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REVIEW

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RNA nanomedicine in liver diseases

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Abstract

The remarkable impact of RNA nanomedicine during the COVID-19 pandemic has demonstrated the expansive therapeutic potential of this field in diverse disease contexts. In recent years, RNA nanomedicine targeting the liver has been paradigm-shifting in the management of metabolic diseases such as hyperoxaluria and amyloidosis. RNA nanomedicine has significant potential in the management of liver diseases, where optimal management would benefit from targeted delivery, doses titrated to liver metabolism, and personalized therapy based on the specific site of interest. In this review, we discuss in-depth the different types of RNA and nanocarriers used for liver targeting along with their specific applications in metabolic dysfunction-associated steatotic liver disease, liver fibrosis, and liver cancers. We further highlight the strategies for cell-specific delivery and future perspectives in this field of research with the emergence of small activating RNA, circular RNA, and RNA base editing approaches.

INTRODUCTION

Over the past few decades, nanomedicine has emerged as a personalizable solution to several diseases owing to its specific targeting capacity, stability, safety, and ability to deliver a wide range of drugs, proteins, and RNA machinery. Among these, the important role of RNA therapeutics during the COVID-19 pandemic cannot be unnoticed. The success

Abbreviations: Ψ, pseudouridine; 10-MWT, 10-meter walk test; AAT, alpha1-antitrypsin; AGO2, Argonaute 2; Apo, apolipoprotein; ASGPR, asialoglycoprotein receptor; CCA, cholangiocarcinoma; CEBPA, CCATT/enhancer binding protein alpha; circRNA, circular RNAs; ConA, Concanavalin A; CRLM, colorectal liver metastasis; DEN, diethylnitrosamine; DMN, dimethylnitrosamine; ECM, extracellular matrix; GalNAc, *N*-acetylgalactosamine; HA, hyaluronic acid; hATTR, hereditary transthyretin-mediated amyloidosis; HSP47, heat shock protein 47; IEM, inborn errors of metabolism; IGFIIR, insulin-like growth factor 2 receptor; IVT, in vitro–transcribed; LDLR, LDL receptor; LM, liver metastasis; LNP, lipid nanoparticle; M6P, Mannose 6 phosphate; miRNA, microRNAs; MMA, methylmalonic acidemia/aciduria; mNIS, modified Neuropathy Impairment Score; MUT, methylmalonyl-CoA mutase; ORF, open reading frame; OTC, ornithine transcarbamylase; OX40L, Oxford 40 ligand; PBAE, poly(β-amino ester); PBGD, porphobilinogen deaminase; PCBP2, poly(rC)-binding protein 2; PCC, propionyl-CoA carboxylase; PDGFR, patient-derived growth factor receptor; PEG, polyethylene glycol; PLK1, polo-like kinase 1; PNA, peptide nucleic acid; PR, partial response; QOL-DN, Quality of Life-Diabetic Neuropathy; RBP, retinol-binding protein; RISC, RNA-induced silencing complex; saRNA, small activating RNA; scAb, single-chain antibody; SD, stable disease; siRNA, small interfering RNA; SNV, single-nucleoside variant; sphk2, Sphingosine kinase 2; SR-B1, scavenger receptor class B type I; TKI, tyrosine kinase inhibitor; TTR, transthyretin; UOx, urinary oxalate; UTR, untranslated region.

of Pfizer-BioNTech and Moderna mRNA-lipid nanoparticle (LNP) COVID-19 vaccines has encouraged researchers to further investigate this niche of therapeutics in other diseases including liver diseases. RNA nanomedicine offers unprecedented advantages of dose titration and personalized therapy based on the patient's liver condition. RNA therapeutics in nanomedicine emphasize the feasibility of rapid optimization and therefore quicker clinical translation. Considering the example of the evolving COVID-19 variants, it was much easier for researchers to modify and optimize the RNA machinery to suit the respective variant of the virus without changing the formulation. This could not have been feasible with other drugs or therapeutic moieties considering the urgency of the situation. This can be potentially applied to treat liver diseases since there are specific signaling pathways either upregulated or downregulated during the disease pathogenesis and targeting core genes or proteins through RNA therapy could provide an optimal therapeutic outcome. Nevertheless, since RNA therapy can be modified without changing the formulation, this offers an unprecedented advantage for personalized treatment depending on the patient's transcriptomic profile. In addition, the unique attribute of most nanoparticles (NP) to inherently accumulate in the liver after systemic delivery possesses an unparalleled approach to liver targeting in these patients with chronic liver disease. This is attributable to the unique feature of lipids used during the synthesis of LNPs allowing liver delivery through their apolipoprotein (Apo) E-binding characteristic that offers a high affinity for LDL receptors (LDLRs) that are abundantly located in the liver. This strategy has been proven by the success of patisiran (Onpattro), a first-in-human FDA-approved RNA nanoformulation for the treatment of hereditary transthyretin-mediated amyloidosis (hATTR), which requires hepatocyte targeting to inhibit transthyretin (TTR), the protein responsible for the amyloidosis.[1–3]

Furthermore, nanomedicine permits dose titration based on individualized liver profiles by developing image-guided nanoformulations. For example, a team of researchers has developed porphysomes and porphyrin-based LNPs that show inherent fluorescence in vivo and are also capable of radio-labeling for effective image-guided therapy in the clinical setting.[4,5] These NPs also offer the advantage of encapsulating a wide range of cargos including RNA machinery and specific liver cell targeting for personalized therapy depending on the patient's needs. Henceforth, image guidance in liver disease treatment has the potential to assist clinicians in better managing the challenges of liver metabolism and its associated toxicities.

RNA MACHINERY FOR LIVER DISEASE TREATMENT

Liver diseases encompass a wide spectrum of conditions, stemming from diverse causes and intricate biological contexts, as well as progressing through varying stages. Consequently, the development of a customizable and universally applicable treatment modality holds tremendous promise in the realm of liver disease management. RNA therapy is a type of medical treatment that uses RNA molecules to modulate gene expression to treat diseases. It has the potential to treat a wide range of diseases, including cancers, infectious diseases, immune diseases, and genetic disorders.[6,7] Most importantly, the goal of RNA therapy is to alter the expression level of specific genes at the RNA or protein level in various liver diseases, without changing the corresponding DNA sequence. This can be achieved through various types of therapeutic RNA molecules (Figure 1), which can be divided into 2 main categories based on their function[8–12]: (1) target silencing, including ASOs, small interfering RNAs (siRNAs), and microRNAs (miRNAs) and (2) protein expression, mostly through mRNAs. Unlike gene editing, RNA therapies do not alter the underlying genetic code, but instead modulate gene expression levels at the RNA or protein level, making these treatments often less invasive, safer, and more reversible than other gene-based therapies.

Target silencing

ASO

ASOs are single-stranded oligonucleotides (~18–30 nucleotides) that bind to target mRNA or pre-mRNA with a complementary base pairing principle.[13,14] Diverse mechanisms of ASO to regulate mRNA expression have been explored, which could be divided into 3 main categories (Figure 1)[11,13,15]: (1) induce degradation of target mRNA sequence through complementary binding; (2) suppress translation of target mRNA through steric blocking; and (3) modulate alternative splicing events of pre-mRNA. The first mechanism is dependent on the endogenous degradation pathways of RNase H, which recognize and degrade the RNA strand of an RNA/DNA duplex (formed by ASO and its target mRNA sequence).[11] Importantly, as RNase H exists in both cytosol and nucleus, ASO can induce both mature mRNA and pre-mRNA degradation through this mechanism.[16] The second mechanism takes place in the cytosol, where ASO and the mRNA molecule form a stable complex that blocks the binding of ribosomes and thereby suppresses subsequent protein translation. The third mechanism occurs in the nucleus, where ASOs bind to the specific splice sites within the intron–exon boundaries of the pre-mRNA, resulting in either skipping or inclusion of the targeted exon/intron and ultimately restoring the normal splicing pattern of the mRNA and producing a functional protein.[14,17] Of note, for the latter two mechanisms, ASOs usually contain 2′

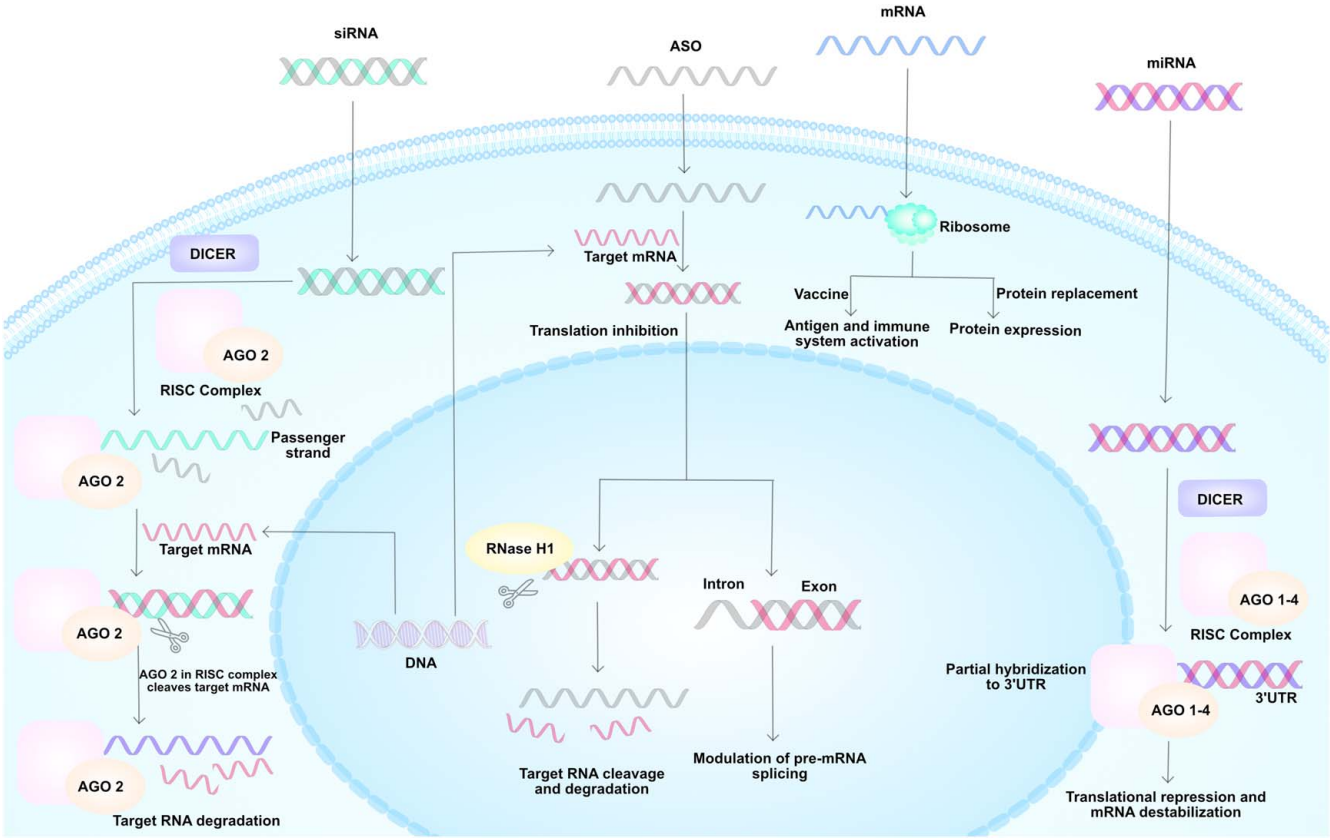

FIGURE 1 Mechanisms of action of different RNAs. Abbreviations: AGO2, Argonaute 2; miRNA, microRNA; RISC, RNA-induced silencing complex; siRNA, small interfering RNAs; UTR, untranslated region.

modified nucleotides (2′-*O*-methyl, 2′-*O*-methoxyethyl, and 2′-fluoro) or phosphorodiamidate-modified morpholino oligomers that prevent the hybrids of these ASO and cellular RNA from being recognized by RNase H and being cleaved.[18] Currently, 2 ASOs based on RNase H–mediated mRNA degradation mechanism have received regulatory approval for treating liver metabolism diseases: mipomersen targeting ApoB-100 for treating hypercholesterolemia (2013) and volanesoren targeting ApoC-III for treating familial chylomicronemia syndrome (2019).[19,20] These currently marketed ASO drugs further provide hope for the development of ASO NPs for the treatment of other chronic liver diseases.

Small interfering RNA

Unlike ASO, siRNAs are dsRNAs typically with 20–24 nucleotides in length (normally 21), which can silence complementary mRNA expression through the RNA interference pathway—a naturally occurring mRNA regulating process in the cell.[21] siRNAs function in the cellular cytosol and involve the following steps (Figure 1)[22]: first, the siRNA molecule is incorporated into the RNA-induced silencing complex (RISC) that unwinds the ds RNA and exposes the antisense strand. This strand is then used by the RISC to find and bind to complementary mRNA molecules. Once the siRNA–RISC complex binds to the target mRNA, the mRNA is cleaved by the RISC's endonuclease activity, resulting in the degradation of the target mRNA and inhibition of subsequent protein production. One of the key advantages of siRNA over other RNA-based therapies is its specificity since siRNA is almost always fully complementary to the target mRNA sequence. This specificity minimizes off-target effects and reduces the risk of toxicity, making it a more attractive approach for therapeutic use compared with other RNA-based therapies.[23–25] In addition, siRNA can be target-delivered to hepatocytes after chemical conjugation with the *N*-acetylgalactosamine (GalNAc) ligand.[26] This has led to the FDA approval of 4 drugs that treat liver-related diseases, including givosiran for acute hepatic porphyria (2019), inclisiran for hypercholesterolemia (2020), lumasiran for primary hyperoxaluria type 1 (2020), and vutrisiran for hATTR amyloidosis (2022).[27–30] Overall, siRNA stands as a very promising RNA therapeutic for treating liver diseases.

MicroRNA

Like siRNAs, miRNAs are also short nucleotides (21–25 bp) and they are naturally occurring molecules

in human cells.[31] Their immature form is processed by dicer and then loaded into RISC to initiate the subsequent cleavage of the mRNA target (Figure 1).[31] The major difference between siRNAs and miRNAs is that the former is mostly fully complementary to its mRNA target, whereas the latter binds to the target mRNA through partial bp matching [usually nucleotides (nt) 2–7 of the 5′ end].[25] Therefore, siRNA has a very high target specificity, while miRNA has a lower target specificity but enables regulating multiple mRNA expressions with the same miRNA sequence. In addition to inducing target mRNA cleavage, endogenous miRNA also has a translational repression function; therefore, they can also serve as drug targets and diagnostic disease biomarkers.[32] Due to the nonspecificity and diverse functional roles of miRNA, the clinical translation of miRNA as a therapeutic agent is much slower compared with siRNA. Currently, there are only 3 miRNA mimics that have entered clinical trials as therapeutic agents, 2 of which are still ongoing (phase I/II trials) while 1 was terminated (MRX 34). MRX 34 is a liposomal mimic of miRNA-34a developed to treat primary liver cancer and several other solid tumors. Although significant clinical activity was observed, the trial was closed early due to the serious immune-mediated adverse events that led to 4 patient deaths.[33] The exact cause of these serious immune-mediated adverse effects was unknown, and the researchers aim to investigate the biology circling these adverse effects, which would be helpful for future drug developments.

Protein expression

mRNA

Contrary to the abovementioned RNA that acts by silencing the target of interest, mRNA is a type of RNA molecule that acts as a blueprint to synthesize proteins in cells. It carries the genetic information from DNA to the ribosome, where it is translated into a functional protein (Figure 1). Under the context of RNA therapy, mRNA refers to in vitro–transcribed (IVT) synthetic RNA molecules with a length varying from a few hundred base pairs to up to 15 kb,[34] which are composed of 5′ cap, 5′-, and 3′-untranslated regions, the coding region, and the poly(A) tail. The crucial role mRNA plays in RNA therapy has led to 2 important treatment avenues[35,36]: (1) protein replacement therapy and (2) mRNA vaccination. For the former application, mRNAs are engineered to replace missing or malfunctioning proteins in the cells. For instance, a lack of ornithine transcarbamylase, a critical urea cycle enzyme in liver cells, can lead to high blood ammonia levels, which can cause diminished cognitive ability, seizures, coma, and death.[37,38] mRNA delivered through LNPs have been investigated in a phase II clinical trial to treat patients with ornithine transcarbamylase deficiency to restore normal ornithine transcarbamylase mRNA expression in the liver (NCT05526066). For the latter application as a vaccine, mRNA uses the host cell translational machinery to produce the targeted antigens in the cellular cytosol that are subsequently presented on the cellular membrane and activate immune cells, eliciting specific adaptive immune responses against infectious diseases and cancers.[34,39,40] As mentioned earlier, the remarkable potential of clinical translation of mRNA vaccines is highlighted by the success of Pfizer-BioNTech's BNT162b2 and Moderna's mRNA-1273 against COVID-19, which showed an extremely rapid timeline in less than a year from the genetic identification of the disease to the regulatory approval.[41] Currently, there are no ongoing mRNA vaccine clinical trials for treating liver diseases; however, preclinical studies in this emerging field of research have demonstrated the immense potential of using mRNA vaccines to protect against HCC and HCV, which could revolutionize the standard of care in liver disease management.[42–44]

As exogenous mRNA, therapeutic IVT mRNA can be recognized by cellular innate immune receptors. Although this property can be advantageous for mRNA vaccine application by providing an additional adjuvant effect, it can also negatively impact protein replacement therapy with an unexpected immune response and reduced mRNA translation.[36,45,46] To minimize the immunogenicity and improve the translation of IVT mRNA, many efforts have been made to optimize its structural elements. One of these elements is the 5′-cap of the mRNA, which is crucial for IVT mRNA translation. The 5′-cap protects the mRNA from degradation by exonucleases and facilitates its recognition by the ribosome.[47] Traditional 5′-capping methods mostly used cap 0, which makes the mRNA very recognizable by pattern recognition receptors that can trigger an undesired innate immune response by activating toll-like receptor 7, 8, RIG-I, or interferon-inducible proteins.[47–49] The latest cotranscriptional capping method such as CleanCap overcomes these limitations by using cap 1, a mimetic of the endogenous mRNA cap, thereby evading pattern recognition receptor recognition and inducing a much lesser innate immune response.[50] The cotranscriptional CleanCap capping also reduces purification steps, results in over 95% capping efficiency, and ultimately leads to improved mRNA translation. Besides 5′-cap, incorporating chemically modified nucleosides can also reduce the immunogenicity of mRNA. Karikó and colleagues first showed that pseudouridine (Ψ)-modification could enable IVT mRNA to resist intrinsic immune responses.[51–53] Ψ-modified mRNA plus HPLC purification achieved up to a 1000-fold increase in mRNA translation in primary human dendritic cells compared with unmodified, unpurified mRNA. Later, researchers

demonstrated that N1-methyl-pseudouridine modification is even better than Ψ in preventing the activation of innate immune sensors.[54,55] Andries and colleagues observed an up to 13-fold increase in luciferase mRNA translation when using a N1-methyl-pseudouridine modification compared with Ψ-modified mRNA in a mouse model through both intramuscular and intradermal injection. Considering the compromised liver metabolic function in many liver diseases, delivering modified mRNA to reduce mRNA immunogenicity and subsequent inflammatory responses in the liver microenvironment would be beneficial.

INTRODUCTION TO NANOCARRIERS FOR RNA DELIVERY

Despite the vast therapeutic potential of RNA-based therapeutics, the targeted and efficient delivery of RNA therapeutic molecules to in vivo gene targets remains a significant challenge. This is due to several key reasons. First, RNA molecules are highly unstable and prone to rapid degradation by ribonucleases in most bodily fluids and tissues.[56] In addition, RNA therapeutic molecules can be recognized by the immune system as foreign invaders, leading to the activation of inflammatory pathways and potentially causing adverse effects.[57] Finally, RNA therapeutic molecules are negatively charged and hydrophilic, which makes it challenging for them to cross cellular membranes and enter the target cells.[21] To address these challenges, researchers have developed several innovative and safe RNA delivery platforms to efficiently deliver RNA molecules to their intended sites of action as shown in Tables 1 and 2, and continue to discover the niche of nanocarriers including LNPs, GalNAc–RNA conjugates, and polymeric NPs for a safe and effective therapy in liver diseases.

Lipid nanoparticles

LNPs are the most advanced and well-established RNA delivery platform available today, with a proven track record of successful clinical translation, demonstrated by patisiran (Onpattro) for siRNA delivery,[1] as well as Pfizer-BioNTech COVID-19 vaccine (Comirnaty) for mRNA delivery.[73] Typically, LNPs are composed of 3 main components: an ionizable lipid, a diffusible polyethylene glycol (PEG)-lipid, and helper lipids (Figure 2). Each component plays a significant role during the RNA loading and delivery.

The ionizable lipids occupy ~50 molar percentage of total LNP components. They usually consist of hydrophobic lipid tails and ionizable headgroups that are typically tertiary amines. During RNA loading, the ionizable lipid headgroups are protonated in the acidic environment, making them positively charged and allowing them to interact with negatively charged RNA molecules. This electrostatic interaction enables ~90%–100% encapsulation of RNA molecules within the LNP.[74,75] Afterward, the LNPs are dialyzed against a neutral buffer to obtain a neutral surface charge, which is crucial for preventing the LNPs from inducing undesired immune responses following i.v. administration.[76] Following intracellular uptake of the LNPs, the ionizable lipids become positively charged in the acidic endosomes and this promotes fusion with anionic endosomal membranes, ultimately leading to cytosolic delivery of the RNA cargo.[77]

In addition, PEG-lipids are crucial in controlling the cellular uptake and transfection efficiency of LNPs, particularly in the liver. Following i.v. administration, the dense PEG layer on the LNP surface hinders the adsorption of serum proteins and the mononuclear phagocyte system, extending LNP circulation time in vivo.[78] However, the shedding of PEG-lipids is essential for subsequent ApoE binding and uptake by hepatocytes. This is largely impacted by the amount and length of PEG-lipids in LNPs. For instance, LNPs with 1.5% PEG-lipids have shown more efficient delivery of the RNA cargo to hepatocytes and significantly improved gene silencing efficacy compared with LNPs with 5% PEG-lipids, despite having shorter blood circulation time.[79] Moreover, LNPs with shorter PEG-lipids (C14 lipid tail) exhibit faster PEG shedding and 2.3-fold higher delivery efficiency to the liver compared with their C18 lipid tail counterparts after 24 hours of i.v. injection.[80]

Helper lipids, including sterols, phospholipids, and glycerolipids, are also essential components of LNP formulations and can have an impact on LNP delivery to the liver. For instance, preclinical studies have revealed that dioleoylphosphatidylethanolamine-containing LNP formulations have stronger interactions with ApoE and stronger mRNA delivery to the liver than identical LNP formulations that substitute dioleoylphosphatidylethanolamine with distearolyphosphatidycholine.[81]

LNP is an ideal RNA delivery system for treating liver diseases due to their liver tropism. Upon i.v. administration, ApoE has been identified as one of the key serum proteins that bind to LNPs, facilitating their subsequent binding to the LDLR expressed on the surface of hepatocytes, thereby enabling selective uptake of LNP into hepatocytes (Figure 3).[26,82,83] In fact, studies have demonstrated that $>80\%$ of LNPs can be distributed to the liver within just 6 hours of i.v. administration in mouse models.[79] In the liver, it is crucial to specifically target the cells of interest since different liver diseases involve specific liver cells, and nonspecific targeting could result in toxic accumulation and significantly lower therapeutic effect. Several recent studies have shown that LNPs can selectively deliver to KCs and hepatic endothelial cells over hepatocytes by

TABLE 1 Currently approved RNA nanotherapeutics for the treatment of liver diseases

Product	Carrier/RNA	Target cell/ gene	Indication	Route/treatment dose	Efficacy
Givosiran (2019)	GalNAc–siRNA	Hepatocytes/ ALAS1	Acute hepatic porphyrias	s.c./2.5 mg/kg, once monthly	A phase III randomized controlled trial involving 94 patients with symptomatic acute hepatic porphyria showed that givosiran reduced the annualized rate of composite porphyria attacks by 74% compared with placebo after 6 mo of treatment. In the givosiran group, the median percent reduction from baseline at 6 mo was 86% for urinary ALA levels and 91% for PBG levels[58]
Inclisiran (2020)	GalNAc–siRNA	Hepatocytes /PCSK9	Hypercholesterolemia	s.c./284 mg, as an initial dose, again at 3 mo, followed by a dose every 6 mo	In the inclisiran-only arm, LDL cholesterol was reduced by 47.5% at day 210 and this reduction was sustained over 1440 d (~4 y). The average mean reduction of LDL-C cholesterol over the 4-y period was 44.2%, with reductions in PCSK9 ranging from 62.2% to 77.8%[59]
Lumasiran (2020)	GalNAc–siRNA	Hepatocytes /glycolate oxidase	Primary hyperoxaluria type 1	s.c. /3 mg/kg monthly for first 3 mo, followed by 3 mg/kg every 3 mo[a]	For the lumasiran/lumasiran group (n = 24), a sustained reduction in 24-h UOx level was observed, with a mean reduction of 66.9% at month 6 and 64.1% at month 12. The placebo/lumasiran group (n = 13) showed a similar trajectory and magnitude of 24-h UOx reduction, with a mean reduction of 57.3% after 6 mo of lumasiran treatment[60]
Vutrisiran (2022)	GalNAc–siRNA	Hepatocytes/ transthyretin	hATTR amyloidosis	s.c. /25 mg, every 3 mo	Vutrisiran met the primary endpoint of change from baseline in mNIS +7 at 9 mo ($p = 3.54 \times 10^{-12}$), and all secondary efficacy endpoints. The drug reduced the Norfolk QOL-DN score by 16.2 points and increased the 10-MWT gait speed by 0.131 m/s[61]
Onpattro (2018)	LNP–siRNA	Hepatocytes/ transthyretin	hATTR amyloidosis	i.v./0.3 mg/kg, once every 3 wk[b]	The median reduction in serum TTR levels during the 18 mo was >80%. Onpattro was superior to placebo on mNIS +7, with a mean difference of −34.0 points (95% CI: −39.9, −28.1; $p < 0.00001$). The drug reduced the Norfolk QOL-DN score by 21.1 points[62]
Tegsedi (2018)	ASO	Hepatocytes/ TTR	hATTR amyloidosis	s.c. /300 mg, once a week	The patients treated with inotersen showed a 75% decrease in TTR levels by week 13, slowed the progression of nerve cell damage, and improved the overall quality of life[63]

Mipomersen (2013)	ASO	Hepatocytes/ ApoB-100	Familial hypercholesterolemia	s.c./200 mg, once a week	Phase III clinical trials showed a 28%–36% decrease in LDL-C levels in patients treated with mipomersen compared with a 13% increase in LDL-C levels in the patients administered with a placebo. No effect was observed on the HDL-C levels. A significant decrease in ApoB, TC, and Lp(a) levels was also noted[64]
Volanesorsen (2018)[c]	ASO	Hepatocytes/ ApoC-III	Familial chylomicronemia syndrome	s.c./300 mg, once a week	Phase III study results showed decreased mean apolipoprotein C-III levels by 84% and 83% after 3 and 6 mo after therapy, respectively. In contrast, patients treated with the placebo showed 6.1% and 5.2% increase in ApoC-III levels 3 and 6 mo after treatment. Also, a 77% decrease in triglyceride levels was observed in patients treated with volanesorsen as opposed to an 18% increase in patients treated with a placebo[65]

[a]For patients weighing $\geq$20 kg.
[b]For patients weighing <100 kg. If >100 kg, recommending 30 mg.
[c]Drug is EU-approved, but not yet FDA-approved.
Abbreviations: ApoB, apolipoprotein B; GalNAc, *N*-acetylgalactosamine; hATTR, hereditary transthyretin-mediated amyloidosis; mNIS, modified Neuropathy Impairment Score; 10-MWT, 10-m walk test; QOL-DN, Quality of Life-Diabetic Neuropathy; siRNA, small interfering ribonucleic acid; TTR, transthyretin; UOx, urinary oxalate.

TABLE 2 Selected RNA nanotherapeutics under clinical trials for the treatment of liver diseases

Product	Indication	Gene target	Type and route	Phase	Clinical trial identifier	Efficacy
ALN-HSD/Alnylam	NASH	HSD17β13	GalNac–siRNA, s.c.	II	NCT05519475	Ongoing study. No results posted yet
ALN-PNP/Alnylam	NASH	PNPLA3	GalNac–siRNA, s.c.	I	NCT05648214	Ongoing study. No results posted yet
ION224/Ionis	NASH	DGAT2	GalNac–ASO, s.c.	II	NCT04932512	Ongoing study. No results posted yet
AZD2693/AstraZeneca	NASH	PNPLA3	GalNac–ASO, s.c.	II	NCT05809934	Ongoing study. No results posted yet
LY3849891/Eli Lily	NASH	PNPLA3	GalNac–siRNA, s.c.	I	NCT05395481	Ongoing study. No results posted yet
TKM-080301/Arbutus	Liver cancer	PLK1	LNP–siRNA, i.v.	I	NCT01437007	Well tolerated between 0.3 and 0.6 mg/kg in patients with advanced HCC; single-agent TKM-080301 did not demonstrate clinically meaningful antitumor activity[66]
ALN-VSP02/Alnylam	Liver cancer	VEGF and KSP	LNP–siRNA, i.v.	I	NCT00882180	Well tolerated between 0.1 and 1.5 mg/kg; 8.3% (1 of 12 pt) at doses $\leq$0.4 mg/kg had SD for at least 2 mo compared with 46.6% (7/15) with SD (n = 6) or PR (n = 1, endometrial cancer with liver metastases) at doses $\geq$0.7 mg/kg[67]
DCR-MYC/Dicerna	HCC	MYC	LNP–siRNA, i.v.	I/II	NCT02314052 (terminated)	No results posted yet
MTL-CEBPA/Mina Alpha	HCC	C/EBP-α	LNP–saRNA (+Sorafenib), i.v.	I/II	NCT02716012, NCT04710641	Well tolerated up to 130 mg/m^2; 4% (1 of 24) had a PR to HCC for over 2 y and 50% (12 of 24) had SD. After discontinuation of MTL-CEBPA, 7 patients were treated with TKIs; 3 patients had a complete response with 1 further PR; and 2 with SD[68]
VIR-2218/Vir Biotechnology	Chronic hepatitis B	HBV mRNAs	GalNac–siRNA, s.c.	II	NCT03672188 NCT04507269	In a 48-wk trial, VIR-2218 was dosed between 20 and 200 mg. Across the treatment arms, 70.8% of patients had more than 1 log IU/mL HBsAg reduction (70.6% of which achieved HBsAg below 100 IU/mL)[69]
AB-729/Arbutus	Hepatitis B	HBsAg	GalNac–siRNA, s.c.	II	NCT04980482	Tested at doses of 60 or 90 mg and dosing intervals of 4, 8, or 12 wk for 24 wk. HBsAg reduction ranged from 1.86 to 2.16 log IU/mL across different treatment arms and lasted for up to 48 wk. HBsAg reduction was associated with lower circulating HBV RNA and HBsAg isoforms, as well as increased HBV-specific T-cell activation markers[69]
Fazirsiran/Takeda and arrowhead	AAT deficiency	AAT	GalNac–siRNA, s.c.	III	NCT05677971	A median reduction of 83% in liver Z-AAT concentrations at week 24 or 48. A reduction of ~90% in serum Z-AAT concentrations at the nadir. A reduction in histologic globule burden (a measure of Z-AAT accumulation in the liver) from a mean score of 7.4 at baseline to 2.3 at week 24 or 48. Reductions in liver enzyme concentrations (a marker of liver inflammation and damage) in all cohorts. Fibrosis regression in 7 of 15 patients and fibrosis progression in 2 of 15 patients after 24 or 48 wk[70]

Fitusiran/Sanofi	Hemophilia A and B	Antithrombin	GalNac–siRNA, s.c.	III	NCT03417245	The estimated mean annualized bleeding rate was significantly lower in the fitusiran prophylaxis group [3.1 (95% CI: 2.3–4.3)] than in the on-demand clotting factor concentrates group [31.0 (21.1–45.5); rate ratio 0.101 (95% CI: 0.064–0.159); $p < 0.0001$]. In the fitusiran group, 40 (51%) of 79 treated participants had no treated bleeds compared with 2 (5%) of 40 participants in the on-demand clotting factor concentrates group. Treatment-emergent serious adverse events were reported in 5 (6%) participants in the fitusiran group[71]
ARCT-810/Arcturus	OTC deficiency	OTC mRNA	LNP–mRNA, i.v.	II	NCT05526066	Ongoing study. No results posted yet
AKCEA-APO(a)-LRx/Akcea, Ionis, Novartis	Cardiovascular disease	Lipoprotein A	GalNac–ASO, s.c.	II	NCT03070782	Dose-dependent decreases in lipoprotein(a) levels were achieved[72]: 35% at a dose of 20 mg every 4 wk, 56% at 40 mg every 4 wk, 58% at 20 mg every 2 wk, 72% at 60 mg every 4 wk. 80% at 20 mg every week, 6% with placebo

Abbreviations: AAT, alpha1-antitrypsin; CEBPA, CCATT/enhancer binding protein alpha; GalNAc, *N*-acetylgalactosamine; LNP, lipid nanoparticle; OTC, ornithine transcarbamylase; PLK1, polo-like kinase 1; PR, partial response; SD, stable disease; siRNA, small interfering ribonucleic acid; TKI, tyrosine kinase inhibitor.

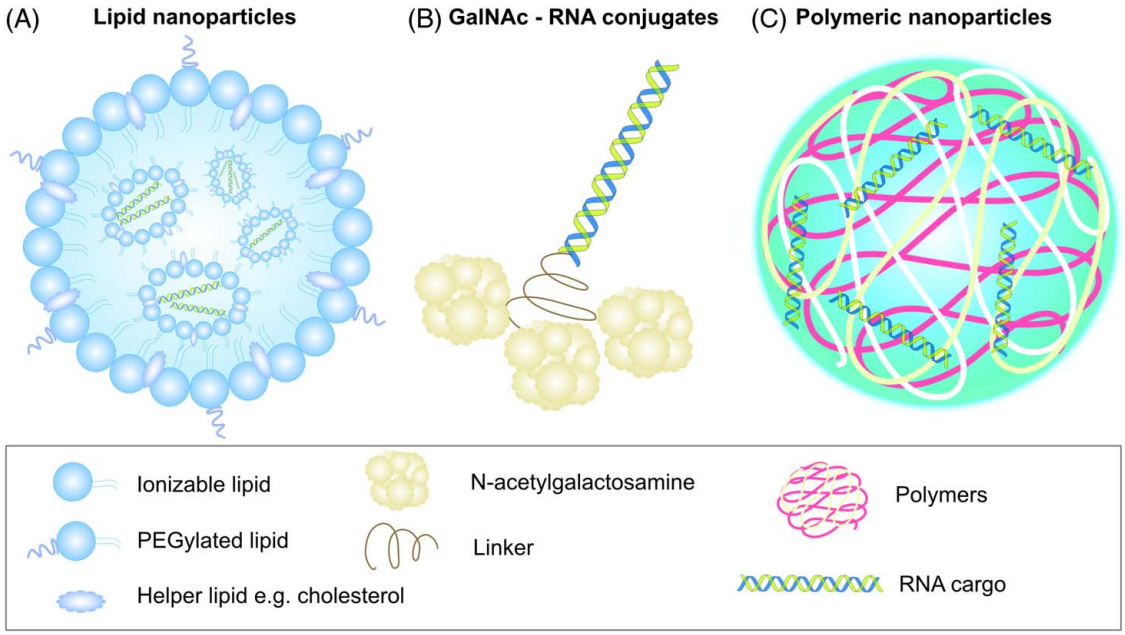

FIGURE 2 Different types of RNA nanocarriers and their compositions. (A) Lipid nanoparticles are composed of three main components: an ionized lipid, a diffusible PEG-lipid, and helper lipids. (B) GalNAc conjugates are composed of an RNA molecule such as siRNA or ASO that is chemically linked to a N-acetylgalactosamine molecule. (C) Polymeric nanoparticles are composed of polymers with ionizable amino groups enabling stable complex formations with RNA through electrostatic interactions. Abbreviations: GalNAc, *N*-acetylgalactosamine; PEG, polyethylene glycol.

changing ionizable lipids and the type of cholesterol in LNPs.[84–86] The size of LNPs can also influence their delivery in the liver. Clinically relevant LNPs typically have a small size of <60 nm. Since the average diameter of liver endothelial fenestrae is 107 ± 1.5 nm,[87] LNPs with smaller sizes can easily pass through and be selectively taken up by hepatocytes.[1,79] Conversely, larger LNPs may have a greater propensity to accumulate in other cells in the liver, such as KCs and LSECs, which is helpful for a therapy-targeting liver fibrosis. Another strategy to enable LNP delivery to specific cell types in the liver is to engineer the LNP with surface-targeting ligands. For example, LSECs express high levels of mannose receptors on their surface, which can be used to selectively deliver LNP-mRNA to these cells by modifying the

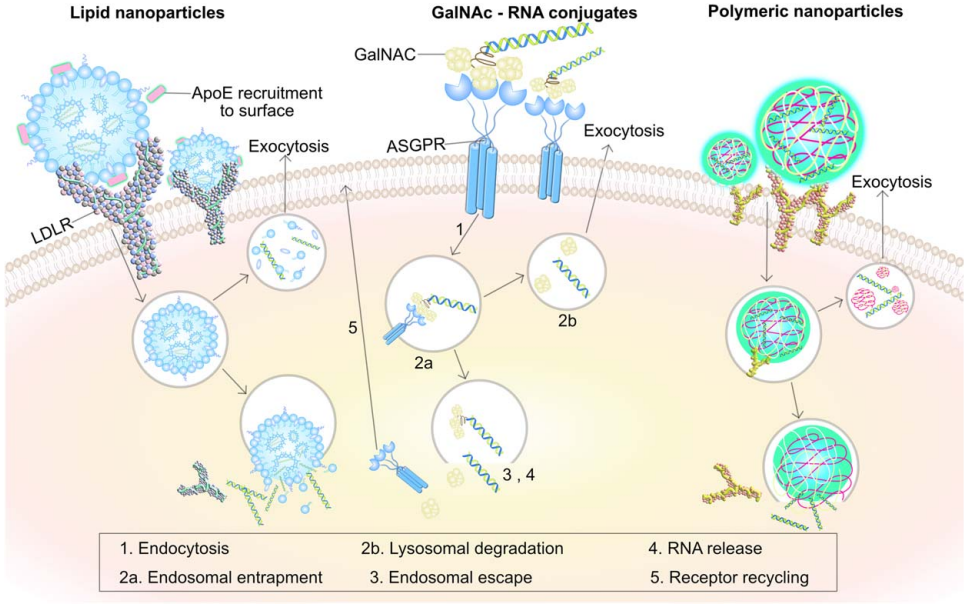

FIGURE 3 Mechanisms of intracellular delivery of various nanocarriers. Abbreviations: ApoE, apolipoprotein E; ASGPR, asialoglycoprotein receptor; GalNAc, *N*-acetylgalactosamine; LDLR, LDL receptor.

LNP surface with mannose.[88] Also, by adding a targeting peptide, LNPs have exhibited higher selectivity to HCC cells over normal liver tissues in an in vitro study.[89] Overall, the growing field of LNPs could accelerate the current challenges in liver disease treatment.

GalNAc–RNA conjugates

Similar to LNPs, GalNAc–RNA conjugates have emerged as another highly promising class of RNA therapeutics that have demonstrated remarkable clinical success in treating liver diseases. Currently, 4 drugs based on GalNAc–RNA conjugates have entered the clinical market. These conjugates are composed of an RNA molecule, such as siRNA or ASO, chemically linked to a GalNAc molecule (Figure 2).[90] The GalNAc molecule acts as a highly efficient ligand for the asialoglycoprotein receptor (ASGPR), which is abundantly expressed on hepatocytes in the liver, with up to 500,000 copies per cell.[91] After the internalization of GalNAc–RNA conjugates by hepatocytes through the ASGPR-mediated endocytosis pathway, the receptor–ligand complex is transported to early endosomes and the ASGPR subsequently recycles back to the cell surface for further uptake of GalNAc–RNA conjugates (Figure 3).[92] Notably, the ASGPR has a rapid recycling rate of <20 minutes.[93] Together with its abundant expression on hepatocyte surfaces, ASGPR enables efficient capture of circulating GalNAc-conjugated siRNA or ASO by hepatocytes.

Despite the strong binding affinity between GalNAc–RNA and hepatocytes, it is necessary to improve the stability and half-life of the RNA molecule in the bloodstream to allow a longer therapeutic window and increase therapeutic efficacy. Thus, several chemical modifications have been applied to GalNAc–RNA conjugates to increase RNA stability during circulation, such as 2′-*O*-methylation,[94] phosphorothioate linkage,[95] and 2′-fluoro modification.[96] These modifications have improved RNAs' resistance to nucleases and binding affinity to their target.

The advancements in GalNAc–RNA chemical modification have significantly reduced the frequency of dosing and enabled s.c. injection. All 4 currently approved GalNAc conjugates are GalNAc–siRNA and are administered s.c. for treating liver-derived diseases. Givosiran is the first GalNAc–siRNA conjugate that was approved by the FDA in 2019, which targets ALAS1 that is involved in acute hepatic porphyria.[27] In a phase III trial, givosiran reduced the annualized rate of porphyria attacks by 74% compared with placebo, and its effects lasted for up to 6 months after the last dose.[58] Similarly, inclisiran, which targets liver PCSK9 mRNA for treating hypercholesterolemia, received FDA approval in 2020. It is recommended to administer a single s.c. injection initially, again at 3 months, and then every 6 months for maintenance.[28] Phase III trials conducted at month 17 demonstrated that inclisiran effectively and sustainably reduced LDL-C by up to 52% compared with placebo and had a safety profile comparable with placebo.[97,98]

Polymeric NPs

Polymeric NPs have emerged as another promising platform for RNA delivery, owing to the diverse chemical structures of polymers that enable tunable biocompatibility, biodegradability, enhanced targeting, and controlled release of RNA payloads.[99–101] Various types of polymers have been investigated for RNA delivery, including synthetic polymers such as branched dendrimers,[102] polyethyleneimine,[103] and poly(β-amino ester)s (PBAEs),[104] and natural polymers such as chitosan and hyaluronic acid.[105,106] These polymers share a common feature of possessing ionizable amino groups that enable the formation of stable complexes with RNA through electrostatic interactions between the positively charged amino groups on the polymer and the negatively charged phosphate groups on the RNA backbone (Figures 2 and 3).

Du et al[107] have used PBAEs to deliver siRNA to silence a newly identified oncogenic circRNA that is significantly upregulated in HCC and is often associated with poor survival in patients with HCC. Their PBAE–siRNA NP formulation effectively inhibited tumor progression in 4 different liver cancer mouse models, including s.c., metastatic, orthotopic, and patient-derived xenograft models, without any observed adverse effects. Recently, another study also used PBAE to deliver a plasmid that only encodes the therapeutic enzyme in the presence of alpha fetoprotein that is highly expressed in HCC cells. This enzyme can further activate the prodrug ganciclovir to kill HCC cells, without causing toxicity to the adjacent healthy liver tissues due to minimal enzyme translation in these cells.[108]

Despite the potential advantages of polymeric NPs as RNA delivery systems for liver disease therapy, none of these platforms have yet entered clinical trials. This is largely due to concerns over the toxicity and biodegradability of polymers. In contrast to LNPs, which closely mimic the biological membranes and can be metabolized and cleared by the body, the high molecular weight and rigid backbone of some polymers may hinder their clearance and promote accumulation in tissues potentially leading to chronic toxicity.[109] Further optimization of their biocompatibility and biodegradability is needed to ensure their safety and efficacy for clinical use.

CURRENT APPLICATIONS OF RNA MEDICINE IN LIVER DISEASES AND HOW NANOTECHNOLOGY CAN BE USED FOR LIVER TARGETING

Success stories of RNA nanomedicine in liver diseases

So far, RNA nanomedicine has achieved exceptional success, particularly in 2 liver diseases: hATTR and alpha1-antitrypsin (AAT) deficiency. In hATTR amyloidosis, liver targeting is required for therapeutic effect since this is a systemic disorder caused by mutations of the TTR gene (chromosome 18q11.2-12.1) in the liver. Such mutations cause the synthesis of misfolded TTR proteins and their deposition as amyloid fibers in various extracellular tissues.[110–112] This further leads to motor, sensory, and autonomic neuropathies that cause cardiac, ocular, renal, and gastrointestinal complications with a fatal outcome within 10 years of disease onset. For a long time, liver transplantation remained the first specific and only treatment option for hATTR until a breakthrough in RNA nanomedicine. In 2018, the first RNA nanomedicine Onpattro (patisiran) was approved by the FDA for the treatment of hATTR. Onpattro acts by inhibiting TTR protein synthesis by delivering LNP–siRNA to hepatocytes.[1–3,113] As a result of the preferential hepatocyte uptake of LNPs and the high specificity of siRNA, patisiran showed exceptionally improved clinical activity during its clinical studies with $>80\%$ of TTR gene knockdown and excellent safety profile with no drug-related adverse effects when the patients received a dose of 0.15–0.5 mg/kg through i.v. administration.[1] These LNPs act through LDLR binding on hepatocytes for their internalization through endocytosis and release siRNA into the cytoplasm for its activity.[1]

Furthermore, AAT deficiency is the most common genetic disease of the liver.[114,115] AAT is a serine protease inhibitor encoded by SERPINA1, which is produced in hepatocytes and secreted systematically. In AAT deficiency, a mutant AAT protein called Z-AAT is produced, which accumulates in hepatocytes, leading to liver fibrosis. The major role of AAT is to protect the lung tissue against enzymatic attack by neutrophil elastase. Any mutations causing the retention of mutated AAT in the liver could lead to a toxic "gain of function" liver disease such as neonatal hepatitis or fibrosis, further progressing to cirrhosis and HCC. Conversely, the deficiency of normal AAT leads to a "loss of function," further causing proteolytic lung damage and panlobular emphysema. To date, the only therapy for "loss of function" AAT deficiency is the administration of i.v. plasma-purified AAT, while the treatment for panlobular emphysema is the same as the treatment for chronic obstructive pulmonary disease.[114,115] On the other hand, there is no specific treatment for liver diseases caused by AAT "gain of function," which results from the accumulation of Z-AAT protein in hepatocytes. Recently, fazirsiran, an siRNA conjugated to GalNAc nanotherapy, has been shown to reduce new Z-AAT synthesis and hence the toxic accumulation of the mutated AAT protein in hepatocytes, further allowing the native liver-restoration processes to function in clinical studies.[70] Although treatment with fazirsiran showed excellent AAT gene knockdown, it did not clinically benefit the patients to prevent regression of fibrosis, and therefore, larger studies are still required to confirm the efficacy of fazirsiran on fibrosis.[116–118] Despite this, the potential of RNA nanotherapy cannot be overlooked and it continues to represent an emerging therapeutic option for liver diseases. Also, the potential of nanotherapy to combine two or more RNA moieties into a single formulation could harness the current challenge with fazirsiran by co-delivering RNA to knock down AAT as well as a core gene or protein involved in liver fibrosis in future studies. Overall, these strategies suggest that LNPs with different RNA machinery can be designed, developed, and optimized for safe and effective liver targeting in different liver diseases.

Liver fibrosis and cirrhosis

Liver fibrosis is a wound-healing response to liver inflammation and injury, which can further progress to cirrhosis and HCC with currently no approved treatment options.[119] Although the uptake of drugs into the liver is usually high, this is not the case in a fibrotic liver.[120] Drug targeting to fibrogenic cells in a fibrotic liver is relatively low and often associated with off-target effects due to compromised hepatic metabolism and excretion, and therefore, researchers are currently in dire need of a breakthrough to safely treat liver fibrosis, which could also reduce its progression to cirrhosis and HCC.[121]

A key event during liver fibrosis is the activation of quiescent HSCs, and therefore, strategies to specifically target these cells are necessary. Furthermore, activated HSCs differentiate into myofibroblasts and lead to excessive extracellular matrix (ECM) secretion and accumulation at all stages of fibrosis and cirrhosis. The accumulation of ECM further causes sinusoidal remodeling by reducing the fenestrations on sinusoidal endothelial cells, and this in return hinders the normal bidirectional metabolic exchange between the hepatic portal vein and hepatocytes along with reduced passive transport of therapeutic drugs as depicted in Figure 4.[122–127] Moreover, this also limits and compromises the RNA-based drug delivery to HSCs for the treatment of liver fibrosis. For RNA therapies to exert their therapeutic effect, NPs need to be taken up by the HSCs and should be capable of cytosolic RNA release. However, in liver fibrosis, the accumulation of ECM

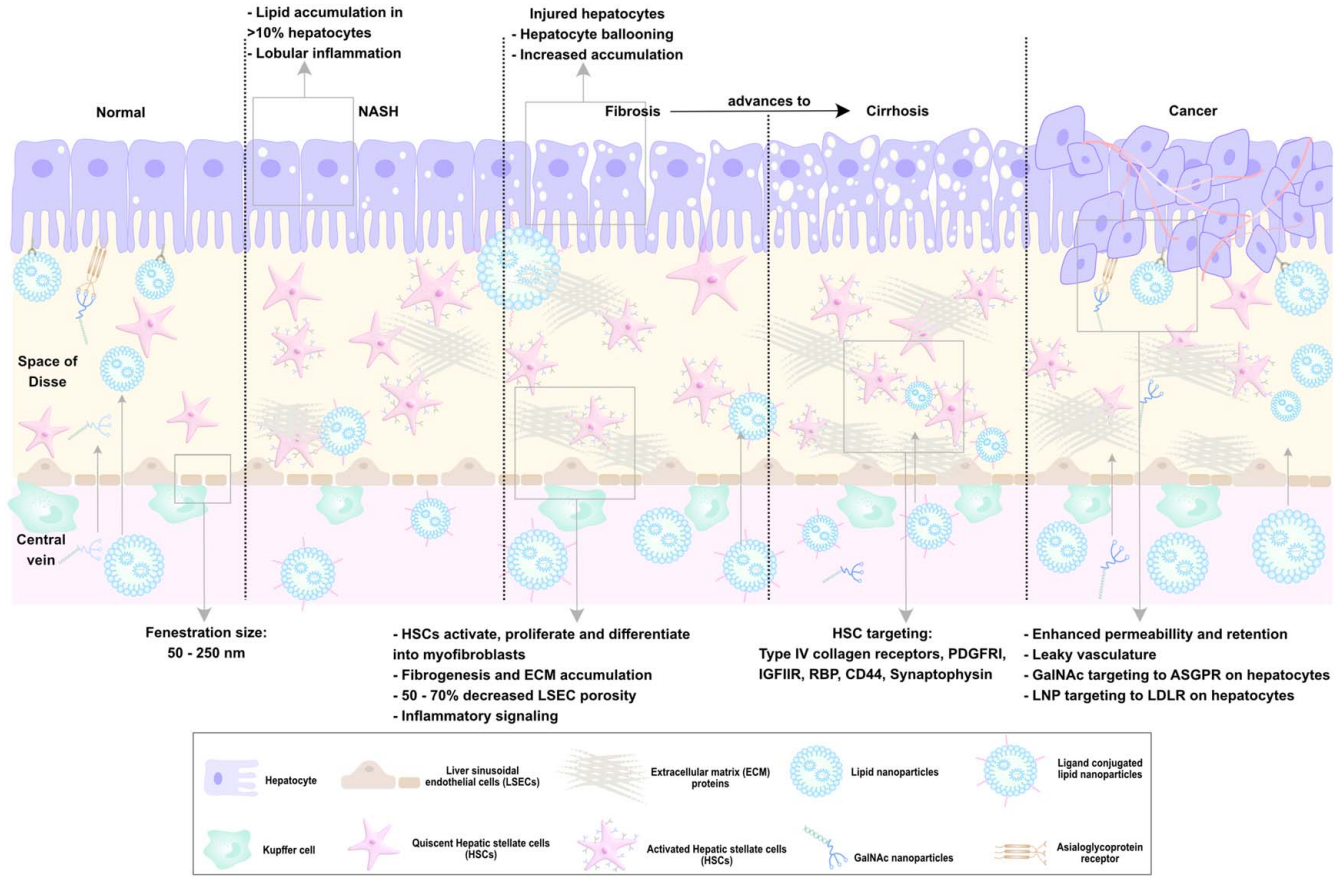

FIGURE 4 Changes in liver microenvironment depending on the liver disease and how nanomedicine can be specifically targeted. Abbreviations: ASGPR, asialoglycoprotein receptor; ECM, extracellular matrix; GalNAc, *N*-acetylgalactosamine; IGFIIR, insulin-like growth factor 2 receptor; LDLR, LDL receptor; PDGFR, patient-derived growth factor receptor; RBP, retinol-binding protein.

hinders and limits the overall NP transport; hence, there is reduced or low NP uptake by the HSCs. Also, considering the stability of the RNA nanoformulations, ECM hindrance can delay the overall cellular transport and lead to the degradation of the RNA nanotherapies. It is therefore of utmost importance to consider these limitations, and therefore, researchers are attempting to target ECM reduction as a potential treatment strategy irrespective of the stimuli causing the disease.[128–130] In return, a successful ECM reduction therapy would not only decrease these hindering ECM fenestrations but also increase cellular uptake of RNA-based therapies for a more stable and efficient therapeutic response in liver fibrosis.

To overcome the current challenges in developing a safe and effective antifibrotic agent, RNA nanomedicine has the potential to specifically target these cells safely and effectively. This is because RNA nanotherapeutics can be designed to specifically target genes or proteins, allowing precise and selective treatment. In response, this approach minimizes any off-target effects, reducing the risk of side effects and improving safety. Also, RNA nanotherapeutics can easily be modified based on the patient's disease state to enhance biodistribution and cellular uptake, thereby increasing therapeutic efficacy.

Recently, Mitchell and colleagues investigated heat shock protein 47 (HSP47) as a novel target for antifibrotic therapy.[131] As mentioned, liver fibrogenesis occurs from the collagen deposition in the ECM, which is mainly produced by HSCs.[119,132] Studies have shown that quiescent HSCs are activated and transdifferentiated into proliferative, profibrogenic, and contractile myofibroblasts during liver injury.[132] These further secrete excessive ECM components, especially collagen that specifically requires HSP47 for its secretion into the ECM.[133,134] Therefore, in this study, LNPs with siRNA against HSP47 were used to reduce collagen production and hence alleviate fibrosis. This was achieved by identifying and optimizing AA-T3A-C12 from a library of anisamide ligand-tethered lipidoids that showed high potency and selectivity to deliver RNA to activated HSCs. Surprisingly, this AA-T3A-C12 lipidoid-synthesized LNPs also showed improved RNA delivery and transfection in HSCs compared with the FDA-approved MC3 ionizable lipid.[131] This is due to the high affinity between anisamide ligands and the overexpressed sigma receptors located on the activated HSCs. Interestingly, the study results also demonstrated that 65% silencing efficacy was achieved with HSP47 siRNA LNPs along with a significant

reduction of collagen deposition and alleviation of liver fibrosis without any noticeable toxicity. These observations are striking and further illustrate that a lipid nanovehicle has the potential to successfully deliver an RNA cargo to the fibrotic liver and holds promise for future antifibrotic drug development research.

Subsequently, while liver fibrosis is still considered a reversible phase of chronic liver injury, depending on the cause of liver damage, environmental factors, and host factors, it becomes inevitably difficult to manage liver fibrosis once it advances to the end stage, also known as cirrhosis. Cirrhosis is characterized by sinusoidal capillarization and hepatocyte islands surrounded by fibrotic septa that are devoid of a central vein.[135] Cirrhosis often impairs liver function with some common complications such as variceal bleeding, ascites, encephalopathy, and spontaneous bacterial peritonitis. Currently, there is no treatment to reverse cirrhosis, and liver transplantation is the only available option for selected patients. However, the elimination of the triggers leading to cirrhosis could probably delay disease progression and reduce further advancement to HCC.

The inclusion of ligands that could target HSCs also confers a high degree of specificity for an antifibrotic agent. Among these, vitamin A has emerged as a promising targeting ligand on nanoformulations for RNA delivery to HSCs. This is by virtue of HSCs being the primary storage site of vitamin A.[129] Studies have shown that vitamin A–conjugated NPs are preferentially taken up by HSCs through their affinity for retinol-binding proteins and are most often used as an anticirrhotic therapy in vivo. Sato et al[136] demonstrated the therapeutic effect of vitamin A–coupled liposomes containing siRNA against a homolog of HSP47 in 3 animal models of liver cirrhosis and the results indicated that vitamin A–coupled liposomes had remarkable uptake into HSCs and further showed significant reduction in liver fibrosis. It was observed that within the cirrhotic rat liver, these vitamin A–coupled liposomes occupied 61.2% of the region stained for α-SMA (indicator for activated HSCs), while unconjugated liposomes showed negligible distribution of 5.6% in α-SMA–positive regions. In addition to this, cirrhotic rats treated with vitamin A–coupled siRNA liposomes showed a dose-dependent increase in survival time reaching a maximum of 83.3% at 0.75 mg/kg i.v. twice per week and 100% when the same dose was administered thrice per week. Anticirrhotic activity was determined by analyzing the collagen deposition in liver sections. Results depicted a 3-fold and 4-fold decrease in the percentage of area positive for collagen I in vitamin A–treated siRNA liposomes as compared with nonconjugated siRNA liposomes and saline control-treated mice, respectively. Furthermore, vitamin A–conjugated siRNA liposome treatment significantly reduced the serum bilirubin, albumin, and alanine aminotransferase levels in cirrhotic rats to a normal rate after 70-day treatment suggesting an improvement in liver functioning.[136] Apart from vitamin A, various other HSC-specific receptors and ligands have also been identified as depicted in Tables 3 and 4. This approach shows proof-of-concept of using cell-specific ligands for developing anticirrhotic therapy. In addition to this, NPs also permit dose titration of the RNA therapeutic considering the severely impaired metabolic function of the cirrhotic liver. NPs can be conjugated to radioisotopes as well as other imaging conjugates to image-guide and quantitatively adjust the treatment provided.[165–168] This allows to manage the therapeutic dose depending on individualized liver function for optimal outcomes while preventing toxicity due to reduced metabolism.

Metabolic dysfunction–associated steatotic liver disease

Although research advances over the past few decades have enhanced our understanding of the disease etiology, pathophysiology, and molecular pathways involved, there are currently no treatment options for metabolic dysfunction–associated steatotic liver disease (MASLD) management. The only therapeutic intervention involves lifestyle changes such as weight loss and management of metabolic diseases, indicating an urgent unmet need for the treatment of MASLD.[169] To overcome the current deficit, researchers are employing RNA nanomedicine as a promising strategy to treat MASLD. Although RNA nanotherapies possess a plethora of advantages, certain potential complications in targeting RNA molecules to hepatocytes in the context of MASLD need to be highly considered while designing an optimal RNA nanotherapy. First, the increased fat accumulation in hepatocytes during MASLD leads to the activation and alteration of the endolysosomal trafficking network that is responsible for regulating lipid metabolism, protein degradation, and signal transduction.[170,171] In addition, this network dysfunction also causes abnormal endocytosis, cargo sorting, and increased degradation of endocytosed cargo such as RNA-based NPs from the cell membrane. In response, this leads to inefficient cellular uptake and internalization of NPs to release RNA and exert their therapeutic effect, and therefore, careful considerations are necessary when designing RNA-based NPs for MASLD. Furthermore, the endolysosomal trafficking also leads to an increased accumulation of lysosomal contents that is responsible for activating other metabolic disease pathways rendering the treatment of MASLD even more difficult.[172,173] To solve this problem, combination therapies may be designed to target more than one target using either the same or different nanoformulations.

TABLE 3 Summary of receptors overexpressed during HSC activation in liver fibrosis and their receptors that have been used for RNA delivery

Receptor	Mechanism of action	Ligands
Type IV collagen receptor	Is a crucial matrix protein for cell adhesion. Is expressed on activated HSCs during liver fibrogenesis but not normal liver cells, therefore is a potential receptor for targeted delivery of antifibrotic agents to HSCs	1. Cyclic arginylglycylaspartic acid (RGD) peptides[137,138]
PDGFR	PDGF plays a crucial role in liver fibrogenesis. It binds to PDGFR for the migration, proliferation, and survival of HSCs. PDGFRs are overexpressed in HSCs and fibrotic livers; however, they are dramatically overexpressed in activated HSCs, and therefore PDGFR-targeted RNA delivery can be used to specifically deliver cargo to a fibrotic liver[122,139–141]	1. Cyclic peptide C*SRNLIDC (* cyclizing cysteine residue)[142,143] 2. Cyclic peptide—pPB[144] 3. PDGFRβ-antibody[145]
IGFIIR	IGFIIR regulates IGFII and also plays a role in the activation of TGF-b. IGFIIR is positively correlated to liver injury and is overexpressed in activated HSCs with 3-fold faster ligand internalization as compared with quiescent HSCs and constitutes an ideal receptor for HSC-targeted RNA delivery	1. IGF2R-specific peptide[146] 2. IGF2R-specific aptamer[147] 3. M6P[148]
LDLR and SR-B1	LDLR and SR-B1 are highly expressed in the liver and responsible for cholesterol homeostasis. These receptors are also located on HSCs and therefore contribute to RNA delivery in a fibrotic liver[149–151]	1. Apolipoprotein E[151] 2. ApoA-1-mimicking peptides[152]
RBP receptor	Quiescent HSCs store 80% vitamin A as retinyl palmitate and are taken up through RBP receptors. Vitamin A is increasingly taken up by activated HSCs and therefore ligands targeting RBP receptors can be used for HSC-specific RNA delivery[136,153]	1. Vitamin A[136] 2. Retinol[154,155] 3. Retinoic acid[156]
CD44	CD44 receptors are overexpressed in liver injury and are mainly present in lymphocytes, KCs, and HSCs. The splice variant CD44v6 is specifically expressed in activated HSCs and can therefore be targeted for RNA delivery to HSCs[157,158]	1. HA[159] 2. CD44-antibody[160]
Synaptophysin	Is a plasma membrane protein expressed in myofibroblasts that are important regulators in liver fibrosis[161]	1. Human recombinant scAb[161]

Abbreviations: ApoA, apolipoprotein A; HA, hyaluronic acid; IGFIIR, insulin-like growth factor 2 receptor; LDLR, LDL receptor; M6P, Mannose 6 phosphate; PDGFR, patient-derived growth factor receptor; RBP, retinol-binding protein; scAb, single-chain antibody; SR-B1, scavenger receptor class B type I.

RNA-based therapeutics offer the potential to modulate key genes and pathways involved in NASH pathogenesis. In addition, the use of nanocarriers for RNA delivery provides stability, enhanced cellular uptake, and targeted delivery to liver cells that are involved at different stages of the disease progression. Several preclinical and clinical studies are using RNA nanomedicine to modulate inflammation and/or inhibit fibrogenesis, and the results highlight the potential of this approach for future translation in NASH therapy. Most recently, Zhou et al[174] studied the therapeutic efficacy of targeting liver macrophages and silencing HMGB1 for NASH treatment. HMGB1 is an inflammatory factor that is synthesized by macrophages following liver injury and plays a critical role in driving the progression of NASH and its subsequent development into liver fibrosis and cirrhosis.[175] In this study, the therapeutic effect of coadministering docosahexaenoic acid, an unsaturated fatty acid, along with HMGB1 siRNA coupled to mannose modification for macrophage targeting was investigated. This combination therapy resulted in rapid recovery of liver function and normalization of hepatic steatosis after 8 weeks. This study not only presents an actively targeted siRNA delivery system that modulates liver macrophage phenotype in NASH but also demonstrates the effectiveness of docosahexaenoic acid coadministration as a therapeutic approach.[174] These findings offer valuable insights and provide a scientific foundation for the development of future therapeutic strategies for NASH RNA nanomedicine.

TABLE 4 Summary of the main results obtained from preclinical studies using RNA medicine in liver fibrosis and cirrhosis

No.	Specific RNA	Delivery vehicle	Preclinical model	Dose (mg/kg) and duration	Summary of results
1	Procollagen a1(I) siRNA	Cationic lipid nanoparticles	CCl_4-induced liver fibrosis	siRNA dose: 100–800 μg/kg, body weight in 100 μL PBS Duration: weekly or biweekly over 2–4 wk	90% knockdown of procollagen a1(I), reduction of septa formation, and 40%–60% decrease in collagen deposition in vivo without any detectable side effects[162]
2	Procollagen a1(I) siRNA	SS-cleavable proton-activated lipid-like materials (ssPalms) containing either myristic acid (ssPalmM), hydrophobic vitamin A (ssPalmA), or vitamin E (ssPalmE)	CCl_4-induced liver fibrosis	siRNA dose: 2 mg/kg, i.v. Duration: 24 h after last CCl_4 dose	Among the 3 LNPs, ssPalmA delivered the highest amount of siRNA selectively to HSCs and this is due to the increased affinity of HSCs toward vitamin A since HSCs produce various intracellular and extracellular vitamin A–binding proteins[163]
3	Procollagen a1(I) siRNA	Cationic nanohydrogel particles	CCl_4-induced liver fibrosis	siRNA dose: 1 and 2 mg/kg, i.v.; Duration: 2 doses 48 h apart and mice killed 48 h after last dose	These cationic nanohydrogel particles showed ideal delivery to the different liver cells especially nonparenchymal (nonhepatocyte) cells devoid of any toxicity. siRNA NPs were taken up by 50% of activated HSCs and myofibroblasts that are the major cell targets for liver fibrosis[164]
4	Gp46 siRNA (a rat homolog of HSP47)	Vitamin A–coupled liposomes	DMN-induced liver cirrhosis rat model	Dose: 0.75 mg/kg siRNA Duration: total 3 doses every other day	These liposomes effectively suppressed collagen through a receptor-specific siRNA delivery and is an excellent strategy to reverse liver cirrhosis[136]
5	PCBP2 siRNA	Multicomponent nanocomplex using siRNA/PNA hybrid	CCl_4-induced rat fibrotic model	Dose: 1 mg/kg siRNA nanocomplex Duration: i.v. injection on days 14, 18, 23, and 27 after induction of CCl_4 injections	PCBP2 is a crucial gene for type I collagen mRNA stability, which further leads to ECM accumulation. This IGF2R-targeted PCBP2 siRNA nanocomplex potently reversed liver fibrosis progression and inhibited type I collagen in vivo[128]

Abbreviations: DMN, dimethylnitrosamine; ECM, extracellular matrix; HSP47, heat shock protein 47; PCBP2, poly(rC)-binding protein 2; PNA, peptide nucleic acid; siRNA, small interfering ribonucleic acid.

Liver cancer (HCC, cholangiocarcinoma, metastatic liver cancer)

Liver cancers are the fourth leading cause of cancer-related mortality worldwide. Liver cancers can be broadly classified as HCC, cholangiocarcinoma (CCA), and liver metastasis (LM). Among these, HCC remains the most common form of liver malignancy with treatments challenged by the disease heterogeneity and common occurrence in the setting of a chronic liver disease such as cirrhosis. Owing to the current pitfalls in HCC management, RNA nanomedicine could bring forward a paradigm shift for a safe targeted therapy in patients with HCC.[176–178]

Recently, Xiao et al[179](p53) demonstrated an interesting breakthrough in mRNA nanomedicine in HCC. They developed a CXCR4-targeted mRNA NP platform to effectively restore p53 expression in HCC models. Since the loss of function of tumor suppressors is a crucial driver of hepatocarcinogenesis and drug resistance, targeting tumor suppressor genes could potentially induce and regulate the immune antitumor effect.[180,181] In this study, the p53 tumor suppressor gene, which is responsible for diverse functions ranging from apoptosis, senescence to the regulation of cell cycle arrest, was used to develop an mRNA NP for p53 therapy.[182,183] The coating of CXCR4-specific targeting peptide CTCE (KGVSLSYR-CRYSLSVGK) has enabled NPs with 15-fold higher cellular uptake to HCC cells in vitro. Moreover, in vivo studies in HCC mouse models showed similar kinetics such that there was significantly higher (1.5–2.7-fold) intratumoral accumulation of CTCE NPs as compared with nontargeted NPs. These CTCE NPs also demonstrated 2–2.5-fold stronger tumor growth inhibition in vivo while enhancing the overall survival of the mice as compared with nontargeted NPs. Overall, these results indicate the immense potential of RNA nanomedicine in transforming the landscape of HCC therapeutics.

To date, 2 RNA-based nanotherapies for advanced HCC have reached the clinical trials—TKM-080301 and DRC-MYC. TKM-080301 uses polo-like kinase 1 siRNA to knock down polo-like kinase 1, which is a validated molecular target in HCC with a crucial role in cell cycle regulation. In this study, 43 patients were given a dose of 0.3 mg/kg siRNA and the results indicated that the siRNA LNPs were safe and well tolerated in patients with advanced HCC; however, the nanomedicine did not show significant antitumor effect limiting its use and advancement to the clinic.[66] Similarly, DRC-MYC is an LNP formulated with dicer substrate siRNA targeting MYC mRNA, which is a well-known tumor driver. This nanoformulation showed promising results in phase Ib/II studies, whereby MYC mRNA silencing efficacy was observed. However, this study was terminated due to the sponsor's decision despite the antitumor potency of DRC-MYC.[184]

The secondmost common primary liver cancer is CCA. This presents with an unfavorable prognosis, low resectability rate, and poor therapeutic outcome due to the associated drug resistance. It is also a highly lethal, epithelial cancer that occurs in the biliary tree or the hepatocyte parenchyma along with characteristics of cholangiocyte differentiation.[177] Considering the current challenges in CCA treatment, increasing studies are using nanoplatforms to enhance specific targeting and reduce toxicities. Although most researchers have been studying the delivery of chemotherapeutic drugs to CCA tumors through nanocarriers, some researchers have investigated the effect of RNA nanomedicine in CCA models and have achieved promising results. Xie et al reported a series of polymeric CXCR4 antagonist NPs capable of delivering miRNA to CCA tumors. In this study, combination therapy was developed using polymeric CXCR4 antagonist-anti miR-210 NPs, which were capable of blocking CXCR4 due to polymeric CXCR4 antagonist and inhibiting hypoxia-inducible miR-210 with the encapsulated miRNA. These NPs were formulated and evaluated in vivo in the Mz-ChA-1 cell–derived xenograft mouse model. The results depicted that this innovative combination NP treatment simultaneously inhibited CXCR4 and miR-210. These NPs were effectively delivered to CCA xenograft tumors and exhibited direct tumor growth reduction (~51%) and chemotherapy sensitization [~79% tumor growth inhibition when NPs were combined with gemcitabine and cisplatin (GEM/CDDP) chemotherapy in this GEM/CDDP-resistant tumor model].[185] Similarly, another study evaluated the antitumor effects of cationic liposome–mediated co-delivery of gemcitabine and aPKC1–siRNA in an EGI-1 cell–derived xenograft mouse model, and the results showed enhanced tumor targeting and a synergistic antitumor effect of the combination therapy. Previous studies report that aPKC1 is responsible for tumor cell survival and gemcitabine resistance in CCA. aPKC1 activation promotes cell survival pathways, counteracting the apoptotic signals triggered by gemcitabine, which in return permits tumor cells to survive. Also, reports indicate that aPKC1 activation is linked to overexpression of multidrug-resistant proteins such as P-glycoproteins that actively contribute to the efflux of gemcitabine from CCA cells leading to drug resistance. Therefore, a combination of gemcitabine with aPKC1–siRNA would not only inhibit aPKC1 gene expression but also permit a synergistic antitumor effect of gemcitabine in a drug-resistant environment.[186] Although several advantages have been reported in RNA delivery through nanocarriers, CCA still presents a challenging tumor to maintain targeting for elevated periods. Therefore, to improve the shortcomings of an effective CCA RNA nanomedicine, the development of surface group modifications and camouflage membrane coating systems could be used, and more research is

needed in this field for a safe and effective CCA nanotherapy.[187]

In addition, due to the rich portal blood supply, the liver presents a harbor for metastases. LMs can be defined as tumors that have spread beyond their site of origin to the liver. The most common cancers that may lead to LM include colorectal, breast, lung, and pancreatic cancers. Considering the heterogeneity of LMs arising from different organs, this presents the challenge of different tumor environments due to varying signaling pathways depending on the tumor origin. The unique features of RNA nanomedicine perfectly fit to address these challenges by virtue of their ability to specifically and effectively target all different metastases as opposed to traditional chemotherapy. Also, with RNA nanomedicine, the delivery profile to the liver is determined by the carrier, which can easily be modified through specific ligands to accommodate different metastases. Furthermore, through combination therapy, it is feasible to treat all different signaling pathways using a similar nanosystem. Among these LMs, colorectal cancer is the most common cause of LM with more than 50% probability that a colorectal tumor will advance to the liver.[177,188,189] Despite the advancements in this field of research, the current chemotherapeutic agents lack effective therapeutic efficacy and safety mostly due to heterogenous tumor response, drug resistance, and microenvironmental factors such as the unique characteristics of the liver, presence of liver-specific immune cells, and immunosuppressive nature of the liver compared to the colon where the tumor originated.[190] RNA therapies provide an alternative to conventional chemotherapy as a molecular tool for silencing genes associated with colorectal cancer. In addition, delivering RNA therapies through nanomedicine provides the advantage of specific targeting and therefore less or no off-target toxicities. Among several molecular targets, the AKT/PI3K pathway largely affects cell proliferation, migration, and survival in colorectal LM (CRLM), and therefore, designing a targeted RNA nanotherapy to deliver inhibitors of this pathway could be a promising strategy.

Recently, Kang et al[191] designed a novel oral gold NP delivery system to encapsulate Akt2 siRNA to treat CRLM. These NPs were synthesized using glycol chitosan-taurocholic acid that facilitates active transport through enterocytes and enhances selective accumulation in CRLM through the enterohepatic recycling process. Briefly, these specifically targeted NPs are absorbed through the apical sodium bile acid transporter receptors from the ileum to the liver through the natural bile acid recycling system. This occurs through a series of processes starting with endocytosis in the ileum through the apical sodium bile acid transporter receptors, followed by endosomal escape or lysosomal degradation and eventually exocytosis through organic solute transporter (OST) peptide receptors to hepatic portal circulation to the liver without disintegration.[192] This study also concluded that this Akt2 siRNA gold nanosystem enhanced siRNA accumulation in the liver (~2-fold) and significantly reduced AKT/PI3K downstream signaling in an orthotopic CRLM mouse model with a significant decrease in tumor nodules compared with nontargeted NPs. This study reflects the magnificent qualities of a targeted RNA nanosystem in reducing tumor growth that could be translatable to the clinic for an effective CRLM therapy.

Similarly, breast cancers are also highly heterogeneous and can spread to the liver.[193,194] Studies have demonstrated that all subtypes of breast cancers can proliferate in the liver; however, among these, HER2-positive tumors have shown the highest percentage of metastasis to the liver. In addition, studies have also shown that patients with LM due to triple-negative breast cancer have worse overall survival than HER2-enriched breast cancer patients.[195–198] Owing to the disease heterogeneity and complexity, currently, there is no defined standard treatment for breast cancer LM and the therapy is often determined through the molecular profile of the metastasis. To overcome the current challenges in breast cancer LM treatment, Liu et al[199] designed siRNA-loaded exosomes as a natural nanocarrier system to deliver siRNA against long noncoding lncRNA DARS-AS1 to triple-negative breast cancer liver metastatic mice. MDA-MB-231 cells transfected with DARS-AS1 or CDKA siRNA (7.92 μg each)-loaded exosomes were injected s.c. into the left breast of the female mice. On days 7 and 14 after injection, the same amount of siRNA-loaded exosomes were injected intratumorally and mice were killed on day 36. Results indicated that DARS-AS1, a newly discovered tumor accelerator, activated HSCs that further facilitated liver colonization and tumor cell growth. It was also reported that DARS-AS1 increased triple-negative breast cancer LM through miR-129-2-3p targeting and activation of NF-kB/STAT3 signaling pathway in MDA-MB-231-tumor bearing mice. Further, DARS-AS1 siRNA nanovesicles substantially inhibited tumor growth (2-fold) and LM in these mice, which rationalizes the potential of RNA nanomedicine in breast cancer LM.

Pancreatic cancer LM also presents a common malignancy due to the proximity of the pancreas to the liver in the body, further increasing the spread of tumors to the liver. RNA nanotherapy has been rarely studied in this cancer; however, many researchers have been successful in reducing tumor growth by using different RNA moieties. A study investigated the efficacy of Nek2 siRNA in a pancreatic cancer liver metastatic rat model.[200] Overexpression of Nek2 has been indicated in several cancers including pancreatic, CCA, breast, and colorectal cancers.[201–203] In this study, Nek2 siRNA was delivered to the tumor using Lipotrust as the lipid nanocarrier through an intraportal catheter that

was initially used to develop the metastatic rat model through an injection of KP4 cells. Interestingly, it was noted that Nek2 siRNA substantially reduced the number and area of LMs in the rats, indicating the efficacy of Nek2 siRNA for pancreatic cancer LM. However, this treatment could be modified by developing a nanosystem to deliver the Nek2 siRNA through NPs or conjugates. This would be clinically applicable as it would not require direct injections into the portal vein and the nanocarrier could be modified to deliver the RNA moiety either systematically or orally. Overall, RNA nanotherapy is being extensively explored in the field of liver cancers and some selected study results are described in Table 5.

Viral hepatitis

To date, limited research has been conducted in the field of RNA nanomedicine for the treatment of viral hepatitis; however, this field of research holds tremendous scope for novel therapies.[212] Hepatitis is a heterogenous syndrome that refers to liver inflammation and damage and can be classified as viral hepatitis, autoimmune hepatitis, drug-induced hepatitis, and alcohol-induced hepatitis.[121,213,214] In the HCV structure, all structural and nonstructural genes are in the same open reading frame, and therefore, designing a specific siRNA or miRNA targeting any portion of the viral sequence could inhibit viral replication.[212] Similarly, considering HBV, which is a circular dsDNA virus as opposed to the RNA HCV virus, modifications and diverse strategies are required to target the 4 different open reading frames.[212] Another important consideration is the everchanging and mutating viral sequence in these viruses. Data indicate that HBV has a mutation rate of 2×10^4 base substitutions/site/year, and therefore, RNA medicine that targets conserved parts of their sequences and/or nanomedicine with multiple RNAi triggers could provide the most optimal therapeutic option.

Recently, Huang and colleagues designed a novel ionizable lipidoid NP (RB131) with a state-of-art lyophilization technology to specifically deliver siRNA targeting apolipoprotein B into hepatocytes. Later, a potent siRNA targeting HBV was encapsulated within these LNPs as a functional cure for hepatitis B. Interestingly, the results of this study indicated that the viral RNA and antigen (HBsAg and HBeAg) expressions were inhibited dose-dependently and time-dependently in vivo when dosed biweekly at 0.04, 0.2, and 1 mg/kg with 99% knockdown at the highest dose, suggesting that RNA nanomedicine could offer a promising strategy for the treatment of hepatitis B.[215] In this study, the siRNA target aims to inhibit HBsAg antigen levels as an effective endpoint for resolved acute HBV since HBeAg and HBsAg are considered to induce T-cell tolerance and T-cell exhaustion, further accelerating viral replication. Researchers are also using combination RNA nanotherapies with antivirals to effectively knock down HBsAg as a therapeutic option for viral hepatitis. Also, this highlights the advantage of nanomedicine that permits combination therapy through diverse cargos. Furthermore, Tan et al[216] have shown that the combination of siRNA and ASO (HBV siRNA ALG-125755 and ASO ALG-020572) in vivo maximized HBsAg reduction and demonstrated additive efficacy, further concluding the effectiveness of a combined RNA nanomedicine for the functional cure of viral hepatitis. These research findings and ongoing preclinical and clinical studies shown in Table 6 demonstrate the potential for RNA nanomedicine to treat hepatitis B.

Liver metabolic diseases

Hepatocytes are the main functional cells of the liver, accounting for about 80% of the liver mass and liver volume.[226,227] They perform various metabolic functions, such as synthesis, storage, and secretion of carbohydrates, lipids, proteins, bile acids, and hormones; detoxification and excretion of drugs, toxins, and bilirubin; and regulation of energy homeostasis, immune response, and inflammation.[228–230] Therefore, hepatocytes are essential for maintaining the metabolic balance of the whole body. However, hepatocytes can also be impaired by rare genetic disorders, known as inborn errors of metabolism (IEMs), which are inherited diseases that affect the metabolism of various substances in the liver and other tissues.[231] IEMs are caused by mutations in genes that encode enzymes or proteins involved in metabolic pathways, resulting in their deficiency or dysfunction. For example, phenylketonuria is an IEM caused by a deficiency of phenylalanine hydroxylase, an enzyme that converts phenylalanine to tyrosine in hepatocytes[232]; glycogen storage diseases are IEMs caused by defects in enzymes that are involved in glycogen synthesis or degradation in hepatocytes.[233] The consequences of these metabolic defects are the accumulation of toxic metabolites or the lack of essential products in hepatocytes and other tissues, which can further cause a variety of symptoms and complications, such as liver failure, neurological damage, kidney damage, developmental delays, and death.[234,235] Therefore, hepatocytes play a critical role in liver metabolic diseases, both as the primary site of metabolic dysfunction and as the potential target of therapeutic intervention.

One of the promising therapeutic interventions for IEM liver diseases is LNP-based mRNA therapy, which can deliver mRNA encoding the missing or defective

TABLE 5 Summary of the main results obtained from selected preclinical studies using RNA medicine in liver cancers

No.	Specific RNA	Delivery vehicle	Preclinical model	Dose (mg/kg) and duration	Summary of results
1	VEGF siRNA	Phenyl b-D-galactoside-decorated LNPs	HCA-1 cell–derived orthotopic mouse model	Dose: 0.7 mg/kg siRNA, 3 times per week for 2 wk	LNPs significantly suppressed the tumor and phenyl galactoside showed excellent HCC-targeting for siRNA delivery in HCC[204]
2	Survivin siRNA	GalNAc-PEG-PLGA nanoconjugates	DEN-induced HCC mouse model	Dose: 150 nM siRNA/200 μL PBS i.v. single daily dose for 10 d	These nanoconjugates were able to specifically bind to ASGPR and showed excellent antitumor effect and siRNA accumulation within the tumor cells[205]
3	Midkine siRNA + sorafenib combination	Ultra small LNPs	HepG2 cell–derived xenograft mouse model	Dose: 0.5 mg/kg siRNA and 2.5 mg sorafenib, i.v. single dose per day at 3-d intervals for total 7 doses	It was demonstrated for the first time that midkine siRNA reverses sorafenib resistance in sorafenib-resistant HCC mice and these LNPs can be potentially useful for designing RNA nanomedicine in drug-resistant HCC[206]
4	Bcl2 siRNA + paclitaxel combination	Hetero assembly of polymeric micelles and liposomes	HepG2 cell–derived xenograft mouse model	Dose: 300 μg siRNA/kg body weight and 1 mg paclitaxel i.v. every 2 d for 7 treatments	This hetero-nanoassembly showed significant intratumoral accumulation and antitumor effect. Also, combination therapy overcame the bcl2-mediated drug resistance during chemotherapy[207]
5	miR-122	Cationic LNPs	Sk-Hep-1 cell–derived xenograft mouse model	Dose: 10 mg miR-122 mimic intratumorally, twice a week for 26 d	LNPs showed significant antitumor effects in vivo demonstrating the potential of miRNA delivery for HCC treatment[208]
6	sphk2 siRNA	LNPs and chitosan NPs	Sk-Hep-1 cell–derived xenograft mouse model	Dose: 1.5 mg/kg siRNA i.v. treated for 21 d	These LNPs and NPs showed significant knockdown of sphk2 in vivo with a potent antitumor effect[209]
7	miR-199a-3p	Arginine a,b-dehydrophenylalanine (RAF) NPs	Huh7 cell–derived xenograft mouse model	Dose: 1 mg/kg miRNA every 3 d for 28 d i.v.	These functionalized RAF NPs showed high stability and specific targetability to HCC tumors. A significant tumor regression was also observed in vivo[210]
8	mRNA encoding costimulatory OX40L	LNPs	H22 cell–derived xenograft mouse model	Dose: 11.5 μg mRNA intratumorally, once every 3 d for a total of 6 injections	mRNA LNPs showed a significant decrease in tumor burden[43]
9	IL-12 mRNA	LNPs	LAP-tTA/tet-O-hMYC transgenic mice	Dose: 0.025 mg/kg weekly i.v. for 3 wk	IL-2 mRNA LNPs were well tolerated and safe. They significantly decreased the tumor burden in vivo and showed increased survival time[211]

Abbreviations: ASGPR, asialoglycoprotein receptor; DEN, diethylnitrosamine; GalNAc, *N*-acetylgalactosamine; LNP, lipid nanoparticle; NP, nanoparticle; OX40L, Oxford 40 ligand; PEG, polyethylene glycol; siRNA, small interfering RNA; sphk2, Sphingosine kinase 2.

TABLE 6 Summary of the main results obtained from selected preclinical and clinical studies using RNA medicine in hepatitis

No.	Specific RNA	Delivery vehicle	Preclinical model/clinical study	Dose (mg/kg) and duration	Summary of results
1	2 siRNAs triggering X ORF of HBV cccDNA	Cholesterol		Clinical study (AR520)—4 mg/kg single dose	The siRNA was well tolerated and produced a reduction in HBeAg levels; however, due to incidences of mortality induced by an excipient in nonhuman primate studies, the study was terminated[217,218]
2	3 siRNA targeting 3 distinct sites on the HBV genome (S and X ORFs)	LNPs		Clinical study (ARB-1467)—0.2 mg/kg and 0.4 mg/kg, monthly for 3 doses	Treatment was well tolerated in both doses with HBeAg antigen inhibition greater in 0.4 mg/kg dose compared with 0.2 mg/kg, supporting further studies[219]
3	dsRNA that cleaves mRNA encoding all forms of HBsAg in hepatocytes	GalNAc		Clinical study (RG-6346)—0.1–12 mg/kg monthly for 4 doses	dsRNA-conjugated GalNAc consistently reduced HBsAg levels regardless of the HBeAg antigen status with no serious adverse effects or dose-limiting toxicities[220]
4	siRNA designed to silence all major HBV transcripts (X ORF)	GalNAc		Clinical study (VIR-2218)—20–200 mg/kg on days 1 and 29	The NPs were well tolerated with dose-dependent decline in HBsAg levels reaching a maximum by week 16[221]
5	siRNA targeting all HBV RNA transcripts (S and X ORF)	GalNAc		Clinical study (JNJ-3989)—100–400 mg/kg doses on days 1, 27, and 57	The NPs were well tolerated in the clinical study with reductions in HBsAg levels. These were linearly correlated to the reduction in HBeAg and HBV RNA levels[222]
6	Fas siRNA	Galactose-conjugated liposomes	Con A–induced hepatitis mouse model	Dose—50 μg siRNA per mouse single i.v. injection	These liposomes showed a decrease in hepatic injury through Fas siRNA specifically delivered to the liver[223]
7	Core-specific HCV siRNA	ApoA1-conjugated cationic liposomes	Transient HCV model using hydrodynamic injection of plasmids	Dose—2 mg/kg siRNA on days 2, 4, and 6	LDLR-targeted liposomes showed high potency and specificity in targeting HCV in the hepatocytes[224]
8	Irf5 siRNA	LNPs	Con A–induced hepatitis mouse model	Dose—5 mg/kg siRNA on days 1, 2, and 7	Irf5 encodes the transcription factor responsible for inflammatory responses in hepatitis. The designed biodegradable LNPs reduced liver enzyme levels and inflammatory cytokines in treated mice[225]

Abbreviations: ApoA, apolipoprotein A; ConA, Concanavalin A; GalNAc, *N*-acetylgalactosamine; LDLR, LDL receptor; LNPs, lipid nanoparticles; NP, nanoparticle; ORF, open reading frame; siRNA, small interfering RNA.

protein to hepatocytes, restoring their normal metabolic function and reducing the symptoms and complications of these diseases.[236,237] LNP-based mRNA is intrinsically a good therapeutic candidate targeting the liver, as LNPs have a natural affinity for hepatocytes following i.v. administration due to their interaction with ApoE and LDLR on the surface of LNP and hepatocytes, which enhances the delivery efficiency and specificity of mRNA therapy. An example of LNP-mRNA therapy for IEM liver diseases is the treatment of acute intermittent porphyria, a rare genetic disorder that causes acute attacks of abdominal pain, vomiting, and neuropsychiatric symptoms due to a deficiency of porphobilinogen deaminase (PBGD) in the hepatocytes.[238] Researchers have loaded human PBGD mRNA into LNP and administered it to acute intermittent porphyria mice at a dose of 0.5 mg/kg. This resulted in a rapid and high therapeutic efficacy, with a gain of ~80% of endogenous PBGD activity 24 hours after administration. The sustained efficacy and tolerability of LNP-hPBGD mRNA was confirmed by repeated administration in mouse, rabbit, and nonhuman primate models.[239] The same approach also worked for variegate porphyria, another liver disease caused by the accumulation of porphyrins and their precursors, in a rabbit model of variegate porphyria.[240] In contrast, methylmalonic acidemia/aciduria (MMA) is a liver disease caused by a deficiency of methylmalonyl-CoA mutase (MUT), a mitochondrial enzyme. Researchers have used LNP to deliver human MUT mRNA to the hepatocytes of mice with MMA and restore MUT in their mitochondria. A single dose of LNP-hMUT mRNA (0.5 mg/kg) reduced plasma MMA levels by 90% within 24 hours. Repeated doses of LNP-hMUT mRNA (0.2 mg/kg, weekly) maintained normal MUT levels and improved survival rates in MMA mice after 6 weeks (100% vs. 17% in control mice).[241] This LNP-mRNA formulation has entered a phase I/II trial by Moderna (NCT04899310). Similarly, LNP-mRNA was used to treat propionic acidemia/aciduria, a liver disease caused by a deficiency of propionyl-CoA carboxylase (PCC), another mitochondrial enzyme. Researchers have used LNP to deliver 2 mRNA molecules encoding both subunits of PCC to the mitochondria of mice with propionic acidemia/aciduria. This dual-mRNA strategy produced higher PCC enzyme activity than single mRNA alone. It also restored functional PCC enzyme in the liver and reduced primary disease-associated toxins in a dose-dependent manner in long-term 3-month and 6-month repeat-dose studies in propionic acidemia/aciduria mice with no toxicity observed.[242]

Overall, LNP-based mRNA therapy has shown efficacy and safety in preclinical models of several liver metabolic diseases by restoring normal protein metabolic functions, highly suggesting its potential to be a transformative treatment for patients with inborn liver metabolic diseases.

FUTURE PERSPECTIVES

While several researchers are currently exploring different RNA machinery for liver diseases, certain recent advances in RNA are yet to be fully explored. Among these, small activating RNA (saRNA) is an intriguing class of noncoding RNA composed of 21-nucleotide dsRNA with an opposite mechanism of action to siRNA and has been recently studied for HCC treatment.[243–246] Unlike siRNA, saRNA only binds to Argonaute 2 protein, and the complex is transported to the nucleus for gene activation by directly targeting the gene promoters. During transcription, the RNA-Argonaute 2 complex recruits proteins such as RNA helicase A, CTR9 homolog (CTR9), and RNA polymerase II-associated factor 1 homolog for gene activation.[247–250] Most RNA medicine has centered around the approach to target gene silencing; however, several liver diseases are also caused by downregulated proteins, and therefore, development of technologies such as saRNA to restore these protein expressions could have a huge impact in the future.

Recently, saRNA that upregulated the transcription factor CCATT/enhancer binding protein alpha (CEBPA) was developed for the treatment of liver cancer. Preliminary studies identified CEBPA as an important gene that is often silenced in HCC and associated with poor survival.[251] Studies have shown that the depletion of CEBPA leads to the dysregulation of liver-specific transcription factors and therefore hinders hepatocyte maturation.[252,253] Therefore, restoration of this gene could serve as a potential therapeutic option for HCC.[254] In this study, an saRNA sequence that upregulated CEBPA mRNA 2.5-fold was designed and evaluated in vitro and in vivo. saRNA treatment showed an antitumor effect through the activation of CEBPA mRNA and its downstream targets in vitro and in vivo. With the exceptional results obtained from preclinical studies, CEBPA saRNA has been encapsulated in a liposomal formulation and is currently being evaluated in patients with HCC as the first-in-human study of an saRNA therapeutic.[68] It can therefore be anticipated that saRNA therapeutics have immense potential and drugs targeting different liver diseases will be developed in the future, transforming the current state of liver disease therapeutics.

Apart from that, circular RNAs (circRNAs) have emerged as a novel class of RNA therapeutics. Traditionally, endogenous circRNAs are considered as noncoding RNA and characterized by a covalently closed loop structure generated by backsplicing.[255–258] Later, researchers discovered that circRNA can act as miRNA sponge, protein sponge, and scaffold for the formation of protein complexes,[259] enabling them as promising therapeutic targets. For example, in the liver, 668 circRNAs have been reported and play a key role in both damage and repair processes during liver diseases.[260] During liver fibrosis, the

major circRNA-mRNA pathways based on a diet-induced NASH model include circ_002581-miR-122-Slc1a5, circ_002581-miR-122-Cpeb1, circ_002581-miR-122-Plp2, and circ_007585-miR-326-UCP2.[261] Also, it has been reported that 179 and 630 circRNAs are upregulated and downregulated in activated HSCs as compared with quiescent HSCs, respectively.[262] As previously discussed, since HSC activation is a crucial process in fibrogenesis, targeting these upregulated or downregulated circRNAs may be promising for the treatment of liver fibrosis.

circRNAs also play a functional role in HCC and function as an miR-7 sponge.[263] Studies have reported that circRS-7 was highly elevated in patients with HCC and this correlated with reduced miR-7 levels. It was also observed that increased ciRS-7 levels increased cell proliferation and invasion of tumor cells by down-regulating miR-7 and its target genes CCNE1 and PIK3CD.[264] Although there is a growing number of studies on circRNAs in liver diseases, there is still an incomplete picture of the underlying mechanisms of circRNAs in these diseases, and extensive research is still required for developing RNA therapies. Recently, exogenous circRNA delivery and translation in vivo have been reported.[265] circRNA translation is extended in adipose tissue in comparison to unmodified and uridine-modified linear mRNAs, highlighting its potential as RNA therapeutics for protein replacement therapy.

Currently, the most recent technique in RNA medicine is RNA base editing for the treatment of different ailments through temporary or permanent RNA base alterations, bringing us closer to precision medicine.[266,267] Base editing refers to the introduction of single-nucleoside variants into RNA in viable cells. Base editors may include RNA-programmable deaminases, mRNAs, etc. For optimal therapy, it is extremely important that the RNA base tool such as mRNA efficiently reaches the site of interest devoid of any off-target side effects.[267] To achieve this, using nanocarriers for specific targeted delivery could overcome the foremost challenges in this RNA delivery. A few studies have investigated the effect of base RNA editing on liver diseases. Most recently, Packer et al[268] developed 2 base editing approaches to treat AAT deficiency through LNPs. A cytosine base editor was used to add a p.Met374Ile compensatory mutation and an adenine base editor was used to correct the major Piz mutation in AAT deficiency. PiZ-transgenic mice were treated with base editing tool encapsulated LNPs and interestingly, it was observed that there was long-term SERPINA1 editing in the liver along with an improvement in liver enzyme levels and overall liver histology. Overall, this RNA nanoformulation was capable of targeting both “loss of function” and “gain of function” complications of AAT deficiency. In addition, as a proof-of-concept, Villiger et al[269,270] have demonstrated cytidine base editing of hepatocytes in vivo without encountering any detectable off-target RNA mutations. This serves as a foundation to discover in-depth effects of RNA base editing in genetic liver diseases and can be investigated in other liver diseases. In this study, intein-split cytidine base editors were encapsulated in LNPs and i.v. administered to mice with phenylketonuria. This led to ~21% of gene editing on hepatocytes with the reversal of the disease phenotype. This is an attractive approach to target genetic variations in liver diseases and promising as a therapeutic option in the clinical setting.

CONCLUSION

In conclusion, RNA nanomedicine presents a promising approach for addressing the complex biological environments associated with various liver diseases. Traditional drug treatments have faced significant challenges due to their nonspecific targeting, limiting their efficacy and leading to potential side effects, especially in the presence of impaired liver function. However, the advent of nanomedicine and RNA-based drugs has revolutionized the field by offering targeted delivery and enhanced therapeutic capabilities. The success of Onpattro has transformed the standard of care for hATTR with specific hepatocyte targeting,[1] highlighting the potential of RNA nanomedicine to bypass the liver’s compromised function, thus increasing treatment effectiveness and reducing off-target effects. Furthermore, the versatility of RNA-based therapies allows for the development of personalized treatments tailored to individual patients, paving the way for more precise and efficient liver disease management. The current clinically approved RNA drugs and the ones in late-stage clinical trials (Tables 1 and 2) have shown a trend that for target silencing applications, GalNAc–RNA (ASO or siRNA) is predominantly developed and potentially the best way ahead, especially for hepatocyte-related diseases. The advantage comes from the clear chemistry, long-lasting effect (stability), and low immune-related adverse effects of GalNAc–RNA conjugates. These properties also enable s.c. delivery, which is much more favorable for patients as compared with i.v. drug administrations. This directional advancement in the field of GalNAc therapeutics is also evident in the product line of Alnylam Pharmaceuticals: since the approval of Onpattro, they have shifted their major focus from LNPs to GalNAc–RNA conjugates and the major progress in gene silencing of hepatocytes is evident using these GalNAc–RNA conjugates. However, to target other cell types in the liver, approaches beyond GalNAc need to be developed and LNP as an RNA carrier can be advantageous in targeting ligand modifications.

Another trend noticed from clinical progress is that LNPs are still the most optimal nanocarrier for protein replacement therapy and vaccine development for liver

diseases using mRNA cargo. This is because in order to modulate the metabolic profile in the liver, mRNA must be delivered to the liver. Unlike siRNA or ASO, chemical modification is sufficient to keep them stable in blood circulation. mRNA is much more fragile; therefore, it has to be protected by a carrier to enable in vivo delivery. As LNP has intrinsic tropism to accumulate in liver and hepatocytes following i.v. administration, most studies have used LNP-mRNA (dosed i.v.) for restoration of functional protein expressions in liver diseases. Moreover, vaccine development studies have shown that LNPs (most likely due to the ionizable lipid component) can serve as an adjuvant to further boost the immune response of the mRNA vaccine, and this also provides an added advantage of using LNP as a nanocarrier to deliver RNA nanomedicine. Although several RNA therapies as discussed in this review have either reached the clinic or are undergoing preclinical/clinical studies in liver disease applications, the complexity and changes in the liver disease microenvironment and the patient's intolerability of therapy still require to be completely understood and focused upon for the successful clinical translation of novel RNA nanotherapies.

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Anita Bakrania and Yulin Mo: conceptualization, writing—original draft, writing—review and editing. Gang Zheng and Mamatha Bhat: writing—review and editing, supervision.

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REFERENCES

1. Akinc A, Maier MA, Manoharan M, Fitzgerald K, Jayaraman M, Barros S, et al. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nat Nanotechnol. 2019;14:1084–7.
2. Hoy, S.M. Patisiran: First Global Approval. Drugs 2018;78: 1625–31.
3. 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. Neurol Ther. 2020;9:301–15.
4. Lovell JF, Jin CS, Huynh E, Jin H, Kim C, Rubinstein JL, et al. Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents. Nat Mater. 2011;10:324–32.
5. Mo Y, Cheng MHY, D'Elia A, Doran K, Ding L, Chen J, et al. Light-activated siRNA endosomal release (LASER) by porphyrin lipid nanoparticles. ACS Nano. 2023;17:4688–703.
6. Wang F, Zuroske T, Watts JK. RNA therapeutics on the rise. Nat Rev Drug Discov. 2020;19:441–2.
7. Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP. The limitless future of RNA therapeutics. Front Bioeng Biotechnol. 2021;9:628137.
8. Davidson BL, McCray PB. Current prospects for RNA interference-based therapies. Nat Rev Genet. 2011;12:329–40.
9. DeWeerdt S. RNA therapies explained. Nature. 2019;574:S2–3.
10. Lu D, Thum T. RNA-based diagnostic and therapeutic strategies for cardiovascular disease. Nat Rev Cardiol. 2019; 16:661–74.
11. Kim Y-K. RNA therapy: rich history, various applications and unlimited future prospects. Exp Mol Med. 2022;54:455–65.
12. Curreri A, Sankholkar D, Mitragotri S, Zhao Z. RNA therapeutics in the clinic. Bioeng Transl Med. 2023;8:e10374.
13. Bennett CF, Swayze EE. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu Rev Pharmacol Toxicol. 2010;50:259–93.
14. Kole R, Krainer AR, Altman S. RNA therapeutics: beyond RNA interference and antisense oligonucleotides. Nat Rev Drug Discov. 2012;11:125–40.
15. Rinaldi C, Wood MJA. Antisense oligonucleotides: the next frontier for treatment of neurological disorders. Nat Rev Neurol. 2018;14:9–21.
16. Crooke ST. Molecular mechanisms of antisense oligonucleotides. Nucleic Acid Ther. 2017;27:70–7.
17. Bennett CF. Therapeutic antisense oligonucleotides are coming of age. Annu Rev Med. 2019;70:307–21.
18. Shen X, Corey DR. Chemistry, mechanism and clinical status of antisense oligonucleotides and duplex RNAs. Nucleic Acids Res. 2018;46:1584–600.
19. Hair P, Cameron F, McKeage K. Mipomersen sodium: first global approval. Drugs. 2013;73:487–93.
20. Paik J, Duggan S. Volanesorsen: first global approval. Drugs. 2019;79:1349–54.
21. Wittrup A, Lieberman J. Knocking down disease: a progress report on siRNA therapeutics. Nat Rev Genet. 2015;16:543–52.
22. de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. Nat Rev Drug Discov. 2007;6:443–53.
23. Pei Y, Tuschl T. On the art of identifying effective and specific siRNAs. Nat Methods. 2006;3:670–6.
24. Eberhardt W, Doller A, Akool ES, Pfeilschifter J. Modulation of mRNA stability as a novel therapeutic approach. Pharmacol Ther. 2007;114:56–73.
25. Lam JKW, Chow MYT, Zhang Y, Leung SWS. siRNA versus miRNA as therapeutics for gene silencing. Mol Ther Nucleic Acids. 2015;4:e252.
26. Akinc A, Querbes W, De S, Qin J, Frank-Kamenetsky M, Jayaprakash KN, et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. Mol Ther. 2010;18:1357–64.
27. Scott LJ. Givosiran: first approval. Drugs. 2020;80:335–9.
28. Lamb YN. Inclisiran: first approval. Drugs. 2021;81:389–95.
29. Scott LJ, Keam SJ. Lumasiran: first approval. Drugs. 2021;81: 277–82.
30. Keam SJ. Vutrisiran: first approval. Drugs. 2022;82:1419–25.
31. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5:522–31.

32. Samad AFA, Kamaroddin MF. Innovative approaches in transforming microRNAs into therapeutic tools. Wiley Interdiscip Rev RNA. 2023;14:e1768.
33. Hong DS, Kang YK, Borad M, Sachdev J, Ejadi S, Lim HY, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. Br J Cancer. 2020;122: 1630–7.
34. Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the messenger: advances in technologies for therapeutic mRNA delivery. Mol Ther. 2019;27:710–28.
35. Weng Y, Li C, Yang T, Hu B, Zhang M, Guo S, et al. The challenge and prospect of mRNA therapeutics landscape. Biotechnol Adv. 2020;40:107534.
36. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—developing a new class of drugs. Nat Rev Drug Discov. 2014; 13:759–80.
37. Brassier A, Gobin S, Arnoux JB, Valayannopoulos V, Habarou F, Kossorotoff M, et al. Long-term outcomes in ornithine transcarbamylase deficiency: a series of 90 patients. Orphanet J Rare Dis. 2015;10:58.
38. Wilson JM. Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. Mol Genet Metab. 2009; 96:151–7.
39. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. Nat Rev Drug Discov. 2018;17:261–79.
40. Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a transformative technology for vaccine development to control infectious diseases. Mol Ther. 2019;27:757–72.
41. Barbier AJ, Jiang AY, Zhang P, Wooster R, Anderson DG. The clinical progress of mRNA vaccines and immunotherapies. Nat Biotechnol. 2022;40:840–54.
42. Vijayamahantesh V, Patra T, Meyer K, Alameh MG, Reagan EK, Weissman D, et al. Modified E2 glycoprotein of hepatitis C virus enhances proinflammatory cytokines and protective immune response. J Virol. 2022;96:e0052322.
43. Deng Z, Hao Y, Yuying T, Zherui L, Fang S, Penghui Y, et al. An OX40L mRNA vaccine inhibits the growth of hepatocellular carcinoma. Front Oncol. 2022;12:975408.
44. Karimkhanilouyi S, Ghorbian S. Nucleic acid vaccines for hepatitis B and C virus. Infect Genet Evol. 2019;75:103968.
45. Pardi N, Hogan MJ, Weissman D. Recent advances in mRNA vaccine technology. Curr Opin Immunol. 2020;65:14–20.
46. Fang E, Liu X, Li M, Zhang Z, Song L, Zhu B, et al. Advances in COVID-19 mRNA vaccine development. Sig Transduct Target Ther. 2022;7:1–31.
47. Ramanathan A, Robb GB, Chan SH. mRNA capping: biological functions and applications. Nucleic Acids Res. 2016;44: 7511–26.
48. Kang DD, Li H, Dong Y. Advancements of in vitro transcribed mRNA (IVT mRNA) to enable translation into the clinics. Adv Drug Deliv Rev. 2023;199:114961.
49. Kwon H, Kim M, Seo Y, Moon YS, Lee HJ, Lee K, et al. Emergence of synthetic mRNA: in vitro synthesis of mRNA and its applications in regenerative medicine. Biomaterials. 2018; 156:172–93.
50. Vaidyanathan S, Azizian KT, Haque AKMA, Henderson JM, Hendel A, Shore S, et al. Uridine depletion and chemical modification increase Cas9 mRNA activity and reduce immunogenicity without HPLC purification. Mol Ther Nucleic Acids. 2018;12:530–42.
51. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005;23:165–75.
52. Karikó K, Weissman D. Naturally occurring nucleoside modifications suppress the immunostimulatory activity of RNA: implication for therapeutic RNA development. Curr Opin Drug Discov Dev. 2007;10:523–32.
53. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008;16:1833–40.
54. Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. J Control Release. 2015;217: 337–44.
55. Nelson J, Sorensen EW, Mintri S, Rabideau AE, Zheng W, Besin G, et al. Impact of mRNA chemistry and manufacturing process on innate immune activation. Sci Adv. 2020;6: eaaz6893.
56. Nair JK, Attarwala H, Sehgal A, Wang Q, Aluri K, Zhang X, et al. Impact of enhanced metabolic stability on pharmacokinetics and pharmacodynamics of GalNAc–siRNA conjugates. Nucleic Acids Res. 2017;45:10969–77.
57. Sioud M. Innate sensing of self and non-self RNAs by Toll-like receptors. Trends Mol Med. 2006;12:167–76.
58. Balwani M, Sardh E, Ventura P, Peiró PA, Rees DC, Stölzel U, et al. Phase 3 trial of RNAi therapeutic Givosiran for acute intermittent porphyria. N Engl J Med. 2020;382:2289–301.
59. Ray KK, Troquay RPT, Visseren FLJ, Leiter LA, Scott Wright R, Vikarunnessa S, et al. Long-term efficacy and safety of inclisiran in patients with high cardiovascular risk and elevated LDL cholesterol (ORION-3): results from the 4-year open-label extension of the ORION-1 trial. Lancet Diabetes Endocrinol. 2023;11:109–19.
60. Hulton SA, Groothoff JW, Frishberg Y, Koren MJ, Overcash JS, Sellier-Leclerc AL, et al. Randomized clinical trial on the long-term efficacy and safety of lumasiran in patients with primary hyperoxaluria type 1. Kidney Int Rep. 2022;7:494–506.
61. Adams D, Tournev IL, Taylor MS, Coelho T, Planté-Bordeneuve V, Berk JL, et al. Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial. Amyloid. 2023;30: 18–26.
62. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med. 2018;379: 11–21.
63. Gales L. Tegsedi (Inotersen): an antisense oligonucleotide approved for the treatment of adult patients with hereditary transthyretin amyloidosis. Pharmaceuticals (Basel). 2019;12:78.
64. Chambergo-Michilot D, Alur A, Kulkarni S, Agarwala A. Mipomersen in familial hypercholesterolemia: an update on health-related quality of life and patient-reported outcomes. Vasc Health Risk Manag. 2022;18:73–80.
65. Witztum JL, Gaudet D, Freedman SD, Alexander VJ, Digenio A, Williams KR, et al. Volanesorsen and triglyceride levels in familial chylomicronemia syndrome. N Engl J Med. 2019;381: 531–42.
66. El Dika I, Lim HY, Yong WP, Lin CC, Yoon JH, Modiano M, et al. An open-label, multicenter, phase I, dose escalation study with phase II expansion cohort to determine the safety, pharmacokinetics, and preliminary antitumor activity of intravenous TKM-080301 in subjects with advanced hepatocellular carcinoma. Oncologist. 2019;24:747–e218.
67. Cervantes A, Alsina M, Tabernero J, Infante JR, LoRusso P, Shapiro G, et al. Phase I dose-escalation study of ALN-VSP02, a novel RNAi therapeutic for solid tumors with liver involvement. JCO. 2011;29(15_suppl):3025.
68. Sarker D, Plummer R, Meyer T, Sodergren MH, Basu B, Chee CE, et al. MTL-CEBPA, a small activating RNA therapeutic upregulating C/EBP-α, in patients with advanced liver cancer: a first-in-human, multicenter, open-label, phase I trial. Clin Cancer Res. 2020;26:3936–46.

69. Hui R, Mak L-Y, Seto W-K, Yuen M-F. RNA interference as a novel treatment strategy for chronic hepatitis B infection. Clin Mol Hepatol. 2022;28:408–24.
70. Strnad P, Mandorfer M, Choudhury G, Griffiths W, Trautwein C, Loomba R, et al. Fazirsiran for liver disease associated with alpha1-antitrypsin deficiency. N Engl J Med. 2022;387:514–24.
71. Srivastava A, Rangarajan S, Kavakli K, Klamroth R, Kenet G, Khoo L, et al. Fitusiran prophylaxis in people with severe haemophilia A or haemophilia B without inhibitors (ATLAS-A/B): a multicentre, open-label, randomised, phase 3 trial. Lancet Haematol. 2023;10:e322–32.
72. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, Tardif JC, Baum SJ, Steinhagen-Thiessen E, et al. Lipoprotein (a) reduction in persons with cardiovascular disease. N Engl J Med. 2020;382:244–55.
73. Lamb YN. BNT162b2 mRNA COVID-19 vaccine: first approval. Drugs. 2021;81:495–501.
74. Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ, et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther Nucleic Acids. 2012;1:e37.
75. Evers MJW, Kulkarni JA, van der Meel R, Cullis PR, Vader P, Schiffelers RM. State-of-the-art design and rapid-mixing production techniques of lipid nanoparticles for nucleic acid delivery. Small Methods. 2018;2:1700375.
76. Guo X, Wang H, Li Y, Leng X, Huang W, Ma Y, et al. Transfection reagent Lipofectamine triggers type I interferon signaling activation in macrophages. Immunol Cell Biol. 2019;97:92–6.
77. Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. Mol Ther. 2017;25:1467–75.
78. Francia V, Schiffelers RM, Cullis PR, Witzigmann D. The biomolecular corona of lipid nanoparticles for gene therapy. Bioconjug Chem. 2020;31:2046–59.
79. Chen S, Tam YYC, Lin PJC, Sung MMH, Tam YK, Cullis PR. Influence of particle size on the in vivo potency of lipid nanoparticle formulations of siRNA. J Control Release. 2016; 235:236–44.
80. Mui BL, Tam YK, Jayaraman M, Ansell SM, Du X, Tam YYC, et al. Influence of polyethylene glycol lipid desorption rates on pharmacokinetics and pharmacodynamics of siRNA lipid nanoparticles. Mol Ther Nucleic Acids. 2013;2:e139.
81. Zhang R, El-Mayta R, Murdoch TJ, Warzecha CC, Billingsley MM, Shepherd SJ, et al. Helper lipid structure influences protein adsorption and delivery of lipid nanoparticles to spleen and liver. Biomater Sci. 2021;9:1449–63.
82. Sabnis S, Kumarasinghe ES, Salerno T, Mihai C, Ketova T, Senn JJ, et al. A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. Mol Ther. 2018;26:1509–19.
83. Chen D, Parayath N, Ganesh S, Wang W, Amiji M. The role of apolipoprotein- and vitronectin-enriched protein corona on lipid nanoparticles for *in vivo* targeted delivery and transfection of oligonucleotides in murine tumor models. Nanoscale. 2019;11: 18806–24.
84. Paunovska K, Da Silva Sanchez AJ, Sago CD, Gan Z, Lokugamage MP, Islam FZ, et al. Nanoparticles containing oxidized cholesterol deliver mRNA to the liver microenvironment at clinically relevant doses. Adv Mater. 2019;31:7.
85. Paunovska K, Da Silva Sanchez AJ, Lokugamage MP, Loughrey D, Echeverri ES, Cristian A, et al. The extent to which lipid nanoparticles require apolipoprotein E and low-density lipoprotein receptor for delivery changes with ionizable lipid structure. Nano Lett. 2022;22:10025–33.
86. Dobrowolski C, Paunovska K, Schrader Echeverri E, Loughrey D, Da Silva Sanchez AJ, Ni H, et al. Nanoparticle single-cell multiomic readouts reveal that cell heterogeneity influences lipid nanoparticle-mediated messenger RNA delivery. Nat Nanotechnol. 2022;17:1–9.
87. Wisse E, Jacobs F, Topal B, Frederik P, De Geest B. The size of endothelial fenestrae in human liver sinusoids: implications for hepatocyte-directed gene transfer. Gene Ther. 2008;15: 1193–9.
88. Kim M, Jeong M, Hur S, Cho Y, Park J, Jung H, et al. Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver. Sci Adv. 2021;7:eabf4398.
89. Younis MA, Khalil IA, Abd Elwakil MM, Harashima H. A multifunctional lipid-based nanodevice for the highly specific codelivery of sorafenib and midkine siRNA to hepatic cancer cells. Mol Pharmaceutics. 2019;16:4031–44.
90. Debacker AJ, Voutila J, Catley M, Blakey D, Habib N. Delivery of oligonucleotides to the liver with GalNAc: from research to registered therapeutic drug. Mol Ther. 2020;28:1759–71.
91. Schwartz AL, Geuze HJ, Lodish HF. Recycling of the asialoglycoprotein receptor: biochemical and immunocytochemical evidence. Philos Trans R Soc Lond B Biol Sci. 1982;300:229–35.
92. D'Souza AA, Devarajan PV. Asialoglycoprotein receptor mediated hepatocyte targeting—strategies and applications. J Control Release. 2015;203:126–39.
93. Schwartz AL, Fridovich SE, Lodish HF. Kinetics of internalization and recycling of the asialoglycoprotein receptor in a hepatoma cell line. J Biol Chem. 1982;257:4230–7.
94. Manoharan M. 2′-Carbohydrate modifications in antisense oligonucleotide therapy: importance of conformation, configuration and conjugation. Biochim Biophys Acta. 1999;1489: 117–30.
95. Braasch DA, Jensen S, Liu Y, Kaur K, Arar K, White MA, et al. RNA interference in mammalian cells by chemically-modified RNA. Biochemistry. 2003;42:7967–75.
96. Foster DJ, Brown CR, Shaikh S, Trapp C, Schlegel MK, Qian K, et al. Advanced siRNA designs further improve in vivo performance of GalNAc-siRNA conjugates. Mol Ther. 2018; 26:708–17.
97. Raal FJ, Kallend D, Ray KK, Turner T, Koenig W, Wright RS, et al. Inclisiran for the treatment of heterozygous familial hypercholesterolemia. N Engl J Med. 2020;382:1520–30.
98. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med. 2020;382:1507–19.
99. Amjad MW, Kesharwani P, Mohd Amin MCI, Iyer AK. Recent advances in the design, development, and targeting mechanisms of polymeric micelles for delivery of siRNA in cancer therapy. Prog Polym Sci. 2017;64:154–81.
100. Hosseinkhani H, Domb AJ. Biodegradable polymers in gene-silencing technology. Polym Adv Technol. 2019;30:2647–55.
101. Jiang X, Abedi K, Shi J. Polymeric nanoparticles for RNA delivery. Encyclopedia of Nanomaterials, 1st edn. Elsevier; 2023:555–73.
102. Palmerston Mendes L, Pan J, Torchilin V. Dendrimers as nanocarriers for nucleic acid and drug delivery in cancer therapy. Molecules. 2017;22:1401.
103. Xue L, Yan Y, Kos P, Chen X, Siegwart DJ. PEI fluorination reduces toxicity and promotes liver-targeted siRNA delivery. Drug Deliv Transl Res. 2021;11:255–60.
104. Karlsson J, Rhodes KR, Green JJ, Tzeng SY. Poly(beta-amino ester)s as gene delivery vehicles: challenges and opportunities. Expert Opin Drug Deliv. 2020;17:1395–410.
105. Lai WF, Lin MCM. Nucleic acid delivery with chitosan and its derivatives. J Control Release. 2009;134:158–68.
106. Serrano-Sevilla I, Artiga Á, Mitchell SG, De Matteis L, de la Fuente JM. Natural polysaccharides for siRNA delivery: nanocarriers based on chitosan, hyaluronic acid, and their derivatives. Molecules. 2019;24:2570.
107. Du A, Li S, Zhou Y, Disoma C, Liao Y, Zhang Y, et al. M6A-mediated upregulation of circMDK promotes tumorigenesis and

acts as a nanotherapeutic target in hepatocellular carcinoma. Mol Cancer. 2022;21:109.
108. Vaughan HJ, Zamboni CG, Hassan LF, Radant NP, Jacob D, Mease RC, et al. Polymeric nanoparticles for dual-targeted theranostic gene delivery to hepatocellular carcinoma. Sci Adv. 2022;8:eabo6406.
109. Zielińska A, Carreiró F, Oliveira AM, Neves A, Pires B, Venkatesh DN, et al. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. Molecules. 2020;25:3731.
110. Gentile L, Russo M, Luigetti M, Bisogni G, Di Paolantonio A, Romano A, et al. Patisiran in hATTR amyloidosis: six-month latency period before efficacy. Brain Sci. 2021;11:515.
111. Ando Y, Coelho T, Berk JL, Cruz MW, Ericzon BG, Ikeda S, et al. Guideline of transthyretin-related hereditary amyloidosis for clinicians. Orphanet J Rare Dis. 2013;8:31.
112. Mazzeo A, Russo M, Di Bella G, Minutoli F, Stancanelli C, Gentile L, et al. Transthyretin-related familial amyloid polyneuropathy (TTR-FAP): a single-center experience in Sicily, an Italian endemic area. J Neuromuscul Dis. 2015;2(suppl 2): S39–48.
113. Ledford H. G ene-silencing technology gets first drug approval after 20-year wait. Nature. 2018;560:291–2.
114. Strnad P, McElvaney NG, Lomas DA. Alpha1-antitrypsin deficiency. N Engl J Med. 2020;382:1443–55.
115. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med. 2009;360:2749–57.
116. Hamesch K, Mandorfer M, Pereira VM, Moeller LS, Pons M, Dolman GE, et al. Liver fibrosis and metabolic alterations in adults with alpha-1-antitrypsin deficiency caused by the Pi*ZZ mutation. Gastroenterology. 2019;157:705–719.e18.
117. Townsend SA, Edgar RG, Ellis PR, Kantas D, Newsome PN, Turner AM. Systematic review: the natural history of alpha-1 antitrypsin deficiency, and associated liver disease. Aliment Pharmacol Ther. 2018;47:877–85.
118. Greene CM, Marciniak SJ, Teckman J, Ferrarotti I, Brantly ML, Lomas DA, et al. α1-Antitrypsin deficiency. Nat Rev Dis Primers. 2016;2:16051.
119. Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol. 2021;18:151–66.
120. Poelstra K, Prakash J, Beljaars L. Drug targeting to the diseased liver. J Control Release. 2012;161:188–97.
121. Salvati A, Poelstra K. Drug targeting and nanomedicine: lessons learned from liver targeting and opportunities for drug innovation. Pharmaceutics. 2022;14:217.
122. Chen Z, Jain A, Liu H, Zhao Z, Cheng K. Targeted drug delivery to hepatic stellate cells for the treatment of liver fibrosis. J Pharmacol Exp Ther. 2019;370:695–702.
123. Tu X, Zhang H, Zhang J, Zhao S, Zheng X, Zhang Z, et al. MicroRNA-101 suppresses liver fibrosis by targeting the TGFβ signalling pathway. J Pathol. 2014;234:46–59.
124. Wang J, Chu ESH, Chen HY, Man K, Go MYY, Huang XR, et al. microRNA-29b prevents liver fibrosis by attenuating hepatic stellate cell activation and inducing apoptosis through targeting PI3K/AKT pathway. Oncotarget. 2015;6:7325–38.
125. Yoon Y, Friedman S, Lee Y. Antifibrotic therapies: where are we now? Semin Liver Dis. 2016;36:87–98.
126. Middleton SA, Rajpal N, Cutler L, Mander P, Rioja I, Prinjha RK, et al. BET inhibition improves NASH and liver fibrosis. Sci Rep. 2018;8:17257.
127. Kang JS, Lee MH, Kang JS, Lee MH. Noninvasive diagnostic and prognostic assessment tools for liver fibrosis and cirrhosis in patients with chronic liver disease. Liver Cirrhosis—Update and Current Challenges. IntechOpen; 2017. doi:10.5772/intechopen.68317
128. Jain A, Barve A, Zhao Z, Fetse JP, Liu H, Li Y, et al. Targeted delivery of an siRNA/PNA hybrid nanocomplex reverses carbon tetrachloride-induced liver fibrosis. Adv Ther (Weinh). 2019;2: 1900046.
129. Friedman SL. Targeting siRNA to arrest fibrosis. Nat Biotechnol. 2008;26:399–400.
130. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev. 2008;88:125–72.
131. Han X, Gong N, Xue L, Billingsley MM, El-Mayta R, Shepherd SJ, et al. Ligand-tethered lipid nanoparticles for targeted RNA delivery to treat liver fibrosis. Nat Commun. 2023;14:75.
132. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol. 2017;14:397–411.
133. Ito S, Nagata K. Biology of Hsp47 (Serpin H1), a collagen-specific molecular chaperone. Semin Cell Dev Biol. 2017;62:142–51.
134. Bellaye PS, Burgy O, Bonniaud P, Kolb M. HSP47: a potential target for fibrotic diseases and implications for therapy. Expert Opin Ther Targets. 2021;25:49–62.
135. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet. 2008;371: 838–51.
136. Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, et al. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol. 2008;26:431–42.
137. Beljaars L, Molema G, Schuppan D, Geerts A, De Bleser PJ, Weert B, et al. Successful targeting to rat hepatic stellate cells using albumin modified with cyclic peptides that recognize the collagen type VI receptor. J Biol Chem. 2000;275:12743–51.
138. Yang J, Hou Y, Ji G, Song Z, Liu Y, Dai G, et al. Targeted delivery of the RGD-labeled biodegradable polymersomes loaded with the hydrophilic drug oxymatrine on cultured hepatic stellate cells and liver fibrosis in rats. Eur J Pharm Sci. 2014;52: 180–90.
139. Campbell JS, Hughes SD, Gilbertson DG, Palmer TE, Holdren MS, Haran AC, et al. Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. Proc Natl Acad Sci USA. 2005;102:3389–94.
140. Borkham-Kamphorst E, Weiskirchen R. The PDGF system and its antagonists in liver fibrosis. Cytokine Growth Factor Rev. 2016;28:53–61.
141. Friedman SL. Liver fibrosis–from bench to bedside. J Hepatol. 2003;38:38–53.
142. Beljaars L, Weert B, Geerts A, Meijer DKF, Poelstra K. The preferential homing of a platelet derived growth factor receptor-recognizing macromolecule to fibroblast-like cells in fibrotic tissue. Biochem Pharmacol. 2003;66:1307–17.
143. Zhang J, Shen H, Xu J, Liu L, Tan J, Li M, et al. Liver-targeted siRNA lipid nanoparticles treat hepatic cirrhosis by dual antifibrotic and anti-inflammatory activities. ACS Nano. 2020; 14:6305–22.
144. Li F, Li Q, Wang J, Zhan C, Xie C, Lu W. Effects of interferon-gamma liposomes targeted to platelet-derived growth factor receptor–beta on hepatic fibrosis in rats. J Control Release. 2012;159:261–70.
145. Ribera J, Vilches C, Sanz V, de Miguel I, Portolés I, Córdoba-Jover B, et al. Treatment of hepatic fibrosis in mice based on targeted plasmonic hyperthermia. ACS Nano. 2021;15: 7547–62.
146. Zhao Z, Li Y, Jain A, Chen Z, Liu H, Jin W, et al. Development of a peptide-modified siRNA nanocomplex for hepatic stellate cells. Nanomedicine. 2018;14:51–61.
147. Chen Z, Liu H, Jain A, Zhang L, Liu C, Cheng K. Discovery of aptamer ligands for hepatic stellate cells using SELEX. Theranostics. 2017;7:2982–95.
148. Bansal R, Prakash J, Post E, Beljaars L, Schuppan D, Poelstra K. Novel engineered targeted interferon-gamma blocks hepatic fibrogenesis in mice. Hepatology. 2011;54:586–96.
149. Van De Sluis B, Wijers M, Herz J. News on the molecular regulation and function of hepatic LDLR and LRP1. Curr Opin Lipidol. 2017;28:241–7.

150. Gao R, Brigstock DR. Low density lipoprotein receptor-related protein (LRP) is a heparin-dependent adhesion receptor for connective tissue growth factor (CTGF) in rat activated hepatic stellate cells. Hepatol Res. 2003;27:214–20.
151. Rudenko G, Henry L, Henderson K, Ichtchenko K, Brown MS, Goldstein JL, et al. Structure of the LDL receptor extracellular domain at endosomal pH. Science. 2002;298:2353–8.
152. Zhao Y, Imura T, Leman LJ, Curtiss LK, Maryanoff BE, Ghadiri MR. Mimicry of high-density lipoprotein: functional peptide-lipid nanoparticles based on multivalent peptide constructs. J Am Chem Soc. 2013;135:13414–24.
153. Higashi N, Sato M, Kojima N, Irie T, Kawamura K, Mabuchi A, et al. Vitamin A storage in hepatic stellate cells in the regenerating rat liver: With special reference to zonal heterogeneity. Anat Rec A Discov Mol Cell Evol Biol. 2005;286: 899–907.
154. Zhang Z, Wang C, Zha Y, Hu W, Gao Z, Zang Y, et al. Corona-directed nucleic acid delivery into hepatic stellate cells for liver fibrosis therapy. ACS Nano. 2015;9:2405–19.
155. Hwang I, Lee EJ, Park H, Moon D, Kim HS. Retinol from hepatic stellate cells via STRA6 induces lipogenesis on hepatocytes during fibrosis. Cell Biosci. 2021;11:3.
156. You DG, Oh BH, Nguyen VQ, Lim GT, Um W, Jung JM, et al. Vitamin A-coupled stem cell-derived extracellular vesicles regulate the fibrotic cascade by targeting activated hepatic stellate cells in vivo. J Control Release. 2021;336: 285–95.
157. Urashima S, Tsutsumi M, Ozaki K, Tsuchishima M, Shimanaka K, Ueshima Y, et al. Immunohistochemical study of hyaluronate receptor (CD44) in alcoholic liver disease. Alcohol Clin Exp Res. 2000;24(suppl):34S–8S.
158. Bakrania AK, Variya BC, Patel SS. Novel targets for paclitaxel nano formulations: hopes and hypes in triple negative breast cancer. Pharmacol Res. 2016;111:577–91.
159. Kim KS, Hur W, Park SJ, Hong SW, Choi JE, Goh EJ, et al. Bioimaging for targeted delivery of hyaluronic acid derivatives to the livers in cirrhotic mice using quantum dots. ACS Nano. 2010;4:3005–14.
160. Wang L, Su W, Liu Z, Zhou M, Chen S, Chen Y, et al. CD44 antibody-targeted liposomal nanoparticles for molecular imaging and therapy of hepatocellular carcinoma. Biomaterials. 2012;33:5107–14.
161. Douglass A, Wallace K, Koruth M, Barelle C, Porter AJ, Wright MC. Targeting liver myofibroblasts: a novel approach in anti-fibrogenic therapy. Hepatol Int. 2008;2:405–15.
162. Calvente CJ, Sehgal A, Popov Y, Kim YO, Zevallos V, Sahin U, et al. Specific hepatic delivery of procollagen α1(I) small interfering RNA in lipid-like nanoparticles resolves liver fibrosis. Hepatology. 2015;62:1285–97.
163. Toriyabe N, Sakurai Y, Kato A, Yamamoto S, Tange K, Nakai Y, et al. The delivery of small interfering RNA to hepatic stellate cells using a lipid nanoparticle composed of a vitamin A-scaffold lipid-like material. J Pharm Sci. 2017;106:2046–52.
164. Kaps L, Nuhn L, Aslam M, Brose A, Foerster F, Rosigkeit S, et al. In vivo gene-silencing in fibrotic liver by siRNA-loaded cationic nanohydrogel particles. Adv Healthc Mater. 2015;4: 2809–15.
165. Smith L, Byrne HL, Waddington D, Kuncic Z. Nanoparticles for MRI-guided radiation therapy: a review. Cancer Nano. 2022;13: 38.
166. Manzari MT, Shamay Y, Kiguchi H, Rosen N, Scaltriti M, Heller DA. Targeted drug delivery strategies for precision medicines. Nat Rev Mater. 2021;6:351–70.
167. Popov Y, Schuppan D. Targeting liver fibrosis: strategies for development and validation of antifibrotic therapies. Hepatology. 2009;50:1294–306.
168. Du S-L, Pan H, Lu W-Y, Wang J, Wu J, Wang J-Y. Cyclic Arg-Gly-Asp peptide-labeled liposomes for targeting drug therapy of hepatic fibrosis in rats. J Pharmacol Exp Ther. 2007;322: 560–8.
169. Parlati L, Régnier M, Guillou H, Postic C. New targets for NAFLD. JHEP Rep. 2021;3:100346.
170. Du J, Ji Y, Qiao L, Liu Y, Lin J. Cellular endo-lysosomal dysfunction in the pathogenesis of non-alcoholic fatty liver disease. Liver Int. 2020;40:271–80.
171. Lu Q, Tian X, Wu H, Huang J, Li M, Mei Z, et al. Metabolic changes of hepatocytes in NAFLD. Front Physiol. 2021;12: 710420.
172. Kwanten WJ, Martinet W, Michielsen PP, Francque SM. Role of autophagy in the pathophysiology of nonalcoholic fatty liver disease: a controversial issue. World J Gastroenterol. 2014;20: 7325–38.
173. Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cell Mol Life Sci. 2018;75:3313–27.
174. Zhou JE, Sun L, Liu L, Jia Y, Han Y, Shao J, et al. Hepatic macrophage targeted siRNA lipid nanoparticles treat non-alcoholic steatohepatitis. J Control Release. 2022;343:175–86.
175. Gaskell H, Ge X, Desert R, Das S, Han H, Lantvit D, et al. Ablation of Hmgb1 in intestinal epithelial cells causes intestinal lipid accumulation and reduces NASH in mice. Hepatol Commun. 2020;4:92–108.
176. Bakrania A, Zheng G, Bhat M. Nanomedicine in hepatocellular carcinoma: a new frontier in targeted cancer treatment. Pharmaceutics. 2021;14:41.
177. Bakrania A, Joshi N, Zhao X, Zheng G, Bhat M. Artificial intelligence in liver cancers: decoding the impact of machine learning models in clinical diagnosis of primary liver cancers and liver cancer metastases. Pharmacol Res. 2023;189: 106706.
178. Bakrania A, To J, Zheng G, Bhat M. Targeting Wnt-β-catenin signaling pathway for HCC nanomedicine. Gastro Hep Advances. 2023;2:948–63.
179. Xiao Y, Chen J, Zhou H, Zeng X, Ruan Z, Pu Z, et al. Combining p53 mRNA nanotherapy with immune checkpoint blockade reprograms the immune microenvironment for effective cancer therapy. Nat Commun. 2022;13:758.
180. Kastenhuber ER, Lowe SW. Putting p53 in context. Cell. 2017; 170:1062–78.
181. Blagih J, Buck MD, Vousden KH. p53, cancer and the immune response. J Cell Sci. 2020;133:jcs237453.
182. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature. 2007;445:656–60.
183. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature. 1991;351:453–6.
184. Chipumuro E, Siddiquee Z, Ganesh S, Shui S, Shah A, Kim B, et al. Abstract 2925: Anti-tumor activity of a MYC-targeting dicer substrate siRNA in combination with BRD4/CDK7 inhibitors. Cancer Res. 2016;76(suppl):2925.
185. Xie Y, Wang Y, Li J, Hang Y, Jaramillo L, Wehrkamp CJ, et al. Cholangiocarcinoma therapy with nanoparticles that combine downregulation of microRNA-210 with inhibition of cancer cell invasiveness. Theranostics. 2018;8:4305–20.
186. Yang T, Deng Z, Xu L, Li X, Yang T, Qian Y, et al. Macrophages-aPKCι-CCL5 feedback loop modulates the progression and chemoresistance in cholangiocarcinoma. J Exp Clin Cancer Res. 2022;41:23.
187. Liu C, Wu K, Li J, Mu X, Gao H, Xu X. Nanoparticle-mediated therapeutic management in cholangiocarcinoma drug targeting: current progress and future prospects. Biomed Pharmacother. 2023;158:114135.
188. Milette S, Sicklick JK, Lowy AM, Brodt P. Molecular pathways: targeting the microenvironment of liver metastases. Clin Cancer Res. 2017;23:6390–9.

189. Tsilimigras DI, Brodt P, Clavien PA, Muschel RJ, D'Angelica MI, Endo I, et al. Liver metastases. Nat Rev Dis Primers. 2021;7:27.
190. Zhou H, Liu Z, Wang Y, Wen X, Amador EH, Yuan L, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Sig Transduct Target Ther. 2022;7:1–25.
191. Kang SH, Revuri V, Lee SJ, Cho S, Park IK, Cho KJ, et al. Oral siRNA delivery to treat colorectal liver metastases. ACS Nano. 2017;11:10417–29.
192. Nurunnabi M, Khatun Z, Revuri V, Nafiujjaman M, Cha S, Cho S, et al. Design and strategies for bile acid mediated therapy and imaging. RSC Adv. 2016;6:73986–4002.
193. Cummings MC, Simpson PT, Reid LE, Jayanthan J, Skerman J, Song S, et al. Metastatic progression of breast cancer: insights from 50 years of autopsies. J Pathol. 2014;232:23–31.
194. Diamond JR, Finlayson CA, Borges VF. Hepatic complications of breast cancer. Lancet Oncol. 2009;10:615–21.
195. Zhao H, Gong Y, Ye F, Ling H, Hu X. Incidence and prognostic factors of patients with synchronous liver metastases upon initial diagnosis of breast cancer: a population-based study. Cancer Manag Res. 2018;10:5937–50.
196. Kennecke H, Yerushalmi R, Woods R, Cheang MCU, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol. 2010;28:3271–7.
197. Horn SR, Stoltzfus KC, Lehrer EJ, Dawson LA, Tchelebi L, Gusani NJ, et al. Epidemiology of liver metastases. Cancer Epidemiol. 2020;67:101760.
198. Rashid NS, Grible JM, Clevenger CV, Harrell JC. Breast cancer liver metastasis: current and future treatment approaches. Clin Exp Metastasis. 2021;38:263–77.
199. Liu X, Zhang G, Yu T, He J, Liu J, Chai X, et al. Exosomes deliver lncRNA DARS-AS1 siRNA to inhibit chronic unpredictable mild stress-induced TNBC metastasis. Cancer Lett. 2022;543:215781.
200. Kokuryo T, Hibino S, Suzuki K, Watanabe K, Yokoyama Y, Nagino M, et al. Nek2 siRNA therapy using a portal venous port–catheter system for liver metastasis in pancreatic cancer. Cancer Sci. 2016;107:1315–20.
201. Kokuryo T, Senga T, Yokoyama Y, Nagino M, Nimura Y, Hamaguchi M. Nek2 as an effective target for inhibition of tumorigenic growth and peritoneal dissemination of cholangiocarcinoma. Cancer Res. 2007;67:9637–42.
202. Tsunoda N, Kokuryo T, Oda K, Senga T, Yokoyama Y, Nagino M, et al. Nek2 as a novel molecular target for the treatment of breast carcinoma. Cancer Sci. 2009;100:111–6.
203. Suzuki K, Kokuryo T, Senga T, Yokoyama Y, Nagino M, Hamaguchi M. Novel combination treatment for colorectal cancer using Nek2 siRNA and cisplatin. Cancer Sci. 2010; 101:1163–9.
204. Huang KW, Lai YT, Chern GJ, Huang SF, Tsai CL, Sung YC, et al. Galactose derivative-modified nanoparticles for efficient siRNA delivery to hepatocellular carcinoma. Biomacromolecules. 2018;19:2330–9.
205. Khan AA, Alanazi AM, Jabeen M, Chauhan A, Ansari MA. Therapeutic potential of functionalized siRNA nanoparticles on regression of liver cancer in experimental mice. Sci Rep. 2019; 9:15825.
206. Younis MA, Khalil IA, Elewa YHA, Kon Y, Harashima H. Ultrasmall lipid nanoparticles encapsulating sorafenib and midkine-siRNA selectively-eradicate sorafenib-resistant hepatocellular carcinoma in vivo. J Control Release. 2021;331:335–49.
207. Yin T, Wang P, Li J, Wang Y, Zheng B, Zheng R, et al. Tumor-penetrating codelivery of siRNA and paclitaxel with ultrasound-responsive nanobubbles hetero-assembled from polymeric micelles and liposomes. Biomaterials. 2014;35:5932–43.
208. Hsu S-H, Yu B, Wang X, Lu Y, Schmidt CR, Lee RJ, et al. Cationic lipid nanoparticles for therapeutic delivery of siRNA and miRNA to murine liver tumor. Nanomedicine. 2013;9: 1169–80.
209. Liang J, Zhang X, He S, Miao Y, Wu N, Li J, et al. Sphk2 RNAi nanoparticles suppress tumor growth via downregulating cancer cell derived exosomal microRNA. J Control Release. 2018;286:348–57.
210. Varshney A, Panda JJ, Singh AK, Yadav N, Bihari C, Biswas S, et al. Targeted delivery of microRNA-199a-3p using self-assembled dipeptide nanoparticles efficiently reduces hepatocellular carcinoma in mice. Hepatology. 2018;67:1392–407.
211. Lai I, Swaminathan S, Baylot V, Mosley A, Dhanasekaran R, Gabay M, et al. Lipid nanoparticles that deliver IL-12 messenger RNA suppress tumorigenesis in MYC oncogene-driven hepatocellular carcinoma. J Immunother Cancer. 2018;6: 125.
212. Wu J, Nandamuri KM. Inhibition of hepatitis viral replication by siRNA. Expert Opin Biol Ther. 2004;4:1649–59.
213. The Lancet. Viral hepatitis elimination: a challenge, but within reach. Lancet. 2022;400:251.
214. Mieli-Vergani G, Vergani D, Czaja AJ, Manns MP, Krawitt EL, Vierling JM, et al. Autoimmune hepatitis. Nat Rev Dis Primers. 2018;4:18017.
215. Huang Y, Zheng S, Guo Z, Mollerat du Jeu X, Liang X-J, Yang Z, et al. Ionizable liposomal siRNA therapeutics enables potent and persistent treatment of Hepatitis B. Signal Transduct Target Ther. 2022;7:38.
216. Tan H, Kang H, Luo M, Nie Y, Pandey R, Montero SM, et al. Combination drug interactions of hepatitis B virus (HBV) small interfering RNA (siRNA) and antisense oligonucleotides (ASO) in vitro and in vivo J Hepatol, 2021;75:S720.
217. Schluep T, Lickliter J, Hamilton J, Lewis DL, Lai CL, Lau JY, et al. Safety, tolerability, and pharmacokinetics of ARC-520 injection, an RNA interference-based therapeutic for the treatment of chronic hepatitis B virus infection, in healthy volunteers. Clin Pharmacol Drug Dev. 2017;6:350–62.
218. Wooddell CI, Yuen MF, Chan HLY, Gish RG, Locarnini SA, Chavez D, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med. 2017;9: eaan0241.
219. Streinu-Cercel A, Gane E, Cheng W, Sievert W, Roberts S, Ahn SH, et al. A phase 2a study evaluating the multi-dose activity of ARB-1467 in HBeAg positive and negative virally suppressed subjects with hepatitis B. J Hepatol. 2017;66((S)(66)):S688–9.
220. Yuen MF, Lim TH, Kim W, Tongkijvonich P, Yoon J-H, Sievert W, et al HBV RNAi inhibitor RG6346 in phase 1b-2a TRIAL was safe, well-tolerated, and resulted in substantial and durable reductions in serum HBsAg levels. AASLD; 2020. Accessed March 5, 2023. https://aasld.confex.com/aasld/2020/meetingapp.cgi/Paper/24466
221. Gane E, Lim TH, Cloutier D, Shen L, Cathcart AL, Ding X, et al Safety and antiviral activity of VIR-2218, an X-targeting RNAi therapeutic, in participants with chronic hepatitis B infection: week 48 follow-up results. J Hepatol. 2021;75:S287–8.
222. Gane E, Locarnini S, Lim TH, Strasser S, Sievert W, Cheng W, et al. Short interfering RNA JNJ-3989 combination therapy in chronic hepatitis B shows potent reduction of all viral markers but no correlate was identified for HBsAg reduction and baseline factors. J Hepatol.;2021;75:S289–90.
223. Jiang N, Zhang X, Zheng X, Chen D, Siu K, Wang H, et al. A novel in vivo siRNA delivery system specifically targeting liver cells for protection of ConA-induced fulminant hepatitis. PLoS One. 2012;7:e44138.
224. Kim SI, Shin D, Lee H, Ahn BY, Yoon Y, Kim M. Targeted delivery of siRNA against hepatitis C virus by apolipoprotein AI-bound cationic liposomes. J Hepatol. 2009;50:479–88.
225. Kawase W, Kurotaki D, Suzuki Y, Ishihara H, Ban T, Sato GR, et al. Irf5 siRNA-loaded biodegradable lipid nanoparticles ameliorate concanavalin A-induced liver injury. Mol Ther—Nucleic Acids. 2021;25:708–15.

226. Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. Hepatology. 2008;47:729–36.
227. Schmucker DL. Liver function and phase I drug metabolism in the elderly. Drugs Aging. 2001;18:837–51.
228. Reddy JK, Sambasiva Rao M. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. Am J Physiol-Gastrointest Liver Physiol. 2006;290:G852–8.
229. Chiang JYL. Bile acid metabolism and signaling. Compr Physiol. 2013;3:1191–212.
230. Rui L. Energy metabolism in the liver. Compr Physiol. 2014;4: 177–97. doi:10.1002/cphy.c130024
231. van den Berghe G. The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. J Inherit Metabol Dis. 1991;14:407–20.
232. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. Lancet. 2010;376:1417–27.
233. Özen H. Glycogen storage diseases: new perspectives. World J Gastroenterol. 2007;13:2541–53.
234. Hansen K, Horslen S. Metabolic liver disease in children. Liver Transpl. 2008;14:391–411.
235. Papanikolaou G, Pantopoulos K. Iron metabolism and toxicity. Toxicol Appl Pharmacol. 2005;202:199–211.
236. Berraondo P, Martini PGV, Avila MA, Fontanellas A. Messenger RNA therapy for rare genetic metabolic diseases. Gut. 2019;68: 1323–30.
237. Córdoba KM, Jericó D, Sampedro A, Jiang L, Iraburu MJ, Martini PGV, et al. Chapter two—Messenger RNA as a personalized therapy: the moment of truth for rare metabolic diseases. Int Rev Cell Mol Biol. 2022;372:55–96.
238. Stein JA, Tschudy DP. Acute intermittent porphyria: a clinical and biochemical study of 46 patients. Medicine. 1970;49:1–16.
239. Jiang L, Berraondo P, Jericó D, Guey LT, Sampedro A, Frassetto A, et al. Systemic messenger RNA as an etiological treatment for acute intermittent porphyria. Nat Med. 2018;24: 1899–909.
240. Jericó D, Córdoba KM, Jiang L, Schmitt C, Morán M, Sampedro A, et al. mRNA-based therapy in a rabbit model of variegate porphyria offers new insights into the pathogenesis of acute attacks. Mol Ther—Nucleic Acids. 2021;25:207–19.
241. An D, Schneller JL, Frassetto A, Liang S, Zhu X, Park JS, et al. Systemic messenger RNA therapy as a treatment for methylmalonic acidemia. Cell Rep. 2017;21:3548–58.
242. Jiang L, Park JS, Yin L, Laureano R, Jacquinet E, Yang J, et al. Dual mRNA therapy restores metabolic function in long-term studies in mice with propionic acidemia. Nat Commun. 2020;11: 5339.
243. Li L-C, Okino ST, Zhao H. Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci USA. 2006;103: 17337–42.
244. Kwok A, Raulf N, Habib N. Developing small activating RNA as a therapeutic: current challenges and promises. Ther Deliv. 2019;10:151–64.
245. Pennacchio LA, Ahituv N, Moses AM, Prabhakar S, Nobrega MA, Shoukry M, et al. In vivo enhancer analysis of human conserved non-coding sequences. Nature. 2006;444: 499–502.
246. Visel A, Prabhakar S, Akiyama JA, Shoukry M, Lewis KD, Holt A, et al. Ultraconservation identifies a small subset of extremely constrained developmental enhancers. Nat Genet. 2008;40: 158–60.
247. Juliano R, Alam MR, Dixit V, Kang H. Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. Nucleic Acids Res. 2008;36:4158–71.
248. Meng X, Jiang Q, Chang N, Wang X, Liu C, Xiong J, et al. Small activating RNA binds to the genomic target site in a seed-region-dependent manner. Nucleic Acids Res. 2016;44: 2274–82.
249. Portnoy V, Lin SHS, Li KH, Burlingame A, Hu ZH, Li H, et al. saRNA-guided Ago2 targets the RITA complex to promoters to stimulate transcription. Cell Res. 2016;26:320–35.
250. Jaehning JA. The Paf1 complex: platform or player in RNA polymerase II transcription. Biochim Biophys Acta. 2010;1799: 379–88.
251. Zhao X, Reebye V, Hitchen P, Fan J, Jiang H, Sætrom P, et al. Mechanisms involved in the activation of C/EBPα by small activating RNA in hepatocellular carcinoma. Oncogene. 2019; 38:3446–57.
252. Akai Y, Oitate T, Koike T, Shiojiri N. Impaired hepatocyte maturation, abnormal expression of biliary transcription factors and liver fibrosis in C/EBPα(Cebpa)-knockout mice. Histol Histopathol. 2014;29:107–25.
253. Tseng HH, Hwang YH, Yeh KT, Chang JG, Chen YL, Yu HS. Reduced expression of C/EBP alpha protein in hepatocellular carcinoma is associated with advanced tumor stage and shortened patient survival. J Cancer Res Clin Oncol. 2009; 135:241–7.
254. Voutila J, Reebye V, Roberts TC, Protopapa P, Andrikakou P, Blakey DC, et al. Development and mechanism of small activating RNA targeting CEBPA, a novel therapeutic in clinical trials for liver cancer. Mol Ther. 2017;25:2705–14.
255. Verduci L, Strano S, Yarden Y, Blandino G. The circRNA–microRNA code: emerging implications for cancer diagnosis and treatment. Mol Oncol. 2019;13:669–80.
256. Ebbesen KK, Hansen TB, Kjems J. Insights into circular RNA biology. RNA Biol. 2017;14:1035–45.
257. Vo JN, Cieslik M, Zhang Y, Shukla S, Xiao L, Zhang Y, et al. The landscape of circular RNA in cancer. Cell. 2019;176: 869–881.e13.
258. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, et al. Circular RNA: a new star of noncoding RNAs. Cancer Lett. 2015;365: 141–8.
259. Santer L, Bär C, Thum T. Circular RNAs: a novel class of functional RNA molecules with a therapeutic perspective. Mol Ther. 2019;27:1350–63.
260. Song M, Xia L, Sun M, Yang C, Wang F. Circular RNA in liver: health and diseases. Circular RNAs Vol 1087 Advances in Experimental Medicine and Biology. Springer; 2018:245–57.
261. Su Q, Kumar V, Sud N, Mahato RI. MicroRNAs in the pathogenesis and treatment of progressive liver injury in NAFLD and liver fibrosis. Adv Drug Deliv Rev. 2018;129:54–63.
262. Chen Y, Yuan B, Wu Z, Dong Y, Zhang L, Zeng Z. Microarray profiling of circular RNAs and the potential regulatory role of hsa_circ_0071410 in the activated human hepatic stellate cell induced by irradiation. Gene. 2017;629:35–42.
263. Xu H, Guo S, Li W, Yu P. The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells. Sci Rep. 2015;5:12453.
264. Zhao ZJ, Shen J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. RNA Biol. 2017;14: 514–21.
265. Wesselhoeft RA, Kowalski PS, Parker-Hale FC, Huang Y, Bisaria N, Anderson DG. RNA circularization diminishes immunogenicity and can extend translation duration in vivo. Mol Cell. 2019;74:508–520.e4.
266. Porto EM, Komor AC, Slaymaker IM, Yeo GW. Base editing: advances and therapeutic opportunities. Nat Rev Drug Discov. 2020;19:839–59.
267. Zhang X, Zhao W, Nguyen GN, Zhang C, Zeng C, Yan J, et al. Functionalized lipid-like nanoparticles for in vivo mRNA delivery and base editing. Sci Adv. 2020;6:eabc2315.

268. Packer MS, Chowdhary V, Lung G, Cheng LI, Aratyn-Schaus Y, Leboeuf D, et al. Evaluation of cytosine base editing and adenine base editing as a potential treatment for alpha-1 antitrypsin deficiency. Mol Ther. 2022;30:1396–406.
269. Villiger L, Rothgangl T, Witzigmann D, Oka R, Lin PJC, Qi W, et al. In vivo cytidine base editing of hepatocytes without detectable off-target mutations in RNA and DNA. Nat Biomed Eng. 2021;5:179–89.
270. Villiger L, Grisch-Chan HM, Lindsay H, Ringnalda F, Pogliano CB, Allegri G, et al. Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. Nat Med. 2018;24:1519–25.

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