Liver-Targeted Delivery of Oligonucleotides with N-Acetylgalactosamine Conjugation

Hao Cui, Xinying Zhu, Shuyue Li, Peipei Wang, and Jianping Fang*

ABSTRACT: The potential therapeutic application of oligonucleotides (ONs) that selectively suppress target genes through antisense and RNA interference mechanisms has attracted great attention. The clinical applications of ONs have overcome multiple obstacles and become one of the most active areas for the development of novel therapeutics. To achieve efficient and specific cellular internalization, conjugation of a variety of functional groups to ONs has been the subject of intensive investigations over the past decade. Among them, a promising liver-targeted N-acetylgalactosamine (GalNAc) ligand has been evaluated in multiple preclinical and clinical trials for improving the cellular uptake and tissue specific delivery of ONs. GalNAc-based delivery relies on the fact that liver hepatocytes abundantly and specifically express the asialoglycoprotein receptor that binds and uptakes circulating glycoproteins via receptor-mediated endocytosis. In recent years, encouraging progress has been made in the field of GalNAc conjugates. This review aims to provide an overview of GalNAc-mediated liver-targeted delivery of small interfering RNA and antisense oligonucleotides, and the immense effort as well as recent advances in the development of GalNAc-conjugated agents are described.

INTRODUCTION

While small molecules and protein drugs are essential to the success of modern medicine, oligonucleotide (ON) therapeutics have the potential to become a third pillar of drug development. Representative ON-based therapeutics include small interfering RNA (siRNA) that targets and degrades disease-causing mRNA through RNA-induced silencing complex (RISC) mediated RNA interference and antisense oligonucleotide (ASO) that binds complementary mRNA and induce sequence-specific cleavage of the RNA by endonuclease RNase H. Unlike small molecular agents, which are lipophilic and readily taken up by tissues and cells, the majority of the ON molecules are more hydrophilic and larger in size, and their cellular uptake remains challenging. It is now well accepted that efficient delivery to the diseased cells is obviously one of the most important limitations for the development of highly potent and safe ON therapeutics.1

Liver is the largest internal organ in human body, playing vital functions in metabolism, detoxification, and iron homeostasis as well as synthesis and secretion of major plasma proteins. Over 80% of the liver mass is composed of hepatocytes. It is therefore not surprising that large amounts of disease targets reside within the liver hepatocytes that are potentially susceptible to ON therapies. However, the main obstacle has been the selective and effective intracellular delivery of the ON compounds to hepatocytes.2

ASIALOGLYCOPROTEIN RECEPTOR (ASGR) AND ITS APPLICATION IN DRUG DESIGN

Over the past decade, encouraging progress has been made in the area of hepatic delivery of ONs. The development of N-acetylgalactosamine (GalNAc) conjugates, which bind to the ASGR, has become a breakthrough approach for targeted delivery of therapeutic ONs to hepatocytes. ASGR is a high-capacity and rapidly internalizing receptor that is abundantly and specifically expressed on the sinusoidal surface of hepatocytes.3 Human ASGR contains two subunits ASGR1 and ASGR2 and facilitates the clearance of desialylated serum glycoproteins containing a terminal galactose or GalNAc.4,5 Cell surface ASGR interacts with GalNAc-conjugated ONs in the presence of Ca2+ at pH > 6 in clathrin-coated vesicles of plasma membrane and internalizes ligands via clathrin-dependent receptor-mediated endocytosis. Following complete internalization, acidification during endosomal maturation dissociates the GalNAc-conjugated ONs from ASGR followed by degradation of GalNAc in the lysosome. Free ASGR further recycles back to the plasma membrane of hepatocyte (Figure

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1) A recent study reported that ASGR1 subunit alone is sufficient for effective in vitro and in vivo uptake of GalNAc-conjugated ONs. Moreover, the heavy blood flow and fenestrated endothelium of the liver allow sufficient uptake and support multimonth efficacy with a single injection of ONs. Owing to these advantages, the GalNAc conjugates are likely to be a clinically dominant approach for ON delivery to liver.

**CHEMICAL OPTIMIZATION OF GALNAC-CONJUGATED ONS**

After several decades of development, great improvements have been achieved by clinically relevant modifications of ONs. A variety of chemical modifications have been explored to improve druglike properties of GalNAc-siRNA and GalNAc-ASO conjugates. The ribosugar moieties of each nucleotide are commonly modified with 2′-deoxy-2′-fluoro (2′-F) or 2′-O-methyl (2′-OMe). Partially modified siRNAs (less than 70% of the total nucleotides) provide some degree of protection against nucleolytic degradation and thus have been extensively used to evaluate the efficacy of various bioconjugates on siRNA distribution and in vivo efficacy. Recent studies indicated that fully chemical modified siRNAs showed higher potency and prolonged duration of gene silencing in vivo and in vitro. In contrast, studies also demonstrated that reduction of 2′-F content (less than 20%) yielded excellent in vitro activity and in vivo performance of GalNAc-siRNA. Incorporation of metabolically stable 5′-(E)-vinylphosphonate to the 5′ end of the antisense strand of siRNA resulted in significantly improved in vitro potency and stability. The incorporation of glycol nucleic acid (GNA) in the antisense strand seed region of GalNAc-siRNA reduced the off-target toxicity while maintaining on-target activity and increased thermal and metabolic stability. Further evaluation showed that the proximity of GalNAc is critical for ASGR recognition, and location of the clustered GalNAc influences the intrinsic potency of the siRNA. An array of nucleosidic GalNAc monomers resembling a trivalent ligand at or near the 3′ end of the sense strand resulted in siRNAs with receptor binding affinities similar to the parent conjugate design. A more recent study by Sharma et al., however, suggested that the di-, tri-, and tetravalent linear assemblies of GalNAc sugars showed potent, dose-dependent gene silencing in the liver, but the divalent GalNAc conjugate was less potent when compared to the tri- and tetravalent conjugates.

ASOs bind to complementary RNA and modulate RNA function to yield pharmacological effects. Early works suggested that the valency and position of the GalNAc conjugate is important for delivery efficacy and potency of GalNAc-linked ASO drugs. Studies demonstrated that introduction of a triantennary GalNAc conjugate enhanced the potency of ASOs 6–10-fold in mouse liver hepatocytes. However, further investigations indicated that conjugation of two and even one GalNAc sugar to chemically modified ASOs is sufficient to enhance potency 5–10-fold in mice. In contrast, studies also noted that two and three GalNAc conjugated ASOs bind the ASGR with the highest affinity and display optimal in vitro and in vivo activities, while single GalNAc conjugated ASOs showed 10-fold reduced receptor binding affinity compared with three GalNAc ASOs but only 2-fold reduced activity in vivo. Taken together, while the trivalent GalNAc conjugate is mostly used, a divalent GalNAc ligand might be sufficient to provide acceptable gene silencing activity. These findings provide a novel strategy for developing GalNAc-ON drugs with simplified chemical structure.

**PHARMACOKINETICS AND SAFETY**

Over the past decade, the pharmacokinetics of GalNAc-ONs has been evaluated extensively in animals and humans. Different from the unconjugated ONs that distribute to a wide range of tissues including liver after systemic administration, GalNAc-conjugated ONs are rapidly and specifically
Table 1. Summary of the Clinical Status of GalNAc Conjugates with Either siRNA or ASO from the Leading Pharma Companies

| company               | name                          | clinical status | type/delivery                  | target                                                                 | indication                                                                 |
|-----------------------|-------------------------------|-----------------|--------------------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Alnylam               | Givosiran (ALN-AS1)          | Registered      | siRNA/(ESC)-GalNAc conjugate   | δ-aminolevulinate synthase 1 (ALAS1)                                   | Acute hepatic porphyria (AHP)                                            |
| Alnylam               | Lumasiran (ALN-GO1)           | Registered      | siRNA/(ESC)-GalNAc conjugate   | Glutamate oxidase (GO)                                                 | Hyperoxaluria type 1 (PH1)                                               |
| Alnylam/Novartis       | Inducan (ALN-PC5sc)           | Registered      | siRNA/(ESC)-GalNAc conjugate   | Propionyl coenzyme A synthetase (PCAS)                                 | Hemophilia/Rare bleeding disorders (RBDs)                                  |
| Alnylam/Sanofi Genzyme | Fitusiran (ALN-AT3)           | Phase 3         | siRNA/(ESC)-GalNAc conjugate   | Antibiotic (AT)                                                        | Hemophilia/Rare bleeding disorders (RBDs)                                  |
| Alnylam               | Vutrisiran (ALN-TTRs02)       | Phase 3         | siRNA/(ESC)-GalNAc conjugate   | Transthyretin (TTR)                                                    | Transthyretin-mediated amyloidosis (ATTR)                                 |
| Alnylam               | Cemdisiran (ALN-CC5)          | Phase 2         | siRNA/(ESC)-GalNAc conjugate   | Complement C5                                                          | Complement-mediated diseases                                              |
| Alnylam               | ALN-AAT02                     | Phase 2         | siRNA/(ESC+)-GalNAc conjugate  | α-1 antitrypsin (AAT)                                                  | α-1 liver disease                                                        |
| Alnylam               | ALN-HBV02                     | Phase 2         | siRNA/(ESC+)-GalNAc conjugate  | Conserved region of hepatitis B virus (HBV)                           | HBV infection                                                            |
| Alnylam               | ALN-AGT                       | Phase 2         | siRNA/(ESC+)-GalNAc conjugate  | Angiotensinogen (AGT)                                                  | Hypertension                                                             |
| Alnylam               | ALN-HSD                       | Phase 2         | siRNA/(ESC+)-GalNAc conjugate  | 17β-Hydroxysteroid dehydrogenase 13 (HSD17B13)                        | Nonalcoholic steatohepatitis (NASH)                                      |
| Arrowhead/Amgen       | AMG-890 (Olpasiran)           | Phase 2/3       | siRNA/GalNAc conjugate         | Lipoprotein (a)                                                        | Cardiovascular disease (CVD)                                              |
| Arrowhead/JNJ         | JNJ-3989 (ARO-HBV)            | Phase 2/3       | siRNA/GalNAc conjugate         | HBV viral proteins                                                     | HBV infection                                                            |
| Arrowhead/Takeda      | ARO-AAT                       | Phase 2/3       | siRNA/GalNAc conjugate         | Mutant α-1 antitrypsin (Z-AAT) protein                                | α-1 Antitrypsin deficiency (AATD)                                         |
| Dicerna/Roche         | Nedosiran (DCR-PHXC)          | Phase 3         | siRNA/GalXC-siRNA              | Lactate dehydrogenase enzyme                                           | Primary hyperoxaluria,2,3 (PH)                                           |
| Dicerna               | Belcesiran (DCR-AIAT)         | Phase 1         | siRNA/GalXC-siRNA              | HBV viral proteins                                                     | HBV infection                                                            |
| Vir biotechnology/Alnylam | VIR-2218                    | Phase 2         | siRNA/(ESC+)-GalNAc conjugate  | Conserved region of HBV                                                | HBV infection                                                            |
| Silence Therapeutics   | SLN-124                       | Phase 1         | siRNA/GalNAc-siRNA              | Transmembrane serine protease 6 (TMFPRSS6)                            | β-Thalassemia/myelodysplastic syndromes (MDS)                             |
| Silence Therapeutics   | SLN-360                       | Phase 1         | siRNA/GalNAc-siRNA              | Lipoprotein (a)                                                        | Cardiovascular disease with high Lp (a)                                  |
| Arbutus               | AB-729                        | Phase 1         | siRNA/GalNAc-siRNA             | HBV viral proteins                                                     | HBV infection                                                            |
| Ionis/Akcea/Novartis   | Pelacarn (AKCEA-APO(α)-Lna)   | Phase 3         | ASO/ASO-GalNAc conjugate       | Apolipoprotein (a)                                                     | CVD                                                                      |
| Ionis                 | IONIS-TTR-LRx (AKCEA-ANGPTL3-Lna) | Phase 3     | ASO/ASO-GalNAc conjugate       | TTR                                                                    | ATTR                                                                     |
| Ionis/Pfizer          | Vupanornen (AKCEA-APOCIII-Lna) | Phase 2       | ASO/ASO-GalNAc conjugate       | Angiopoietin-like 3 protein (ANGPTL3)                                  | CVD                                                                      |
| Ionis                 | IONIS-APOCIII-LRx (AKCEA-APOCIII-Lna) | Phase 2     | ASO/ASO-GalNAc conjugate       | Apolipoprotein C-III (ApoC-III)                                       | CVD                                                                      |
| Ionis/Roche           | IONIS-FB-Lna                  | Phase 2         | ASO/ASO-GalNAc conjugate       | Complement factor B (FB)                                               | Immunoglobulin A nephropathy (IgAN)/Age-related macular degeneration      |
| Ionis                 | IONIS-AGT-Lna                 | Phase 2         | ASO/ASO-GalNAc conjugate       | Angiotensinogen                                                        | Resistant hypertension                                                   |
| Ionis/Bayer           | IONIS-FXI-Lna                 | Phase 2         | ASO/ASO-GalNAc conjugate       | Factor XI                                                              | Clotting disorders                                                       |
| Ionis/AstraZenea       | ION449 (IONIS-AZ4-2.5Lna)     | Phase 2         | ASO/ASO-GalNAc conjugate       | PCSK9                                                                  | CVD                                                                      |
| Ionis                 | IONIS-GHR-Lna                 | Phase 2         | ASO/ASO-GalNAc conjugate       | Growth hormone receptor (GHR)                                         | Acromegaly                                                               |
| Ionis                 | IONIS-PKK-Lna                 | Phase 2         | ASO/ASO-GalNAc conjugate       | Prekallikrein (PKK)                                                    | Hereditary angioedema                                                     |
| Ionis                 | IONIS-TMPRSS6-Lna             | Phase 2         | ASO/ASO-GalNAc conjugate       | TMFRSS6                                                                | β-Thalassemia                                                           |
| Ionis/AstraZenea       | ION839 (IONIS-AZ6-2.5Lna)     | Phase 1         | ASO/ASO-GalNAc conjugate       | Patatin-like phospholipase domain-containing protein 3 (PNPLA3)        | NASH                                                                     |
| Ionis                 | ION224                        | Phase 1         | ASO/ASO-GalNAc conjugate       | Diacylglycerol acyltransferase 2 (DGAT2)                                | NASH                                                                     |
| Ionis                 | ION904                        | Phase 1         | ASO/ASO-GalNAc conjugate       | Angiotensinogen                                                        | Treatment resistant hypertension (TRH)                                    |
taken up by liver hepatocytes via a ligand/receptor sorting system. Following subcutaneous injection, GalNAc-ASOs are rapidly absorbed with peak plasma concentration being achieved between 15 and 60 min in mice, 1 and 4 h in monkey, and 0.5 and 5 h in human.13−16 After absorption, the majority of GalNAc-ASOs bind to plasma protein with over 98% in monkey and human, slightly lower in mice, over 97%.13 The extent of plasma protein binding by GalNAc-ASOs is similar to the unconjugated ASOs.13 Two comprehensive animal studies by Yu et al. reported that GalNAc-conjugated ASOs are relatively stable in plasma. Fully conjugated intact GalNAc-ASOs accounts for 60−90% of total full-length ONs detected at maximum plasma concentration (Cmax) in mice, and over 70% in monkey. However, metabolites with GalNAc sugar deletion or unconjugated ASOs are also detected in the blood (less than 13% and 17% of the total fully conjugated ASOs in monkey and mice, respectively). Moreover, after 24 h, the naked ASOs become the major ON species in blood, reflecting a plasma–tissue equilibrium is reached.15,16

The rapid plasma clearance of GalNAc-ASO drugs is attributed to tissue distributional clearance. The rate of plasma clearance of GalNAc-ASOs is approximately 5-fold higher than unconjugated ASOs, reflecting a more rapid distribution from plasma to tissues.13 After subcutaneous administration, GalNAc-ASOs mainly distribute to liver and also kidney, which occurs concurrently with rapid decrease of plasma concentration. Once in tissues, GalNAc conjugates are rapidly cleaved within minutes to hours by lysosomal hydrolase and release the unconjugated ASO, which subsequently bind to target mRNA and yield pharmacological response (Figure 1). Parent unconjugated ASO is metabolized slowly in tissues via nuclease-mediated metabolic pathway with elimination half-life in weeks.13 Recent mechanism studies of extended duration revealed that a slow release of ONs from the acidic compartments of cells enables prolonged target silencing activity.17 Conjugation of GalNAc to ASOs results in significantly improved gene silencing activity in hepatocytes compared to unconjugated ASOs, as approximately 80% of the total drug in the liver is delivered to hepatocytes for GalNAc conjugates, in contrast to approximately 12% with the unconjugated ASOs.18 With hepatocyte targeted delivery, the ED50 for the naked ASOs ranges from 120 to 210 mg/week, whereas the ED50 for the GalNAc conjugated ASOs ranges from 4 to 10 mg/week.18 The increased potency gives an improved safety margin and allows less frequent dosing for GalNAc-conjugated drugs.

Toxicity of GalNAc-mediated drugs is generally caused by on-target side effects, chemical modifications, ON degradation products, and hybridization-mediated off-target effects, and the majority of the undesirable effects are nonadverse, often partially or completely reversible.1,19 However, the discontinued study of revusiran, the first-generation GalNAc conjugate targeting hereditary transthyretin-mediated amyloidosis.
dosis with cardiomyopathy, was a clinical setback. During a multicenter, randomized, placebo-controlled, double-blind phase 3 ENDEAVOR study, an imbalance in mortality in the revusiran arm was observed compared with placebo and further development of this compound has been discontinued. The underlying mechanism for the imbalance of mortality is not yet known. Comprehensive nonclinical safety assessments including safety pharmacology, acute and repeat-dose toxicity, genotoxicity, and carcinogenicity revealed that revusiran has a favorable nonclinical safety profile. However, unknown drug-mediated toxic effects cannot be excluded.

CURRENT PROGRESS OF GALNAC-CONJUGATED THERAPEUTICS

Recently, GalNAc-conjugated therapeutics targeting hepatocyte expressed target genes are progressing rapidly and emerged as a novel treatment approach for treating a variety of liver diseases with high unmet medical need. To date, GalNAc-based therapeutics feature prominently in the drug development pipelines of several pharma companies, and three GalNAc-based drugs have achieved their global approval and advanced to clinical applications over the past two years (Table 1).

The first registered GalNAc-conjugated ON drug is givosiran (GIVLAARI) from Alnylam Pharmaceuticals (Figure 2A). Givosiran was approved for use in the United States on November 20, 2019 and in the European Union on March 3, 2020 for the treatment of acute hepatic porphyria (AHP). AHPs are rare inherited disorders involving deficiencies in the pathway responsible for heme synthesis in liver hepatocytes. Upregulation of hepatic ubiquitously expressed s-aminolevulinate synthase I (ALAS1) is a common feature of all AHPs. The overexpression of ALAS1 combined with downstream enzyme deficiencies results in the overproduction and accumulation of toxic heme intermediates including δ-aminolevulinic acid (ALA), porphobilinogen (PBG) and porphyrins. The presenting complaint of AHP during the acute attack is usually abdominal pain accompanied with mainly nausea, vomiting, and constipation. The current goal of treatment for AHPs is to downregulate hepatic ALAS1 and thus reduce production and accumulation of the toxic byproducts. Givosiran is a synthetic double-stranded siRNA that covalently conjugates to three GalNAc residues (Figure 2D) and specifically targets ALAS1 mRNA in the hepatocytes. Preclinical studies in animals demonstrated that subcutaneous dosing with givosiran decreased ALAS1 transcript level in serum, urine and liver. In a phase 1 trial of givosiran in patients with acute intermittent porphyria, once-monthly injections of givosiran resulted in sustained reduction in ALAS1 mRNA, ALA, and PBG levels to near normal and largely mild adverse events. In a double-blind, placebo-controlled, phase 3 trial, a total of 94 patients were enrolled and randomly assigned (48 in the givosiran group and 46 in the placebo group) to receive either monthly givosiran (2.5 mg/kg) or placebo for 6 months. Treatment of givosiran demonstrated a 74% reduction of the mean annualized attack rate and led to lower levels of urinary ALA and PBG, fewer days of hemin use, and better daily scores for pain compared to placebo. Adverse events were more frequent in the givosiran group than in the placebo group. Treatment of givosiran resulted in higher serum aminotransferase and creatinine levels, reduced estimated glomerular filtration rate (<60 mL/min per 1.73 m²) and increased injection-site reaction rate. The mechanism for these changes is unknown.

The second registered GalNAc-based ON drug is lumasiran (OXLUMO) produced by Alnylam Pharmaceuticals (Figure 2B). Lumasiran approved in the EU on November 19, 2020 and in the USA on November 23, 2020 for the treatment of patients with primary hyperoxaluria type 1 (PH1). PH1 is an inherited, rare, life-threatening disease of glyoxylate metabolism resulting in increased production of oxalate due to the mutations of alanine-glyoxylate aminotransferase. Increased oxalate production results in formation and deposition of toxic calcium oxalate crystals mainly in the kidney and urinary tract causing serve multiorgan dysfunction. Lumasiran targets the mRNA of hydroxycid oxidase 1 gene (HAO1) encoding glycolate oxidase, the key enzyme involved in hepatic oxalate synthesis, and thereby inhibits the synthesis of oxalate with increased serum glycolate concentration. Preclinical studies in animals demonstrated that subcutaneous administration of lumasiran dose-dependently lowered the mRNA level of glycolate oxidase and reduced urinary oxalate concentration up to 50% after a single dose and up to 98% after multiple doses. In a randomized, double-blind, placebo-controlled multinational phase 3 ILLUMINATE-A study (N = 39, 54 months dosing period), treatment with lumasiran resulted in a 53% mean reduction in urinary oxalate relative to placebo. At month 6, the majority (84%) of patients in the lumasiran group achieved significant reduction of urinary oxalate levels. In a single arm, open-label, multinational phase 3 ILLUMINATE-B study in patients with PH1 under the age of six (N = 18), treatment with lumasiran led to a 72% mean reduction in spot urinary oxalate:creatinine ratio averaged across months 3–6. In addition, an ongoing phase 3 ILLUMINATE-C trial is evaluating the safety, efficacy, pharmacokinetics, and pharmacodynamics of lumasiran in PH1 patients with advanced renal disease including patients on dialysis, with an estimated study complete date on July 2025. The most common drug-related side effect of lumasiran is injection site reactions, and there were no serious or severe adverse events reported during the clinical studies, which is encouraging.

The third registered GalNAc-based ON drug is inclisiran (Leqvio) developed by Alnylam Pharmaceuticals and licensed to The Medicines Company that recently acquired by Novartis (Figure 2C). Inclisiran received its first global approval in the EU on December 11, 2020 for the treatment of adults with hypercholesterolemia or mixed dyslipidemia, alone or in combination with other lipid-lowering agents. It is known that high low-density lipoprotein-cholesterol (LDL-C) level significantly increases the risk of atherosclerotic cardiovascular disease. Most LDL-C is removed from the circulation by LDL receptors expressed on hepatocytes via receptor-mediated endocytosis. Proprotein convertase subtilisin-kexin type 9 (PCSK9), a serine protease mainly produced by hepatocytes, binds to LDL receptors and targets the receptor for lysosomal degradation. Inclisiran is a long-acting, synthetic siRNA conjugated to triantennary GalNAc units that targets PCSK9 mRNA. The reduction of hepatic PCSK9 results in increased LDL receptor levels and promotes the clearance of plasma LDL-C, thus reducing the incidence of cardiovascular risks.

In the double-blind, placebo-controlled phase 3 ORION 9 trial, 482 patients with heterozygous familial hypercholesterolemia were randomized 1:1 to inclisiran sodium (at a dose of 300 mg on days 1, 90, 270, and 450) or placebo. Subcutaneous dosing with inclisiran led to a 39.7% reduction of the LDL-C
level versus placebo. The ORION 10 and 11 phase 3 trials for inclisiran were randomized, double-blind, placebo-controlled study on over 3000 patients with atherosclerotic cardiovascular disease who had elevated LDL-C levels. Patients were randomly assigned in a 1:1 ratio to receive either inclisiran (284 mg) or placebo, administered by subcutaneous injection on day 1, day 90, and every 6 months thereafter over a period of 540 days. At day 510, inclisiran reduced LDL-C by 52.3% in the ORION-10 study and by 49.9% in the ORION-11 study. In all three phase 3 trials, no serious drug-related adverse events were reported after administration of inclisiran. The frequency of adverse event of inclisiran was similar to that of placebo groups, although more injection-site adverse events occurred with inclisiran than with placebo in three trials.

Furthermore, numerous promising GalNAc-based therapeutics are now in phase 3 clinical trials for liver diseases including hepatitis B virus infection, transthyretin-mediated amyloidosis, and α1-antitrypsin deficiency. Besides, more than 30 GalNAc-based ONs are in the early stages of clinical trials (Table 1). With this number of agents in the clinical development, one can imagine that a number of new GalNAc-conjugated therapeutics for a wide range of liver-based diseases will gain market authorization in the near future.

### CONCLUSION

Owing to its high potency, better tolerability, and excellent safety profile, ON therapy has potential to be a next-generation platform for drug discovery. The conjugation of GalNAc moieties to ONs is a great breakthrough and represents a powerful, long-lasting, and safe approach for liver-targeted delivery of siRNA and ASO therapeutics. Over the past few years, three GalNAc-conjugated drugs have registered, and a robust pipeline of GalNAc-conjugated therapeutics is progressing into clinic. However, the chemical structure as well as safety profile have yet to reach maturity. Further investigations of pharmacokinetics in patients and regulation mechanisms of ASGR-mediated uptake are ongoing. Taken together, GalNAc-based delivery systems open a world of possibilities for the development of potent and liver-targeted ON therapies for both rare diseases and common diseases from genetic disorders.

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**Notes**

The authors declare no competing financial interest.

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