urine	16% renal clearance as inclisiran
Adverse effects	No observed serious events (54)	On chronic use, increase creatinine level in serum and	No serious adverse effects except respiratory tract	Chances of adverse effects on long term use (73)

		decrease in GFR (59)	infection (65).	
Drug-Drug interaction	Not observed (45)	Not observed	No such as inducer or inhibitor of CYP450 (64)	Not observed

Summary and conclusion:

By using the naturally occurring phenomenon of siRNA&RNAi, mutated gene were targeted and achieved enormous success to treat mostly the rare, inherited, autosomal diseases. Till now, fourdrugs are marketed to treat haTTR (patisiran), AHP (givosiran), PH_1 (Lumasiran) and hypercholesterolemia (inclisiran) and all were developed by targeting specific mRNA in hepatocytes. Patisiran with its unique mode of action make an advancement to treat amyloidosis which requires less safety monitoring than other available therapeutic options (TTR stabilizer) (80). Though vitamin A level should be examined before starting treatment with patisiranand daily supplement of vitamin A is recommended. Patients should be aware about mild to moderate infusion related adverse effects and peripheral edema(45). Givosiran, the second successful candidate in this siRNA run, wassuccessful in treating the acute hepatic porphyria. Previously available therapy was mostly targeted on symptomatic relief or administration of haem injection. Discovery of givosiran abolish the need for liver transplantation on the progression of AHP(81).Liver function should be checked before administering givosiran due to elevation of alanine transaminase enzymes and serum creatinine level should also be monitored(57). Like givosiran, lumasiran is also associated with elevated liver enzyme, so should check the glycolate level. Inclisiran is the last candidate, associated with some adverse effects but no history of discontinuation has been found. Long term clinical trial is suggested and can continue with statin therapy (73).

All developed siRNAs were targeted to liver to treat disease of various organs including kidney, heart, central nervous system, and others as it is the epicenter of enzyme synthesis. The main challenges in developing these novel therapeutics were escaping from immune system recognition and retaining potency. To escape from this, siRNAs were chemically modified which additionally help to improve selective delivery and stability. Even the successful siRNA carriers were originated and found to be non-genotoxic & safe. Another challenge was short circulation time and clearance by renal filtration, for reducing this hindrance, plasma protein binding was targeted to improve. A furthertarget was selective cellular uptake and escaping from endosomal degradation. Combination of methods was developed to formulate these four successful siRNAs by eradicating all barriers.

Beyond all success, injectable route always has some complications regarding injection related reactions and convenience of patients. As dosage regimen is quite favorable, not frequent than one month administration, it could be adjustable comparing with greatest curability. Expenses are another triggering factor for siRNA. Sequential analysis will be helpful to reduce the economy as these rare diseases are increasing in prevalence.Life-risk adverse events were absent in approved siRNAs, but post market surveillance would ensure future safety and monitoring.It would be a scope for future work and make the profile of these therapeutics more informative.

Ethics approval statement: Not required

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

References:

1. Brosius J, Raabe CAJRb. What is an RNA? A top layer for RNA classification. RNA biology. 2016;13(2):140-4.
2. Li J, Liu CFig. Coding or noncoding, the converging concepts of RNAs. Frontiers in genetics 2019;10:496.
3. Hubé F, Francastel C. Coding and non-coding RNAs, the frontier has never been so blurred. Frontiers in genetics. 2018;9:140.
4. Brosius J, Raabe CARb. What is an RNA? A top layer for RNA classification. RNA biology. 2016;13(2):140-4.
5. Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nature reviews Drug discovery. 2013;12(11):847-65.
6. Neumeier J, Meister GJFiPS. siRNA specificity: RNAi mechanisms and strategies to reduce off-target effects. Frontiers in Plant Science. 2021;11:526455.
7. Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JSJTJoPAJotPSoGB, Ireland. Non-coding RNAs: regulators of disease. The Journal of Pathology: A Journal of the Pathological Society of Great Britain. 2010;220(2):126-39.
8. Zhang P, Wu W, Chen Q, Chen M. Non-coding RNAs and their integrated networks. Journal of integrative bioinformatics. 2019;16(3).
9. Phillips T. Small non-coding RNA and gene expression: Nature Education 2008 [cited 2022 23/7]. 1(1):115:[Available from: https://www.nature.com/scitable/topicpage/small-non-coding-rna-and-gene-expression-1078/.
10. Lim LP, Lau NC, Weinstein EG, Abdelhakim A, Yekta S, Rhoades MW, et al. The microRNAs of Caenorhabditis elegans. Genes development. 2003;17(8):991-1008.
11. Aalto AP, Pasquinelli AEJCoicb. Small non-coding RNAs mount a silent revolution in gene expression. Current opinion in cell biology. 2012;24(3):333-40.
12. Cai A, Hu Y, Zhou Z, Qi Q, Wu Y, Dong P, et al. PIWI-Interacting RNAs (piRNAs): Promising Applications as Emerging Biomarkers for Digestive System Cancer. Frontiers in Molecular Biosciences. 2022;9.
13. Thomson T, Lin HJAroc. The biogenesis and function PIWI proteins and piRNAs: progress and prospect. Annual review of cell developmental biology. 2009;25:355.
14. Höck J, Meister G. The Argonaute protein family. Genome biology. 2008;9(2):1-8.
15. Neumeier J, Meister G. siRNA specificity: RNAi mechanisms and strategies to reduce off-target effects. Frontiers in Plant Science. 2021;11:526455.

16. Agarwal S, Simon AR, Goel V, Habtemariam BA, Clausen VA, Kim JB, et al. Pharmacokinetics and pharmacodynamics of the small interfering ribonucleic acid, givosiran, in patients with acute hepatic porphyria. Clinical Pharmacology & Therapeutics. 2020;108(1):63-72.
17. Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y, et al. Therapeutic siRNA: state of the art. Signal transduction and targeted therapy. 2020;5(1):101.
18. Kim DH, Rossi JJJB. RNAi mechanisms and applications. Biotechniques. 2008;44(5):613-6.
19. Juliano C, Wang J, Lin H. Uniting germline and stem cells: the function of Piwi proteins and the piRNA pathway in diverse organisms. Annual review of genetics. 2011;45.
20. Bumcrot D, Manoharan M, Koteliansky V, Sah DW. RNAi therapeutics: a potential new class of pharmaceutical drugs. Nature chemical biology. 2006;2(12):711-9.
21. Koutsilieri E, Rethwilm A, Scheller C. The therapeutic potential of siRNA in gene therapy of neurodegenerative disorders. Neuropsychiatric Disorders An Integrative Approach. 2007:43-9.
22. Gavrilov K, Saltzman WM. Therapeutic siRNA: principles, challenges, and strategies. The Yale journal of biology

medicine. 2012;85(2):187.
23. Lares MR, Rossi JJ, Ouellet DL. RNAi and small interfering RNAs in human disease therapeutic applications. Trends in biotechnology. 2010;28(11):570-9.
24. Chevalier R. si RNA Targeting and Treatment of Gastrointestinal Diseases. Clinical Translational Science. 2019;12(6):573-85.
25. Burnett JC, Rossi JJ, Tiemann K. Current progress of siRNA/shRNA therapeutics in clinical trials. Biotechnology journal. 2011;6(9):1130-46.
26. Milhavet O, Gary DS, Mattson MPJPr. RNA interference in biology and medicine. Pharmacological reviews. 2003;55(4):629-48.
27. Dana H, Chalbatani GM, Mahmoodzadeh H, Karimloo R, Rezaiean O, Moradzadeh A, et al. Molecular mechanisms and biological functions of siRNA. International journal of biomedical science: IJBS. 2017;13(2):48.
28. Lam JK, Chow MY, Zhang Y, Leung SW. siRNA versus miRNA as therapeutics for gene silencing. Molecular Therapy-Nucleic Acids. 2015;4:e252.
29. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005;123(4):631-40.
30. Preall JB, Sontheimer EJ. RNAi: RISC gets loaded. Cell. 2005;123(4):543-5.
31. Leuschner PJ, Ameres SL, Kueng S, Martinez J. Cleavage of the siRNA passenger strand during RISC assembly in human cells. EMBO reports. 2006;7(3):314-20.
32. Chiu Y-L, Rana TM. siRNA function in RNAi: a chemical modification analysis. Rna. 2003;9(9):1034-48.
33. Noland CL, Ma E, Doudna JA. siRNA repositioning for guide strand selection by human Dicer complexes. Molecular cell. 2011;43(1):110-21.
34. Müller M, Fazi F, Ciaudo C. Argonaute proteins: from structure to function in development and pathological cell fate determination. Frontiers in Cell and Developmental Biology. 2020;7:360.
35. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136(4):642-55.
36. Dong Y, Siegwart DJ, Anderson DG. Strategies, design, and chemistry in siRNA delivery systems. Advanced drug delivery reviews. 2019;144:133-47.
37. Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y, et al. Therapeutic siRNA: state of the art. Signal transduction and targeted therapy. 2020;5(1):1-25.
38. Setten RL, Rossi JJ, Han S-p. The current state and future directions of RNAi-based therapeutics. Nature Reviews Drug Discovery. 2019;18(6):421-46.

39. Subhan MA, Torchilin V. siRNA based drug design, quality, delivery and clinical translation. Nanomedicine: Nanotechnology, Biology and Medicine. 2020;29:102239.
40. Salzano G, F Costa D, P Torchilin V. siRNA delivery by stimuli-sensitive nanocarriers. Current pharmaceutical design. 2015;21(31):4566-73.
41. Luigetti M, Romano A, Di Paolantonio A, Bisogni G, Sabatelli M. Diagnosis and treatment of hereditary transthyretin amyloidosis (hATTR) polyneuropathy: current perspectives on improving patient care. Therapeutics and Clinical Risk Management. 2020;16:109.
42. Wood H. FDA approves patisiran to treat hereditary transthyretin amyloidosis. Nature Reviews Neurology. 2018;14(10):570-.
43. Kristen AV, Ajroud-Driss S, Conceição I, Gorevic P, Kyriakides T, Obici L. Patisiran, an RNAi therapeutic for the treatment of hereditary transthyretin-mediated amyloidosis. Neurodegenerative disease management. 2019;9(1):5-23.
44. Urits I, Swanson D, Swett MC, Patel A, Berardino K, Amgalan A, et al. A review of patisiran (ONPATTRO®) for the treatment of polyneuropathy in people with hereditary transthyretin amyloidosis. Neurology and therapy. 2020;9(2):301-15.
45. Hoy SM. Patisiran: first global approval. Drugs. 2018;78(15):1625-31.
46. Zhang X, Goel V, Attarwala H, Sweetser MT, Clausen VA, Robbie GJ. Patisiran pharmacokinetics, pharmacodynamics, and exposure-response analyses in the phase 3 APOLLO trial in patients with hereditary transthyretin-mediated (hATTR) amyloidosis. The Journal of Clinical Pharmacology. 2020;60(1):37-49.
47. Kachur E. Novel Therapies for Transthyretin Amyloidosis. US Pharm. 2019;44(8):8-10.
48. Titze-de-Almeida SS, Brandão PRdP, Faber I, Titze-de-Almeida R. Leading RNA interference therapeutics part 1: silencing hereditary transthyretin amyloidosis, with a focus on patisiran. Molecular diagnosis & therapy. 2020;24(1):49-59.
49. Zhang X, Goel V, Robbie GJ. Pharmacokinetics of Patisiran, the first approved RNA interference therapy in patients with hereditary transthyretin-mediated amyloidosis. The Journal of Clinical Pharmacology. 2020;60(5):573-85.
50. Müller ML, Butler J, Heidecker B. Emerging therapies in transthyretin amyloidosis–a new wave of hope after years of stagnancy? European Journal of Heart Failure. 2020;22(1):39-53.
51. Harikrishnan P, Yandrapalli S, Aronow WS, Lanier GM, Jain D. Novel drug therapies for cardiac amyloidosis. Expert Opinion on Investigational Drugs. 2019;28(6):497-9.
52. Rizk M, Tüzmen Ş. Update on the clinical utility of an RNA interference-based treatment: focus on Patisiran. Pharmacogenomics and personalized medicine. 2017;10:267.
53. Yang J. Patisiran for the treatment of hereditary transthyretin-mediated amyloidosis. Expert review of clinical pharmacology. 2019;12(2):95-9.
54. Shahryari A, Nazari Z, Jazi MS, Hashemi-Shahraki F, Wißmiller K, Xu W, et al. Pharmacological Aspects of Clinically Approved Gene Therapy Drugs and Products. 2022.
55. Honor A, Rudnick S, Bonkovsky H. Givosiran to treat acute porphyria. Drugs of Today (Barcelona, Spain: 1998). 2021;57(1):47-59.
56. Gonzalez-Aseguinolaza G. Givosiran—running RNA interference to fight porphyria attacks. Mass Medical Soc; 2020. p. 2366-7.
57. Scott LJ. Givosiran: first approval. Drugs. 2020;80(3):335-9.
58. Syed YY. Givosiran: a review in acute hepatic porphyria. Drugs. 2021;81(7):841-8.
59. Majeed CN, Ma CD, Xiao T, Rudnick S, Bonkovsky HL. Spotlight on Givosiran as a Treatment Option for Adults with Acute Hepatic Porphyria: Design, Development, and Place in Therapy. Drug Design, Development and Therapy. 2022;16:1827.

60. Alexandre J. DJV, Matthew Catley, David Blakey, Nagy Habib. Delivery of Oligonucleotides to the Liver with GalNAc: From Research to Registered Therapeutic Drug. Molecular Therapy. 2020;28(8):1759-71.
61. Li J, Liu J, Zhang X, Clausen V, Tran C, Arciprete M, et al. Nonclinical Pharmacokinetics and Absorption, Distribution, Metabolism, and Excretion of Givosiran, the First Approved N-Acetylgalactosamine–Conjugated RNA Interference Therapeutic. Drug Metabolism and Disposition. 2021;49(7):572-80.
62. Frishberg Y, Deschênes G, Groothoff JW, Hulton S-A, Magen D, Harambat J, et al. Phase 1/2 study of lumasiran for treatment of primary hyperoxaluria type 1: a placebo-controlled randomized clinical trial. Clinical Journal of the American Society of Nephrology. 2021;16(7):1025-36.
63. Garrelfs SF, Frishberg Y, Hulton SA, Koren MJ, O'Riordan WD, Cochat P, et al. Lumasiran, an RNAi therapeutic for primary hyperoxaluria type 1. New England journal of medicine. 2021;384(13):1216-26.
64. Scott LJ, Keam SJ. Lumasiran: first approval. Drugs. 2021;81(2):277-82.
65. D'Ambrosio V, Ferraro PM. Lumasiran in the Management of Patients with Primary Hyperoxaluria Type 1: From Bench to Bedside. International Journal of Nephrology and Renovascular Disease. 2022;15:197.
66. Zhang MM, Bahal R, Rasmussen TP, Manautou JE, Zhong X-b. The growth of siRNA-based therapeutics: Updated clinical studies. Biochemical Pharmacology. 2021;189:114432.
67. Bacchetta J, Lieske JC. Primary hyperoxaluria type 1: novel therapies at a glance. Clinical Kidney Journal. 2022;15(Supplement_1):i17-i22.
68. Hulton S-A. Lumasiran: expanding the treatment options for patients with primary hyperoxaluria type 1. Expert Opinion on Orphan Drugs. 2021;9(7-10):189-98.
69. German CA, Shapiro MD. Small interfering RNA therapeutic inclisiran: a new approach to targeting PCSK9. BioDrugs. 2020;34(1):1-9.
70. Lamb YN. Inclisiran: first approval. Drugs. 2021;81(3):389-95.
71. Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M, et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. N Engl J Med. 2017;376:1430-40.
72. Bege M, Borbás A. The Medicinal Chemistry of Artificial Nucleic Acids and Therapeutic Oligonucleotides. Pharmaceuticals. 2022;15(8):909.
73. Merćep I, Friščić N, Strikić D, Reiner Ž. Advantages and disadvantages of inclisiran: a small interfering ribonucleic acid molecule targeting PCSK9—a narrative review. Cardiovascular Therapeutics. 2022;2022.
74. Warden BA, Duell PB. Inclisiran: a novel agent for lowering Apolipoprotein B–containing lipoproteins. Journal of Cardiovascular Pharmacology. 2021;78(2):e157-e74.
75. Paul A, Muralidharan A, Biswas A, Kamath BV, Joseph A, Alex AT. siRNA therapeutics and its challenges: Recent advances in effective delivery for cancer therapy. OpenNano. 2022:100063.
76. Sirbu CA, Ivan R, Authier FJ, Ionita-Radu F, Jianu DC, Vasiliu O, et al. Orphan Drugs in Neurology—A Narrative Review. Journal of Personalized Medicine. 2023;13(3):420.
77. Tschöpe C, Elsanhoury A. Treatment of transthyretin amyloid cardiomyopathy: The current options, the future, and the challenges. Journal of Clinical Medicine. 2022;11(8):2148.
78. Friedrich M, Aigner, A. Therapeutic siRNA: State-of-the-Art and Future Perspectives. BioDrugs. 2022. doi: https://doi.org/10.1007/s40259-022-00549-3.
79. Chiou TT, Tomasi K, Taub PR, Wilkinson MJ. Inclisiran creates unique opportunities and challenges for patient access to therapy: early experience in a United States Lipid Clinic. Journal of Clinical Lipidology. 2022.
80. Gertz MA, Mauermann ML, Grogan M, Coelho T. Advances in the treatment of hereditary transthyretin amyloidosis: a review. Brain and behavior. 2019;9(9):e01371.

81. Wang B, Rudnick S, Cengia B, Bonkovsky HL. Acute hepatic porphyrias: review and recent progress. Hepatology communications. 2019;3(2):193-206.