# Intelligent Delivery of Oligonucleotide Drugs

In recent years, the development of RNA-silencing oligonucleotide therapies is emerging as a hot spot for new drug development. **Oligonucleotides can be mainly classified into antisense oligonucleotides(ASO), RNA interference (RNAi) and aptamer RNAs** according to their molecular types. Currently, several small interfering RNA (siRNA) and antisense oligonucleotide (ASO) therapies have been approved for marketing by the FDA. However, the delivery of this type of drug is still in urgent need of further breakthroughs.

Due to physicochemical properties, **unmodified free oligonucleotides are not only quickly cleared by the body after drug delivery, but also have the risk of off-target and toxic side effects.** Therefore, nucleic acid drugs require both chemical modification and suitable delivery systems to achieve therapeutic efficacy. Chemical modifications play an important role in the delivery of oligonucleotide drugs, but it is still difficult for small interfering RNA (siRNA) drugs to reach the target site through chemical modifications alone. Delivery of antisense oligonucleotide (ASO) drugs is also poor, making delivery systems critical for success in oligonucleotide drug development. At present, the leading companies in siRNA drug development have their own or licensed delivery technology platforms. **siRNA drug delivery systems are relatively mature in various types of nucleic acid drug development, and this article will discuss the delivery technology of nucleic acid drugs from the delivery systems of siRNA drugs.**

## Delivery System of Marketed siRNA Drugs

To date, **four siRNA therapeutics have been approved for commercial application**, including ONPATTRO®, GIVLAARI™, OXLUMO™ and Leqvio® .

| # | FDA Approval | Drug name | Company | Indication | Delivery System |
|---|---|---|---|---|---|

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|---|---|---|---|---|---|
| 1 | Aug 10, 2018 | Onpattro (Patisiran) | Alnylam Pharmaceuticals | Familial Amyloid Neuropathies | Lipid Nanoparticle Delivery Systems |
| 2 | Nov 20, 2019 | Givlaari (Givosiran) | Alnylam Pharmaceuticals | Acute Hepatic Porphyria | GalNAc-siRNA Conjugates |
| 3 | Nov 23, 2020 | Oxlumo (Lumasiran) | Alnylam Pharma | Primary hyperoxaluria type 1 | GalNAc-siRNA Conjugates |
| 4 | Dec 22, 2021 | Leqvio (Inclisiran) | Novartis | lower low-density lipoprotein cholesterol | GalNAc-siRNA Conjugates |

FDA Approved siRNA drugs

We can divide the in vivo process of siRNA drugs into three main phases, in each of which the body sets up a different biological barrier for siRNA drugs.

- **Arrival at the Target Tissue:** After administration, siRNA has to escape degradation by nucleases in plasma and tissues and capture by the immune system to arrive at the target tissue smoothly.

- **Entry into Cells:** Due to the large molecular weight and negative surface charge of the siRNA drug, it cannot pass freely through the cell membrane even if it arrives at the target tissue and needs to enter the cell through cytokinesis.

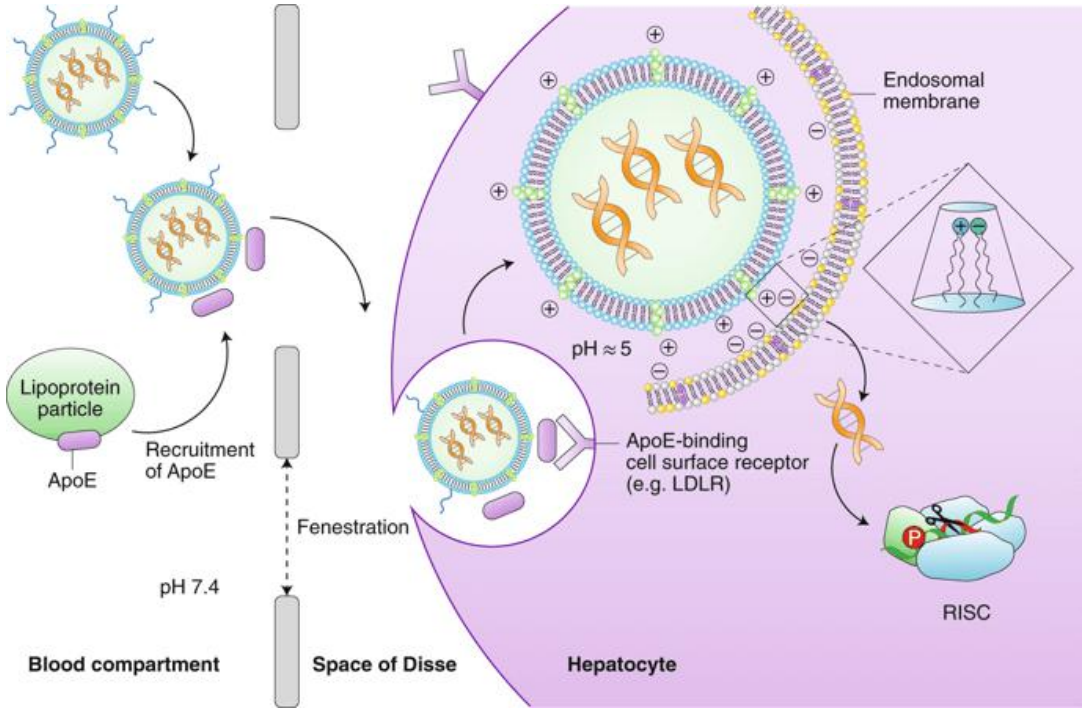
- **Endo-lysosomal Escape:** siRNA has to achieve escape before endosomal binding to lysosomes, entering the cytoplasm, and binding to the target mRNA to achieve gene silencing. [1]

The main mission and responsibility of the delivery system are to protect the siRNA to cross all biological barriers and reach the cytoplasm smoothly to bind to the target mRNA, which in turn silences the gene to exert its drug effect.

## Lipid Nanoparticle Delivery System

Due to the inherent charge characteristics of siRNA, cationic liposomes appear to be the best candidates for delivery systems. However, the toxicity of traditional cationic liposomes has hindered the development of siRNA drugs. Alnylam has brought a breakthrough in the successful delivery of oligonucleotides through the use of Arbutus' ionizable cationic lipids. **In 2018, Onpattro, the first siRNA drug using lipid nanoparticles as a delivery system, was approved for marketing for the treatment of polyneuropathy caused by hereditary transthyretin amyloidosis (hATTR).**

The key to the success of these lipid nanoparticle delivery systems is the use of **ionizable cationic lipids**. These lipid materials exhibit different charging characteristics at different pH conditions, being positively charged at acidic pH conditions and essentially neutral at physiological pH conditions. The ionizable properties of lipids provide intelligent protection for siRNA across the biological barrier [2] (Figure 1).




Integrated model of lipid nanoparticle (LNP)-mediated delivery of siRNA to hepatocytes in vivo. Image Source: Reference [2]

Firstly, the production process of siRNA drugs needs to be done at low pH conditions. During the production process, the ionizable lipids are positively charged to achieve a

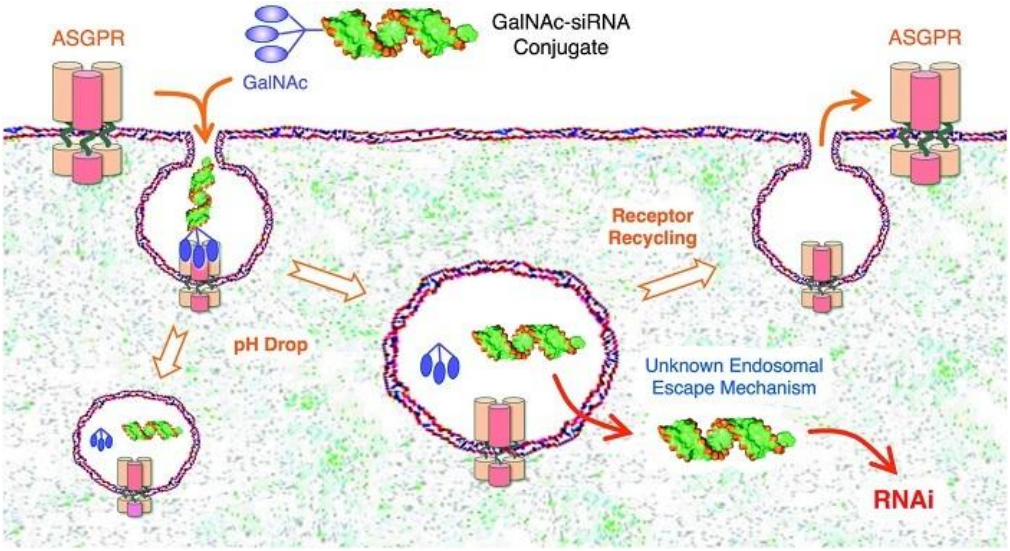
stable and optimal siRNA drug encapsulation rate. Liposomes with a particle size of 100 nm or smaller can be obtained by adding PEGylated lipids to the liposomes. The size of liposomes (20 nm-100 nm) can be regulated by varying the ratio of PEGylated lipids to other lipids in the liposome.

After administration, in plasma, the liposome delivery system presents an essentially neutral charge surface that, together with the PEGylated lipids, helps the siRNA drug to evade capture by the immune system and degradation by nucleases.

As the PEGylated lipids dissociate from the liposome surface, the siRNA-carrying liposomes enter the cell via endocytosis. Due to the low pH of endosomes, the ionizable lipids are gradually protonated and positively charged, thus destabilizing the structure of phospholipid bilayer and enabling the escape of siRNA endosomes. The escaped siRNA drug enters into the cytoplasm and binds to the target mRNA to achieve gene silencing.

## GalNAc-siRNA Conjugates

The delivery technology of GalNAc-siRNA conjugates has attracted much attention in recent years due to its efficient liver targeting and good safety. N-acetylgalactosamine (GalNAc) is a ligand for the asialoglycoprotein receptor (ASGPR), which is highly specifically expressed on the membrane surface of hepatocytes (approximately 500,000 ASGPR per cell). GalNAc-siRNA conjugates transport siRNA from the cell surface into cells under endocytosis by specifically binding to ASGPR. Subsequently, the GalNAc-siRNA conjugate is separated from ASPGR, ASPRGR returns to the cell surface and the GalNAc-siRNA conjugate is further dissociated, and the released free siRNA is effective in silencing genes in the cytoplasm (Figure 2) [3, 4] . The 3' end of the siRNA sense strand is combined with GalNAc with a trivalent structure through chemical bonds, and the stability of GalNAc-siRNA conjugates is enhanced by ESC (enhanced stabilization chemistry) technology to increase liver exposure and prolong gene sinking time [ 4,5].




GalNAc-siRNA conjugate delivery process, source: Reference [3]

# Effects of delivery systems on the pharmacokinetics of siRNA drugs

The delivery system escorts the siRNA drug from the site of administration all the way to the cell. Before leaving the delivery system, siRNA mostly exists as a complex with the delivery system. Therefore, the delivery system influences the in vivo process and biodistribution of siRNA drug**s. It has been shown that the delivery system can achieve targeting and effectively prolong the in vivo retention time of siRNA drugs by altering the tissue distribution characteristics of free siRNAs.**

**It is generally believed that lipid nanoparticle delivery systems have passive liver targeting, while GalNac-siRNA conjugated delivery systems achieve active liver targeting through the ligand GalNac of the asialoglycoprotein receptor.** Adjusting the structure, ratio, or particle size of each component in the delivery system, as well as modifying the oligonucleotide structure, may produce significant changes in the tissue distribution and gene silencing effect of siRNA drugs.

# Prospects of Oligonucleotide Drug Delivery Systems

At present, the indications of siRNA drugs that have been marketed or entered the clinical research stage are **mostly focused on liver or liver-related diseases**, which is inseparable from the successful development of liver-targeted delivery systems. **Expanding the indication field and effectively targeting other tissues and organs** is the focus of oligonucleotide drug development companies at this stage. It has been shown that siRNA can achieve distribution and release in different tissues such as heart, lung, fat, and muscle by coupling different lipids (saturated fatty acids, unsaturated fatty acids, cholesterol, vitamins, etc.).

There is no lack of commonality in the development of various nucleic acid drugs, and in recent years, delivery systems for siRNA drugs have also received attention from miRNA, ASO, mRNA and other nucleic acid drug development companies. By coupling with delivery systems, the efficacy of ASO can be significantly enhanced; several GalNac-ASO conjugated drugs have already entered the clinical research phase. Lipid nanoparticle delivery systems have also been successfully used by Pfizer and Moderna for the mRNA vaccine against COVID-19 .

# Conclusion

In 1978, Zamecnik and Stephenson published the first research on antisense oligonucleotides for therapeutic purposes, and 20 years later, in 1998, the first ASO drug was approved for marketing. In 1998, Fire and Mello discovered RNA interference, and again after 20 years, in 2018 the first siRNA drug was approved for marketing. It seems that with every 20 years, there is a breakthrough in the development of oligonucleotide drugs. At present, oligonucleotide drug development has entered a brand new 20 years, and with the continuous maturation of research technology for this type of drugs, it is

believed that these 20 years will be a brand new era of accelerated development of oligonucleotide drugs.

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References:

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