Gene Therapies in the Fight Against Liver Disease: A Comprehensive Review

Abstract:

Introduction:

The field of hepatology is rapidly evolving, with new treatments being investigated using gene therapy. Gene therapy is the concept of using a patient's genetic code and altering it to create a desired phenotype/genotype.

Areas covered:

Different methods of gene therapy include gene editing, gene silencing, gene transfer, and mRNA therapy. These genetic modulations are achieved through several modalities. Examples include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), cluster-regulated interspaced short palindromic repeats with associated cast 9 proteins (CRISPR/Cas9), SiRNA particles, antisense oligonucleotides (ASO), and synthetically engineered mRNA. Initially, the method for gene therapy included injecting genetic code, usually in a vector, to create phenotypic production in gene-deficient states.Recently, gene therapy has includedmore precise editing of the genome with technologies such as CRISPR-Cas9 and silencing of pathogenic genes.

Expert opinion:

Within this emerging domain, there is a multitude of delivery mediums used to alter genes. It is essential to understand the mechanisms of genetic therapies and the risks and benefits associated with them. This review aims to breakdown the mechanisms of action of gene therapies, describecurrent research that has been conducted, and discuss future implications for the field of hepatology.

Keywords : Gene Therapies, Vectors, CRISPR-Cas9, TALENs, ZFNs, ASOs, mRNA therapy, Liver Disease

I. Introduction

The field of hepatology has drastically changed over the last two decades with rapid growth in treatment options for chronic liver diseases such as hepatitis C, and new pathogenic processes being identified including the genetic basis for several diseases. The field continues to push the envelope of treatments with gene therapy which is the concept of using a patient's genetic code and altering it to create a desired phenotype/genotype. This method of therapy has been growing in the scientific community with numerous proof-of-concept studies being completed in the last decade and current clinical trials underway. Within this emerging domain, there is a multitude of delivery mediums used to therapeutically alter one’s genetic material. Starting with vectors: there are viral vectors as well as nonviral vectors to help deliver genetic code into cells. Viral vectors include adenoviral vectors, adeno-associated viral vectors, and lentiviral vectors. There are also various nonviral factors, including lipid-nanoparticles and cation polymers.[1-12]Additionally, there are methods of delivering naked genetic material using hemodynamic injections. The listed vectors above are being used to deliver genetic material and gene-modifying molecules to promote specific desired genotypical/phenotypical outcomes in cells. Delivery vesicles can also be modified to target liver-specific cells.[7,11] Specificity for target molecules decreases the off-target effects from gene therapy. Off-target effects of gene therapy and immune reactions secondary to vectors are serious and potentially deadly consideration associated with these new therapy modalities.

Once the genetic material/modifying molecules have been delivered to the hepatocytes, various molecular mechanisms are utilized to address disease. Gene editing, gene silencing, gene transfer, and mRNA therapy are different methods of achieving the desired phenotype/genotype. The field has focused mostly on gene editing with the use of zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and cluster regulated interspaced short palindromic repeats with associated cast 9 proteins (CRISPR/Cas9). ZFNs are nucleases that use multiple base pairs to bind to specific parts of DNA and cause double-strand breaks, where these breaks occur, DNA can be introduced.[13]TALENs use a similar method as ZFNs with a locus that binds to DNA and causes double-stranded breaks through a FokI nuclease domain, the difference is TALENs use TALE proteins to bind to DNA. CRISPR/Cas9 is a strand of DNA derived from bacteria that uses specified proteins on its peripheries to target specific codons, and allows for rapid splitting of DNA, creating a binding location for new DNA. Gene silencing uses SiRNA particles, and antisense oligonucleotides (ASO). SiRNA particles and ASOs are similar in the sense they bind to mRNA and activate a molecule to silence the mRNA strand, the difference is ASOs are single-stranded siRNAs are double-stranded, and siRNA activates RNA-induced silencing complex (RISC) and ASOs activate RNase H endonucleases. Gene transfer is used for gene-deficient states, where genetic code is injected, usually in a vector, to create a phenotypic production of the desired protein. This method does not create a strong response but is effective in disease states that require minimal amounts of genes to suppress/create a desired reaction. mRNA therapy is a treatment that has gained a lot of traction recently as a gene modifying therapy. mRNA therapy in a simplified manner, is synthetically engineered mRNA which is injected into cells to allow for the replication of specific proteins. The goals of these therapies in hepatology are broad, ranging from treatment for hepatitis B virus (HBV), rare genetic liver diseases, to providing a possible cure for hepatocellular carcinoma (HCC). This paper aims to simplify complex but pertinent gene therapies that are currently being studied and provide an overview of current trials. A fundamental understanding of these future therapies is important for clinicians and researchers involved in taking care of liver diseases.

II. Delivery Vectors

Viral Delivery Vectors

Adenoviral vector:

Adenoviruses are non-enveloped double-stranded DNA vectors that have been used in the field of oncology.[1] Their maximum packaging capacity is anywhere from 36-37 kb.[1,8] The advantage of using adenovirus vectors is their efficient transduction into liver cells.[1] The disadvantages to adenoviral vectors include the possible elicitation of a strong immune response, the presence of serotype-dependent pre-existing immunity, and the development of acute inflammatory responses.[1,8] The utility of adenoviral vectors has been discussed in several studies examining the safety and efficacy of such a medium. One study displayed successful lacZ gene (gene that creates β-galactosidase which cleaves lactose, a disaccharide, into glucose and galactose) transfer via adenoviral vectors to normal and cirrhotic livers in a mouse model. Histochemical evaluation revealed marked transgene expression even in fulminant hepatitis mouse livers without significant differences in cellular or humoral immune response among normal, cirrhotic, and hepatitis mouse livers.[14] A similar study portrayed the therapeutic potential of adenoviral vectors, specifically in the role against hepatocellular carcinoma. In-vivo and In-vitro suppression of hepatocellular carcinoma was achieved through delivery of adenoviral vector Ad-ECRG2.[15] Esophageal cancer-related gene 2 is critical in carcinogenesis.

Ad-ECRG2 successfully suppressed the invasion and adhesion of cancer cells while altering the expression of multiple key cancer-related molecules.[15]

Adeno-associated virus derived vector:

Adeno-associated viruses (AAV) are non-enveloped single-stranded DNA vectors that can act as a delivery system for genetic material into cells.[1]The maximum package size for AAV vectors is 47 KB.[1]The advantages of AAVs are that they create a low inflammatory response, and have high liver transduction, making them the most commonly used vectors for gene delivery.[1,8] However, these viruses remain mostly episomal in cells which prevents endo-nucleation and integration into the cellular DNA.[1] This may lead to loss of therapeutic effect especially in young patients due to natural cell division. Another downside associated with using this vector is serotype dependent pre-existing immunity in some individuals given the fact that up to 30-80% of humans are exposed to natural AAV infection early in their lives.[1,8] Utilization of this type of viral vector in theory would require serotype testing prior to initiation of treatment to determine efficacy.

There has been a proof-of-concept study completed using AAV's with the induction of hepatitis B virus (HBV). The study implemented AAV8 to carry HBV genotype D into mouse cells to stimulate a chronic infection. A similar study was completed in rhesus monkeys, a species that is not naturally capable of being infected with HBV, where the monkeys were given the HBV specific receptor hNTCP carried in AAV8. These Rhesus monkeys were then infected with HBV and showed viral replication up to 24 weeks after inoculation.[6] AAV vectors are currently being utilized in several clinical trials and are well tolerated. This vector has been utilized in Phase I studies targeting Hemophilia B,[16-26] Hemophilia A,[27-32] Ornithine Transcarbamylase(OTC) deficiency,[33-36] Phenylketonuria,[37,38]Acute Intermittent Porphyria (AIP),[39]Methylmalonic Acidemia,[40]Familial Hypercholesterolemia,[41]FabryDisease,[42-44]Mucopolysaccharide Syndrome (MPS) I, MPS II, MPS IV,[21,45-47]Wilsons Disease,[48]and Crigler Najjar.[49,50] Phase II studies are ongoing targeting several of these pathological conditions.

Another study using adeno-associated vector encoding human ATP7B cDNA in the hepatocytes of mouse model with Wilson disease demonstrated long term correction of copper metabolism and prevented liver injury.[51] In addition, a shorter vector coding for a mini ATP7B protein was also shown to be effective in providing long-term correction of copper metabolism in mouse models regardless of sex or stage of Wilson disease.[52]

Lentiviral vector:

Lentiviral vectors are enveloped, short-stranded RNA vectors that can carry up to 10 KB of material.[1] Factors like long-term gene expression due to integration into the host genome and low pre-existing immunity in humans are thought to be major advantages for the utilization of Lentiviral vectors.[1,8] Their use in in-vivo studies are poorly characterized at this time.[2] One important consideration with lentiviral vectors is their possible genotoxicity associated with factor designs.[1] Two studies have successfully displayed the role of lentiviral vectors against hereditary tyrosinemia type 1 (HT-1). The first study conducted by Kaiser et. al. utilized lentiviral vectors in a unique manner for gene delivery via ex-vivo hepatocyte transduction in an attempt to cure HT-1.[53] Once these hepatocytes had been corrected, they were then injected via the portal vein back to the autologous donor. In a porcine model, this study displayed high levels of success without progression of cirrhosis and HCC and the complete reversal of early fibrosis along with no detectable uncorrected cells threeyears post-therapy.[53] A recent in-vivo study with a similar objective presented a cure of HT-1 via lentiviral vector specifically targeting

the expression of a human fumarylacetoacetate hydrolase (*FAH*) gene within a porcine model.[19] The in-vivo lentiviral vector administration was deemed to be superior to alternate in-vitro therapies while also having a benign profile and similar rates of fibrosis/tumorigenicity as wild-type animals.[54] Lentiviral gene transfer that integrates the ATP7B gene into the genome was shown to be effective for prevent disease progression in a rat model of Wilson disease.[55]

Nonviral Vectors:

Nonviral vectors are advantageous compared to viral vectors due to their ability to be mass produced, production cost, longer shelf-life, larger carrying capacity, and better safety profile.[5] The disadvantages to nonviral vectors include inefficiency at translocating into the nucleus, and maintaining a sustained response.[8] The suboptimal ability to enter the nucleus is a large limitation but has shown some promise of integration when dealing with enterocytes due to their rapid nuclear breakdown in rodents.[5] Two of the most efficacious nonviral vectors are cation polymers and lipid nanoparticles.

Cation Polymers:

Cation polymers interact with nucleic acids on the cell membrane using electrostatic interactions to allow passage of the vesicle. A common cation polymer used is polyethylenimine due to its high transduction rate.[7] One of the more efficient transductions documented is glycosylated polyethylenimine targeting the asialoglycoprotein receptor, which has been studied in cell lines and mice/rat livers.[7]

Lipid Nanoparticles:

Lipid nanoparticles are manufactured similarly to cell membranes to allow for passage through the membrane. They are composed of amphiphilic lipids creating a micelle with genetic material trapped on the inside with a hydrophilic layer, and outside coated hydrophobic layer. The bio-similarity to cell membranes gives the lipid nanoparticle low toxicity, low immunogenicity, and structural flexibility.[8] Other advantages that lipid nanoparticles provide are their ability to be mass produced and their inherent biodegradability.[8] The disadvantage of using lipid nanoparticles is their lack of specificity for cells. This problem has been combated by binding N-acetylgalactosamine(GalNAc) clusters to the lipid nanoparticles to help target cells more specifically.[8] Another method to help specificity of lipid nanoparticles is the use of polyethelyne glycol (PEG). This was shown in a medication approved by the FDA in 2018 for TTR-type familial amyloid polyneuropathy. The lipid nanoparticle used a PEG siRNA system that targeted transthyretin.[11] The lipid nanoparticle is taken up via endocytosis by the low-density lipoprotein receptor on cell membranes. Lipid nanoparticle therapies are being utilized in clinical trials utilizing mRNA therapy and the CRISPR/Cas9 gene editing technique.

Alternative methods:

An alternate nonviral method of carrying genetic material is through the delivery of naked material using hydrodynamic injection. Hydrodynamic injection is the rapid injection of fluid with genetic material to increase venous pressure within the liver. This allows for the dissemination and cellular entry of the desired genetic material.[2,9] This method is not particularly feasible in humans, but multiple animal studies have been conducted using hydrodynamic injections with partial catheterization and have displayed some success.[2,9] Another method that has been studied is the use of electroporation or ultrasound, which has shown some effect for Alpha-1 Antitrypsin (AAT) deficiency in mice and pigs.[2,9] Electroporation/ultrasound method works by using nanobubbles to carry medications to help them seed into tissues. Ultrasound waves are then used to release medication from the microbubbles.[2] These methods are employed using stabilization sequences, transposons, or

nucleases. Stabilization sequences are scaffold matrix attachment regions, DNA sequences that bind chromatin to matrix during interphase. Stabilization sequences have shown good success in mice and pig trials.[4] Transposons are sequences of repeats that can be inserted into DNA and are cleaved by transposase enzymes at the desired location. This method is currently being looked into for AAT deficiency, and mucopolysaccharidosis.[3,10]

III. Therapies

Gene Editing

Gene editing is the process of targeting specific sections of DNA and altering or replacing those sequences for the desired product. There are two main classes of editing: nuclease-guided and nuclease-free editing.[12] The three main nucleases used in editing are zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats with associated Cas9 protein (CRISPR/Cas9).[1,8] These techniques are combined with either non-homologous end-joining, or homology-directed repair. These repair mechanisms allow for insertion or deletion of desired genetic code. The most common target is the albumin locus in the liver, given its high transcriptional capabilities.[12] Non-homologous end-joining allows for insertion or deletion of variable lengths, which tends to cause frameshift mutations and can lead to gene knockout.[56] RNA-guided nuclease editing tends to have higher efficiency of integration.[57]Nuclease-facilitated editing also has the potential for off-target effects secondary to Cas9. Due to this problem, nuclease-free editing was developed based on homology-directed repair but it is less efficient.[58]

Zinc-Finger Nucleases:

Zinc-finger nucleases (ZFNs) are nucleases that use multiple base pairs to bind to specific parts of DNA and cause double-strand breaks.[13] ZFNs are made up of 30 amino acids bound to amolecule of zinc.[13,59] There are five amino acids that do not fold around the zinc molecule, which act as a link between the zinc-fingers.[60] The amino acids bind to 3-4 complementary nucleotides of dsDNA that they have specificity towards.[59] The ZFN will bind to one strand of DNA using multiple base pairs, with a cleavage domain (FokI nuclease) at the end, while another ZFN group of amino acids will attach to the other strand of dsDNA, creating a double-strand break**(Figure 1).** The locations of the breaks can then be targeted with newly introduced genetic code. There have been two phase II trials using ZFNs directed at the albumin locus of hepatocytes targeting Alpha-L-Iduronidase and Iduronate 2 sulfatase in MPSI and MPSII, respectively. Both of these trials have been rolled to long-term follow-up studies.[61,62]Previous trials also have used adeno-associated viral vectors modified with ZFN to target Factor IX at the albumin locus in Hemophilia B.[17,21]

Transcription Activator-Like Effector Nucleases:

Transcription activator-like effector nucleases (TALENs) are similar to ZFNs by the fact that they use a FokI nuclease domain to create the double-strand breaks**(Figure 2)**. TALE proteins occur naturally in the bacteria xanthomonads, and have been shown to attach FokI domain to alter the genes of plants.[63] This domain attached to the FokI domain is the transcription activator-like effector repeat domain. This set of protein codes is flanked by a C-terminal domain and an N-terminal domain, which allows for the replacement of genetic code with complementary pairing to attach at the C-terminus and N-terminus. These terminus amino acids allow for more cost-effective and efficient production over ZFNs. This also allows TALENs to be specific on where the double-strand break will occur. Similar to ZFNs, two TALENs are needed to cause the double-strand DNA break.[56] This gene therapy has been shown to be effective in mouse liver models of chronic HBV, where markers of viral replication were

inhibited after the introduction of TALENs targeting the four HBV-specific sites within the viral genome.[64]

CRISPR/Cas9:

The CRISPR/Cas9 system is the immunologic system of prokaryotes. The CRISPR sequence is a strand of DNA that encodes protein production for the Cas9 system. The Cas9 system is a set of proteins that break down bacteriophage DNA upstream from the CRISPR sequence. The CRISPR sequence has a region of protospacer adjacent motifs (PAMs) on both sides **(Figure 3)**.[65] The PAM region has an allosteric effect that helps the Cas9 region break down DNA.[65]All together the bacteria will detect foreign DNA, and break it down using Cas9 proteins. These broken DNA pieces attach to the PAM region and are sent to incorporate into the CRISPR sequence. The CRISPR sequence will then replicate and send RNA to the Cas9 region to create protein complexes specific for that broken DNA**(Figure 4)**. This allows the PAM to bind specific DNA and the Cas 9 proteins to efficiently break down that specific foreign DNA quickly. This is currently the favored genetic therapy technique.[8] Cas9 can be guided using engineered short RNA to help it bind with higher specificity to a desired locale of DNA, allowing for decreased off-target effects. After the DNA break occurs, the strand can be repaired through multiple mechanisms that are inherent to cells. These mechanisms are non-homologous end joining, innate homology repair, or artificially stimulated using specific genome templates in homology-directed repair. The CRISPR/Cas9 system cannot enter cells with high efficiency, so if this technique is implored, it is usually encapsulated using a viral or nonviral vector to allow for entry into cells. There has been recent research using CRISPR/Cas9 via AAVs with anti-HBV DNA. There have been new discoveries using AAVs to avoid second-strand synthesis, which decreases transgene expression. This is done by mutating the terminal resolution site (trs) site on the AAVs, allowing for hairpin loops to form reducing its packaging size, and are called self-complementary AAVs (scAAVs).[66] With this smaller packaging size the scAAVs studies were able to use a Staph Aureus Cas9 with multiple cccDNA targets through RNAi targeting HBV and Argonaut2 in mice with chronic HBV.[67] These studies showed a significant decline in HBV replication in both cultures and mice.[68] There is an active phase I clinical trial using lipid nanoparticles as a vector for the CRISPR/CAS9 gene editing technology targeting the transthyretin protein, present predominantly in the liver, which is misfolded and accumulates in this condition.[69]

Nuclease Free:

Nuclease-free gene editing was first described using an AAV vector carrying a promoterless factor IX sequence flanked by homologous sequences to the targeted albumin locus for treatment of hemophilia B in mice. This proved to be an effective way to create ample coagulation factors in the mice.[70] The advantages to nuclease-free gene editing is the avoidance of off-target modification.[12] This method was designed to have homology for the 3’ end of the albumin locus and is called the GeneRide system.[12] The design is intended to integrate upstream from the stop codon with a self-cleaving protease sequence. There have been studies using the GeneRide system with CRISPR/Cas9 targeting the insertion site. This led to improving the editing efficiency by 20 to 50-fold in a mouse model studying Crigler-Najjar syndrome.[71] Apoa1 has also been targeted with similar strategies and shown effective in mouse models for hereditary tyrosinemia type 1.

Gene Silencing

Gene silencing identifies an unwanted DNA sequence and effectively stops cells from replicating this sequence, which can be done by targeting mRNA using small interfering RNA (siRNA) or

antisense oligonucleotides (ASO). siRNA and ASOs are similar in concept; they both use Watson-Crick base pairing to attach to host mRNA and suppress replication. [72]

Small Interfering RNA:

siRNA is a double-stranded RNA that causes "RNA interference," which is double-stranded RNA binding complementary mRNA and silencing the gene.[72] This process is done by cleavage of the double-stranded RNA by RNase III-like enzyme. The more stable siRNA binds to the mRNA and activates a protein called RNA-induced silencing complex (RISC). This RISC causes the mRNA to be cleaved and effectively silenced**(Figure 5).**[73] The use of siRNA with a naked delivery system was discussed in a previous section, but siRNA can be delivered using any traditional method weighing the pros and cons, i.e. nonviral vectors, viral vectors, or naked. Using the nonviral mechanisms has its main limitation of having to re-administer doses after a period time, it is not a sustained response.[1] Specifically for the delivery of siRNA to hepatocytes, recent development of a polymer-siRNA conjugate called Dynamic PolyConjugates (DPC) has shown promise. The study demonstrated effective knockout of apolipoprotein B and proliferator-activated receptor alpha.[74] GalNAc and siRNA have been a focus of research with FDA-approved drugs Givosiran for acute hepatic porphyria, [75,76] and Lumasiran for the treatment of primary hyperoxaluria type 1. [77] There are currently ongoing studies looking at GalNAc-modified siRNA for HBV, NASH, GSD1a, and hereditary hemochromatosis.[8,78-81]There is currently a siRNA-LNP that has completed a phase III trial for familial hypercholesterolemia targeting PCSK9 and apoB.[54,82,83] This vector method is also being explored for chronic hepatitis B with a recently completed Phase II clinical trial.[84] There is also a completed phase II trial involving the development of a siRNA targeting polo-like kinase 1 (PLK 1), which is overexpressed in hepatocellular carcinoma. The specific targets for this therapy are oncogene MYC and polo-like kinase-1.[85]

Antisense Oligonucleotides:

There are multiple types of ASOs that are all single-stranded antisense molecules. The original ASOs consisted of a phosphodiester backbone and were called unmodified ASOs. Issues associated with unmodified ASOs included being readily degraded by nucleases and poor cell penetrance due to the large size. ASOs, like siRNA target mRNA, one difference is that ASOs are single-stranded deoxyribonucleotides that act within the nucleus. The other difference is in the mechanism of action, ASOs have multiple mechanisms of action to attempt to silence genes, through the activation of RNase H endonucleases, inhibition of 5' cap, altering the ability to splice, or by utilizing steric hindrance. The most common method utilized is the induction of RNase H endonucleases**(Figure 6)**.[86]Recent investigations have been using different types of modifications to ASOs with different benefits. The list of modified ASOs include: phosphorothioates (PS), phosphorodiamidate morpholinos (PMO), peptic nucleic acids (PNA), locked nucleic acids (LNA), 2'-O-methyls, 2'-O-methoxyethyls, 2' fluoros, 5' methylcytosines, and G-clamps. PS have a modification to the phosphate group, allowing for enzymatic stability. PMOs and PNAs have sugar phosphate modifications, PMOs have the benefit of improved solubility and binding affinity; PNAs have increased enzymatic stability, better binding affinity, and do not activate the immune system. LNAs, 2'-O-methyls, 2'-O-methoxyethyls, and 2'fluoros have sugar modification. LNAs have stability and high binding affinity. 2'-O-methyls and 2'-O-methoxyethyls have high binding affinity, stability, and decreased immune reactions.[87] 2'fluoros have high binding affinity. 5'methylcytosine and G-clamp use a nucleobase modification. Both 5'methylcytosine and G-clamp have high binding affinity, and 5'methylcytosine has no immune reaction.[87]There is another genetic treatment in progress for

NASH using GalNAc-modified ASOs.[88] This treatment seeks to target serine/threonine protein kinase or fat-specific protein 27. ASO therapy has already been approved for the treatment of homozygous familial hyperlipidemia with the drug Mipomersen.[88]Other trials utilizing antisense oligonucleotides have been studied in transthyretin TTR amyloidosis. There is an ongoing phase III clinical trial of a ligand-conjugated antisense oligonucleotide against the TTR protein.[89]

mRNA Therapy

The rationale for mRNA therapy centers on synthetically engineered mRNA injected into cells to allow for replication of specific proteins. Though oversimplified, this allows an understanding of the concept for a more in-depth discussion of the process and future implications. mRNA therapy creates a transient effect in protein production. This has a benefit of allowing for dose control but requires the need for repeat dosing for a sustained effect.[1] The synthetic mRNA structurally is a 5' cap, 5' to 3' untranslated regions, kozak sequences, and poly-A tails.[12]This structure is designed to resemble a naturally occurring mature mRNA found within the cytoplasm. It was discovered that certain RNA nucleoside modifications led to better control of duration, kinetic profile and decreased immunogenicity.[90] This was a major breakthrough in the development of this method. The modified mRNA is negatively charged and is unable to freely cross the cell membrane into cells. This has led to the use of multiple methods to impregnate cells with synthetic mRNA, similar to other genetic editing mechanisms. Eukaryotic cells have the ability to actively transport these negatively charged particles across the cell membrane with the use of endocytosis.[12] This process is mediated by the scavenger receptor, which will endocytose the mRNA into lysosomes then subsequently out to the cytosol. Endocytosis has minimal uptake and tends to have minimal effect on the host cells.[12] Given that endocytosis has poor uptake, methods previously discussed i.e. LNPs, and viral/nonviral vesicles are commonly used.[1,5,12] The first trials with LNPs led to liver damage and an immunogenic response. Moderna developed a version of LNP that was specific for LDL and apo-E receptors.[12] This LNP was fully metabolized after six hours, reached sinusoidal cells after two hours, hepatocytes by six hours, and had lasting effects up to 24 hours in these cells.[91] It was shown that these LNPs reached 100% of hepatocytes in animal models.[12]. The safety profile of mRNA therapy has been promising with one study showing mild inflammatory infiltrate and increased in mitotic activity.[12]. There is an ongoing phase I/II clinical trial targeting methylmalonic acidemia utilizing mRNA. Another active phase I/II trial utilizing mRNA is recruiting for propionic acidemia.[12]

V. Expert Opinion

Genetic therapies seem to be on the forefront of the hepatology world. Hundreds of animal studies and clinical trials are currently underway for a variety of liver diseases. Most of these trials are in the starting phase one and phase two, but there are also gene therapiesthat are currently FDA-approved and being used in clinical practice.

Adenoviral vector gene transfer is well described in previous clinical trials and in the literature. AAV therapy has promising results in treatment for Hemophilia A. The liver is the site of production for clotting factors (liver parenchymal cells produce coagulation factors involving generation of fibrin clot, except factor VIII which was synthesized by hepatic and extrahepatic endothelial cells) and can serve as a site for AAV therapy.[93] A multiyear follow-up study was conducted after a single administration of AAV5 genetic transfer normal VIII factor which resulted in a sustained, clinically relevant benefit in 15 participants.[94] In seven participants, bleeding events decreased from a mean of 16.3 events per year to 0.7 events per year at the end

of year 3, a 96% reduction.[94] In six participants bleeding events decreased from a mean of 12.2 events per year to 1.2 events per year at the end of year 2, a 92% decrease.[94]A phase III trial was conducted with valoctocogeneroxaparvovec, a B-domain-deleted factor VIII coding sequence with AAV genetic transfer.[29,95] In this study 134 men were included and the mean annualized treated bleeding rate decreased by 84.5% (p=<0.001) from baseline.[95] During the time of the study there were no serious adverse events related to the treatment.[95]AAV therapy has also shown to be effective in the treatment of Hemophilia B. A phase III trial was conducted which administered one infusion of AAV5 vector containinga Padua factor IX variant.[16] It was found that annualized bleeding rate decreased from 4.19 to 1.51 during months 7 through 18 after treatment (p=<0.001).It was concluded that etranacogenedezaparvovec gene therapy was superior to standard prophylactic factor IX, and showed a favorable safety profile.[96]Another phase I clinical trial targeting porphobilinogen deaminase haploinsufficiency, an enzyme deficient in AIP showed promising results for AAV gene therapy. In this clinical trial two patients had a positive clinical outcome that resulted in the cessation of hematin treatment.[97]AAV genetic transfer therapy is promising for pathologies in which a genetic derangement results in decreased activity of an essential enzyme. However, the division of cells over time causes vector dilution and repeat vector therapy that may be ineffective due to the immune response of the patient.[98]This therapy has been used in pathologies such as Fabry disease, Crigler Najjar, AIP, familial hypercholesterolemia, hemophilia A and B, OTC deficiency, phenylketonuria, and Wilson's disease. Adenoviral vectors have also shown to be effective as vectors for more advanced genetic therapies involving gene editing such as zinc finger mediated nucleases and CRISPR/CAS9.

Gene editing therapy was developed to help combat the gradual diminishing of normal gene expression or difficulty in achieving an initial level of transgene expression that would correlate to clinical benefits. The first modality for gene editing was zinc finger mediated nucleases in which double-stranded DNA breaks are used to replace defective genes with a genetic sequence that encodes a normal function protein. The first clinical trials involving in-vivo genome editing in humans were the EMPOWERS and CHAMPIONS trialsin patients with Mucopolysaccharide Syndrome I and II. They utilized zinc finger-mediated nucleases targeting the two defective genes in these pathologies, Alpha-L iduronidase and Iduronate 2 sulfatase, respectively. A therapeutic transgene was inserted into the albumin locus, a gene that is abundantly present in the liver. These genetic therapies were tolerated well in these trials but resulted in low transgene expression. Elevation of transgene expression can overcome this obstacle but increases the risk of toxicity and increased costs of genetic therapies.[99,100] This was a promising first step for genome editing in humans. In an effort to increase transgene expression, another mouse trial was conducted in mice with MPS I using CRISPR/Cas9.[101] In this study, IDUA enzyme activity in the brain significantly increased and storage levels normalized.[101] Histological analysis revealed the absence of foam cells in the liver and vacuolation in the neuronal cells.[101] This study and many other animal trials will pave the way for human clinical trials utilizing CRISPR/CAS9.

Specifically, for A1AT deficiency, there are promising SiRNA trials currently in phase two. Fazirsiran, an siRNA, has shown promising results in an active phase II clinical trial where results showed a significant reduction in the alpha 1 antitrypsin Z mutant protein.[102]

Current hepatitis B trials seem to be focused on mostly the use of siRNA targeting HBV RNA, with multiple trials in phase 1. These studies work to decrease the expressionof hepatitis B surface antigen messenger mRNA in liver hepatocytes. One study used DCR-HBVS which is a

synthetic RNAi which is conjuges to GalNAc ligands.[81] Another study utilized AB-729, a siRNA inhibitor of HBV, in combination with vebicorvir, a novel core inhibitor.[103]
There are currently two NASH trials using ASO. One trial in phase I, using ASOs to target the PNPLA3 gene.This mouse trial has shown to reduce liver steatosis, inflammation, and fibrosis by lowering the mRNA expression of PNPLA3. [104] Another trial in phase II is using ASOs targetingdiacylglycerol acetyltransferase 2 (DGAT2), an enzyme that catalyzes the final reaction in the synthesis of triglycerides. This antisense nucleotide works to reduce the production of DGAT2 and therefore decrease triglyceride synthesis in the liver. [105,106]
The field of genetic therapies continues to grow rapidly in all fields as it does in the sphere of hepatology. The continued success of genetic therapies in other fields of medicine will fuel the growth in hepatology. There is no definitive endpoint to genetic therapy research. There are limitations to the field of genetic therapies not only on the technical/research and development side, but also in funds of knowledge of providers prescribing these medications, and public distrust in genetic therapies. A meta-analysis reported patients were more comfortable with gene therapies if patients discussed risks and benefits with their patients. [107]
The authors of this review speculate 5 years from publication of this article some of the trials outlined will complete their respective phases of trials with a handful reaching FDA approval for treatment options. We expect there to be an exponential growth in clinical trials as more genetic therapies are approved by the FDA for use.

Figure 1:The Action of Zinc-Finger Nucleases. The Zinc-Finger Nuclease (ZFN) binds to one strand of DNA using multiple base pairs, with the FokI nuclease used as a cleavage domain. Dimerization occurs when another ZFN attaches to the complementary DNA strand, creating a double stranded break.

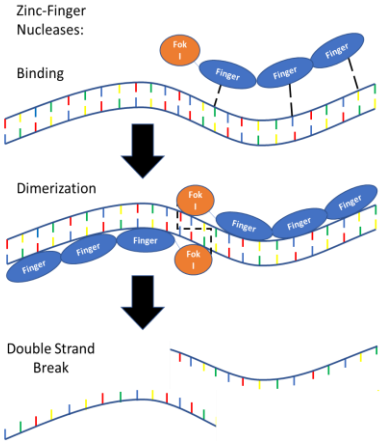

Figure 2:Transcription activator-like effector nucleases (TALENs): Transcription activator-like effector nucleases (TALENs) are a set of protein codes flanked by a C-terminal domain and an N-terminal domain and attached to a FokI domain. One TALEN attaches to a strand of DNA and the FokI nuclease is used as a cleavage domain. Another TALEN attaches the complementary DNA strand, dimerization occurs and creates a double stranded break.

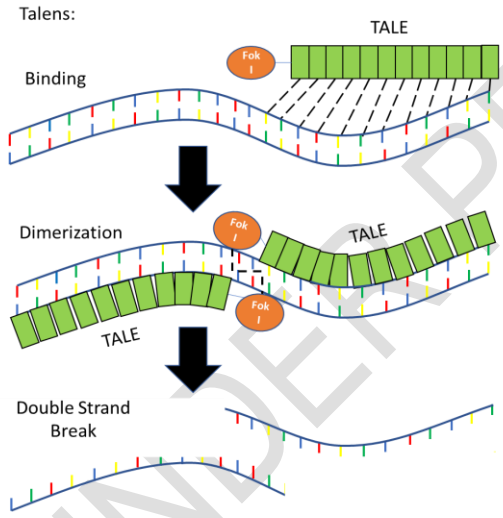

Figure 3: cluster regulated interspaced short palindromic repeats with associated cast 9 proteins (CRISPR/cas9):CRISPR sequences are associated protospacer adjacent motif (PAM) regions used to help break down DNA, with associated Cas9 adjacent.The CRISPR sequence is used to create protein complexes to identify specific DNA sequences. After the complimentary region of DNA is identified, the Cas9 protein creates a double-strand break. After the DNA break occurs, the strand is repaired through non-homologous end joining (NHEJ) or innate homology repair (HR).

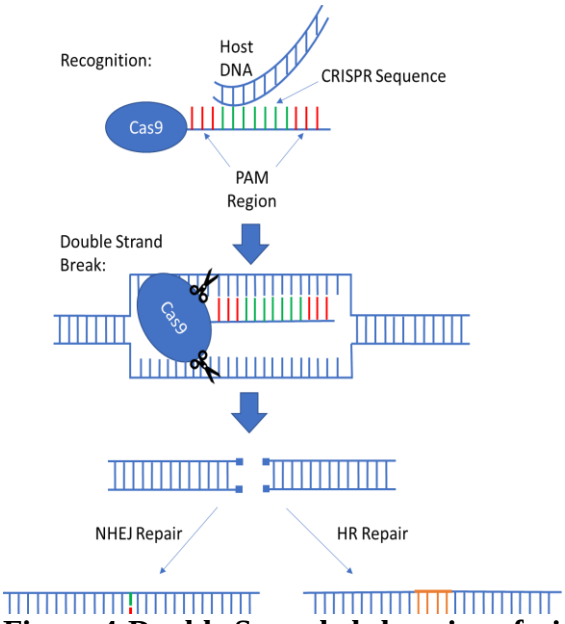

Figure 4:Double Stranded short interfering RNA (siRNA): Double stranded RNA enters the cell and an Rnase III-like enzyme cleaves the double stranded RNA into the single strand siRNA. siRNA then binds to mRNA an RNA-induced silencing complex is recruited once binding is accomplished. A double-stranded DNA break is created, resulting in silencing of the mRNA.

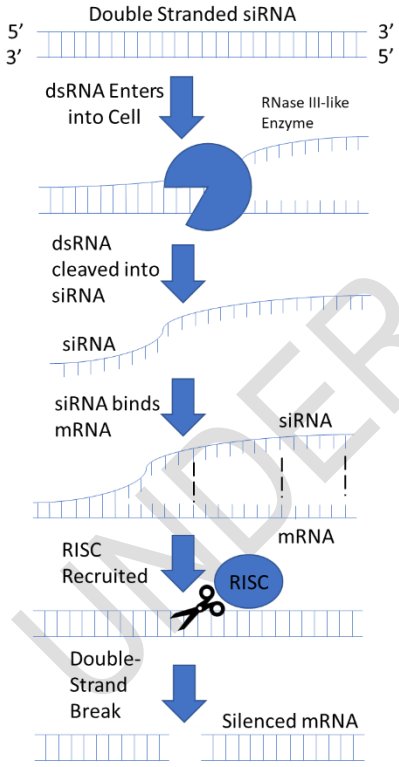

Table 1: List of Genetic Therapy Clinical Trials for Liver Diseases

Disease	Study #	Vector	Modification type	Molecule	Target	Clinical phase	Route	Ref
A1AT liver disease	NCT03946449	cholesterol -conjugated siRNA	siRNA	Inhibits AAT mRNA	AAT mRNA	II (active)	subcutaneous	[107, 108]
	NCT02363946	siRNA	Dynamic polyconjugate	Silence AAT gene expression	AAT gene	I (terminated)	IV infusion	[108]
	NCT04764448	GalNAc-siRNA	siRNA	siRNA targeting SERPINA1		II (recruiting)	subcutaneous	[109]
	NCT02503683					I (Terminated)		[110]
	NCT04174118	GalNAc-siRNA	siRNA			(I) active	subcutaneous	[111]
Acute hepatic porphyria	NCT03338816	GalNAc-siRNA conjugate	siRNA	siRNA against ALAS1	ALAS1	III (completed)	IV infusion	[75]
Acute intermittent porphyria	NCT02082860	AAV5	Gene transfer	Liver specific-promoter for Porphobilinogen deaminase expression	Porphobilinogen deaminase	I (completed 2014)	IV infusion	[39]
Acute intermittent porphyria	NCT02452372	GalNAc-siRNA conjuga	siRNA	Inhibitor of hepatic aminolevulinic acid	Delta-aALAS1	I (completed)	Subcutaneous	[76]

		te		synthase 1 (ALAS1)				
Chronic HBV	NCT02826018	GalNAc-siRNA conjugate	siRNA		HBV mRNAs	I (terminated)		[112]
	NCT03772249	GalNAc-siRNA conjugate	siRNA			I (Complete)	subcutaneous	[81]
	NCT03672188	GalNAc-siRNA conjugate	siRNA		HBV transcripts			[84]
	NCT02981602		ASO		HBV messenger RNAs	II (Completed)	subcutaneous	[80]
	NCT03365947	GalNAc-siRNA conjugate	siRNA		HBV mRNAs	I/II (completed)	subcutaneous	[113]
Crigler Najjar	NCT03466463	AAV	Gene transfer	UDP glucuronosyltransferase 1 (UGT1A1) transgene	UGT1A1	N/a (recruiting)	IV infusion	[49]
	NCT03223194	AAV8	Gene transfer	UGT1A1 gene	UGT1A1	I (terminate due to sponsor decision)	IV infusion	[50]
Fabry	NCT04040049	AAV	Gene transfer	alpha galactosidase gene	alpha galactosidase	I/II (recruiting)	IV infusion	[43]

	NCT04046224	AAV 2/6	Gene transfer	cDNA of Alpha galactosidase	Alpha galactosidase	I/II (recruiting)	IV infusion	[42]
	NCT04519749	AAV	Gene transfer	Codon-optimized full length human GLA transgene driven by CAG promoter	Alpha galactosidase	I/II (recruiting)	IV infusion	[44]
Familial hypercholesterolemia	NCT00004809	Autologous hepatocytes	Gene transfer	Low-density lipoprotein (LDL) receptor gene	LDL receptor	I (completed)	Inferior mesenteric vein infusion	[114]
	NCT02314442	GalNAc-siRNA conjugate	siRNA	PCSK9 inhibitor	PCSK9	I (completed)	Subcutaneous	[115]
	NCT03851705	GalNAc-siRNA conjugate	siRNA	PCSK9 inhibitor	PCSK9	III (completed)	Subcutaneous	[82]
	NCT02651675	AAV8	Gene transfer	Human low-density lipoprotein receptor gene	Low Density Lipoprotein Receptor	I/II (completed)	IV infusion	[41]
	NCT02709850			Was discontinued due to phase 2b results				[116]
Glycogen Storage Disease Type 1a (GSD1a)	NCT05095727		mRNA	mRNA encoding Glucose 6-phosphatase	Glucose 6-phosphatase	I (recruiting)	IV infusion	[117]
Hemophilia A	NCT02576795	AAV5	Gene transfer	B domain deleted Factor VIII	Factor VIII	I/II (ongoing)	IV infusion	[27]

	NCT03370913	AAV	Gene transfer	B-domain-deleted factor VIII	Factor VIII	III (active)	IV infusion	[29]
	NCT03003533	AAV	Gene transfer	B domain deleted Factor VIII	Factor VIII	I/II (recruiting)	IV infusion	[28]
	NCT03061201	AAV 2/6	Gene transfer	cDNA containing B domain deleted Factor VIII	Factor VIII	II (ongoing)	IV infusion	[31]
	NCT03588299	AAV	Gene transfer	B domain deleted Factor VIII	Factor VIII	I/II (ongoing)	IV infusion	[32]
	NCT03370172	AAV8	Gene transfer	B domain deleted Factor VIII	Factor VIII	I/II (ongoing)	IV infusion	[30]
Hemophilia A and B	NCT03549871	GalNAc-siRNA	siRNA	siRNA targeting antithrombin	Antithrombin	III (completed)	subcutaneous	[118]
	NCT03754790	GalNAc-siRNA	siRNA		antithrombin	III (active)	subcutaneous	[119]
	NCT03417245	GalNAc-siRNA	siRNA		antithrombin	III (active)	subcutaneous	[120]
	NCT03417102	GalNAc-siRNA	siRNA		antithrombin	III (active)	subcutaneous	[121]
Hemophilia B	NCT00076557	AAV	Gene transfer	Human Factor IX	Factor IX	I/II was terminated with no results	Hepatic artery injection	[25]

	NCT03569891	**AAV5**	**Gene transfer**	**Padua variant of a codon optimized factor IX gene**	**Factor IX**	**III (active)**	**IV infusion**	**[16]**
	NCT00979238	**AAV**	**Gene transfer**	**codon-optimized factor IX transgene**	**Factor IX**	**I (ongoing)**	**IV infusion**	**[19]**
	NCT02396342	**AAV5**	**Gene transfer**	**Codon optimized human factor IX gene**	**Factor IX**	**I/II Completed**	**IV infusion**	**[26]**
	NCT01687608	**AAV8**	**Gene transfer**	**Factor IX gene**	**Factor IX**	**I/II ongoing**	**IV infusion**	**[23]**
	NCT03489291	**AAV5**	**Gene transfer**	**Padua variant of a codon optimized factor IX gene**	**Factor IX**	**IIb**	**IV infusion**	**[18]**
	NCT03369444 NCT03641703	**AAV**	**Gene transfer**	**Transgene cassette including liver-specific promoter (FRE1) and a partially codon-optimized gene encoding factor IX with a gain of function**	**Factor IX**	**I/II (terminated) Long term observational study currently active**	**IV infusion**	**[20,22]**
	NCT02618915	**AAVRH10**	**Gene transfer**	**Factor IX**	**Factor IX**	**I/II terminated due to sponsor decision**	**IV infusion**	**[24]**

	NCT02695160 NCT04628871	**AAV6**	**Zinc Finger mediated**	**Factor IX**	**Factor IX at albumin locus**	**I (terminated)**	**IV infusion**	**[17,21]**
Hepatocellular Carcinoma	**NCT02191878**	**Stable nucleic acid lipid particle**	**siRNA**	**Decreased expression of polo-like kinase 1 (PLK 1) expression**	**PLK1**	**I/II (completed)**	**IV infusion**	**[122]**
	NCT03780049	**AAV5**		**Oncolytic activity**		**III (recruiting)**	**IV infusion**	**[123]**
Hereditary Transthyretin amyloidosis with polyneuropathy	**NCT04601051**	**Lipid nanoparticles (LNPs)**	**CRISPR/CAS9**	**Decreased production of both wild-type and mutant transthyretin (TTR) protein**	**TTR in hepatocytes**	**I (recruiting)**	**IV infusion**	**[69]**
Methylmalonic acidemia	**NCT03810690**		**mRNA**			**Study withdrawn before dosing**		**[124]**
	NCT04581785	**AAV**	**LK03 capsid**	**Methylmalonyl-CoA mutase gene at albumin locus**		**I/II (recruiting)**	**IV infusion**	**[40]**
	NCT04899310 NCT05295433	**Lipid nanoparticle**	**mRNA**	**Methylmalonyl-coenzyme A (CoA) mutase (MUT) gene**	**MUT**	**I/II (recruiting)**	**IV infusion**	**[125, 126]**
MPS I	**NCT02702115 NCT04628871**	**AAV8**	**Zinc finger nuclease**	**Alpha-L-Iduronidase transgene**	**Alpha-L-iduronidase gene at the albumin locus**	**I/II (subjects rolled over to long-term follow up**	**IV infusion**	**[21,45]**

						study)		
MPS II	**NCT03041324** **NCT04628871**	**AAV**	**Zinc finger nuclease**	**Iduronate 2 – sulfatase gene**	**Iduronate 2 – sulfatase gene at the albumin locus**	**I/II(subjects rolled over to long-term follow up study)**	**IV infusion**	**[21,46]**
MPS IV	**NCT03173521**	**AAV8**	**Gene transfer**	**Liver specific thyroxine binding globulin promoter**	**Arylsulfatase B gene**	**I/II (active)**	**IV Infusion**	**[47]**
NASH	**NCT04932512**	**Ligand conjugated**	**ASO**		**Diacylglycerol acyltransferase 2**	**II (recruiting)**	**Subcutaneous**	**[104]**
	NCT04483947	**Ligand conjugated**	**ASO**	**Patatin-like phospholipase domain containing protein 3**	**mRNA expression of PNPLA3**	**I (recruiting)**	**subcutaneous**	**[103]**
OTC deficiency	**NCT03767270**	**Lipid based nanoparticles**	**mRNA**	**Ornithine transcarbamylase gene**	**OTC**	**I/II withdrawn (program discontinued)**		**[127]**
	NCT00004498	**AAV**	**Gene transfer**	**Ornithine transcarbamylase gene**	**Ornithine transcarbamylase**	**I (terminated)**	**Hepatic Artery**	**[35]**
	NCT00004386	**AAV**	**Gene transfer**	**Ornithine transcarbamylase gene**	**Ornithine transcarbamylase**	**I (terminated)**	**Intrahepatic infusion**	**[34]**

	NCT02991144	AAV8	Gene transfer	Ornithine Transcarbamylase gene	Ornithine transcarbamylase	I/II (completed)	IV infusion	[36]
	NCT05345171	AAV8	Gene transfer	Ornithine Transcarbamylase gene	Ornithine transcarbamylase	III (recruiting)	IV infusion	[33]
Phenylketonuria	NCT03952156	AAVHSC15	Gene transfer	Functional copy of phenylalanine hydroxylase gene	Phenylalanine hydroxylase	I/II (recruiting)	IV infusion	[38]
	NCT04480567	AAV5	Gene transfer	Phenylalanine hydroxylase gene	Phenylalanine hydroxylase	I/II (active)	IV infusion	[37]
Primary hyperoxaluria	NCT05001269	GalNAc-siRNA conjugate	siRNA	siRNA against hepatic LDH	Hepatic lactate dehydrogenase (LDH)	II (recruiting)	subcutaneous	[128]
	NCT03681184	GalNAc-siRNA	siRNA	Decreased hepatic oxalate production	Glycolate oxidase	III (active) approved for sale	Subcutaneous injection	[77]
Propionic acidemia	NCT04159103		mRNA			I/II (recruiting)	IV infusion	[129]
Transthyretin TTR amyloidosis	NCT03728634	GalNAc-ASO conjugate	Antisense oligonucleotide	ASO against TTR mRNA	TTR mRNA	I/II (completed)	IV infusion	[130]
	NCT05071300	Ligand-conjugated antisense	Antisense oligonucleotide	ASO against TTR	TTR protein	III (recruiting)	Subcutaneou	[89]

	NCT03759379	GalNAc-siRNA conjugate	siRNA	siRNA against transthyretin protein	Transthyretin protein	III (active)	subcutaneous	[131]
	NCT04153149			May not be relevant as this is cardiac amyloidosis				[132]
Wilson's disease	NCT04884815	AAV9	Gene transfer	ATP7B Gene	Copper transporting ATPase 2	I/II (recruiting)	IV infusion	[48]

Table 2: List of Animal Studies Using Genetic Therapy for Liver Diseases

Disease	Target Organ	Vector	Modification type	Molecule	Target	Route	Ref
AATD	Mouse liver	AAV	CRISPR/Cas9	Guide RNA	hSERPINA1	Hydrodynamic tail vein injection	[133]
HBV	Mouse liver	TALEN expressing plasmid	TALEN	HBV target DNA and pairs of left and right TALEN expressing plasmids	Sites within the S/pol, C/pol, and pol ORFs of HBV genome	Hydrodynamic injection	[64]
Hemophilia A and B	Mouse liver	AAV8	ZFN	Human F9 Gene	Intron 1 of Human F9 Gene	IV infusion	[134]
	Mouse liver	AAV8	ZFN		Albumin locus	IV infusion	[135]
	Mouse liver	AAV8	ZFN		Human IDS at the albumin locus		[136]
Hemophilia B		AAV9	CRISPR/Cas9	Guide RNA	Murine Factor IX	Hydrodynamic tail vein	[137]

					gene	**injection**	
HTI	**Mouse liver**	**pX330 plasmid**	**CRISPR/Cas 9**	**pX330 plasmids expressing Cas9 and a single guide RNA**	**Fumarylacetoacetate hydrolase (fah) locus**	**Hydrodynamic tail vein injection**	**[138]**
	Mouse liver	**Lipid nanoparticle and AAV**	**CRISPR/Cas 9**	**U6-sgRNA, homology directed repair template**	**Exon 8 of Fumarylacetoacetate hydrolase (fah)**	**IV injection**	**[139]**
	Mouse liver	**pX330**	**CRISPR/Cas 9**	**Guide RNA sequences**	**Introns adjacent to exons 3 and 4 of hydroxyphenylpyruvate dioxigenase gene**	**Hydrodynamic tail vein injection**	**[140]**
	Mouse liver	**Plasmid DNA**	**CRISPR/Cas 9**	**Adenine base ditor and a single guided RNA**	**Exon 8 of Fumarylacetoacetate hydrolase (fah)**	**Hydrodynamic tail vein injection**	**[141]**
MPS I	**Mouse liver**	**AAV8**	**CRISPR/Cas 9**	**Albumin locus of hepatocytes**	**Alpha-L-iduronidase**		**[91]**
MPS II	**Mouse liver**	**AAV2/AAV8**	**ZFN**	**Human IDS coding sequence**	**Intron 1 of the albumin locus**	**IV infusion?**	**[61]**
Transthyretin amyloidosis	**Mouse liver**	**Lipid nanoparticles**	**CRISPR/Cas 9**	**Cas9 mRNA with single guide RNA**	**Transthyretin gene**	**Hydrodynamic tail vein injection**	**[142]**

Abbreviations: ATD, alpha-1 antitrypsin deficiency; AAV, Adeno-associated vector; ABE, adenine base editing; Ad, adenovirus; ALS, amyotrophic lateral sclerosis; CAR, chimeric antigen receptor; CRISPR/Cas, clustered regularly interspaced short palindrome repeats-associated Cas nuclease; dCas9, dead Cas9; DMD, Duchenne muscular dystrophy; gRNA, guide RNA; HBV, hepatitis B virus; HDR, homology-directed repair; HIV, human immunodeficiency virus; HITI, homology-independent targeted integration; HR, homologous recombination; HTI, hereditary tyrosinemia; LCA, Leber's congenital amaurosis; LNP, lipid nanoparticles; NHEJ, nonhomologous end-joining; PNA, peptide nucleic acids; RNP, ribonucleoprotein; SCD, sickle cell disease; sgRNA, single-guide RNA; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease.

IV. **Conclusion**

The future implications for genetic therapies have no bounds. A majority of trials outlined in this article are either animal studies or phase 1 and phase 2 trials. Most modalities have passed animal studies showing safety and efficacy. These data points will encourage future research into different liver diseases. The advent of FDA-approved drugs helps to show the current efficacy of these treatment modalities. Two drugs on the market include givosiran, [75,76] an siRNA for acute hepatic porphyria and lumasirin, an siRNA for hyperoxaluria. [77] Another genetic therapy that has gained traction is PCSK9s. PSCK9s use siRNA and are one of the main therapies for hyperlipidemia, it is being compared to the current gold standard of statin therapy.[92] SiRNA seems to be the forefront of therapy modalities in trials currently. There are multiple animal trials associated with the CRISPR/Cas9 system, but very few clinical trials at this time. The animal trials for CRISPR/Cas9 have been showing promising results, and once the technology has more research to back the concept, this will most likely become the common mechanism of action for future therapies. Even with all of this promising data, and studies to give validity to these concepts, there has been a lot of public mistrust over the recent years with genetic therapies, especially mRNA therapies with the advent of the COVID-19 vaccines. These therapies have been proven to be effective in multiple clinical trials, but proper counseling on safety and efficacy should be discussed with patients so they can understand the risks and benefits and clear up misconceptions about genetic therapies. Possible future problems for gene therapy include the management of off-target effects and specifying delivery particles to decrease this effect. There still are leaps-and-bounds to be made on the efficacy and penetrance of genetic therapies, and normalizing these types of therapies to the public.

Gene therapy is most likely the future of medicine across the board. These concepts can be difficult to comprehend not only for patients but for clinicians that are not specialized in genetics/biochemistry. There is a need to understand these basic concepts of genetic therapy so clinicians can perform their due diligence to explain how medications work, and to give hope for patients that are struggling with liver diseases that don’t have effective treatment modalities today.

References

1. Cozmescu AC, Counsell J, Gissen P. Gene therapies targeting the liver. J Hepatol. 2021 Jan;74(1):235-236.
2. Endo-Takahashi Y, Negishi Y. Microbubbles and Nanobubbles with Ultrasound for Systemic Gene Delivery. Pharmaceutics. 2020 Oct 14;12(10).
3. Hackett PB, Jr., Aronovich EL, Hunter D, et al. Efficacy and safety of Sleeping Beauty transposon-mediated gene transfer in preclinical animal studies. Curr Gene Ther. 2011 Oct;11(5):341-9.
4. Heng HH, Goetze S, Ye CJ, et al. Chromatin loops are selectively anchored using scaffold/matrix-attachment regions. J Cell Sci. 2004 Mar 1;117(Pt 7):999-1008.
5. Kruse RL, Huang Y, Kumbhari V. How to Embrace Gene Therapy in Gastroenterology. Gastroenterology. 2022 Apr;162(4):1019-1023.
6. Limani SW, Mnyandu N, Ely A, et al. In Vivo Modelling of Hepatitis B Virus Subgenotype A1 Replication Using Adeno-Associated Viral Vectors. Viruses. 2021 Nov 9;13(11).
7. Lungwitz U, Breunig M, Blunk T, et al. Polyethylenimine-based non-viral gene delivery systems. Eur J Pharm Biopharm. 2005 Jul;60(2):247-66.
8. Maestro S, Weber ND, Zabaleta N, et al. Novel vectors and approaches for gene therapy in liver diseases. JHEP Rep. 2021 Aug;3(4):100300.
9. Noble-Vranish ML, Song S, Morrison KP, et al. Ultrasound-Mediated Gene Therapy in Swine Livers Using Single-Element, Multi-lensed, High-Intensity Ultrasound Transducers. Mol Ther Methods Clin Dev. 2018 Sep 21;10:179-188.
10. Podetz-Pedersen KM, Olson ER, Somia NV, et al. A Broad Range of Dose Optima Achieve High-level, Long-term Gene Expression After Hydrodynamic Delivery of Sleeping Beauty Transposons Using Hyperactive SB100x Transposase. Mol Ther Nucleic Acids. 2016 Jan 19;5:e279.
11. Xue L, Yan Y, Kos P, et al. PEI fluorination reduces toxicity and promotes liver-targeted siRNA delivery. Drug Deliv Transl Res. 2021 Feb;11(1):255-260.
12. Zabaleta N, Torella L, Weber ND, et al. mRNA and gene editing: Late breaking therapies in liver diseases. Hepatology. 2022 Sep;76(3):869-887.
13.Carroll D. Genome engineering with zinc-finger nucleases. Genetics. 2011 Aug;188(4):773-82.
14.Nakatani T, Kuriyama S, Tominaga K, et al. Assessment of efficiency and safety of adenovirus mediated gene transfer into normal and damaged murine livers. Gut. 2000 Oct;47(4):563-70.
15.Song H, Song C, Wang H, et al. Suppression of hepatocarcinoma model in vitro and in vivo by ECRG2 delivery using adenoviral vector. Cancer Gene Ther. 2012 Dec;19(12):875-9.
16.Pipe S. HOPE-B: Trial of AMT-061 in Severe or Moderately Severe Hemophilia B Patients. https://clinicaltrials.gov/ct2/show/NCT03569891; 2018.
17.Ascending Dose Study of Genome Editing by Zinc Finger Nuclease Therapeutic SB-FIX in Subjects With Severe Hemophilia B. https://ClinicalTrials.gov/show/NCT02695160; 2022.
18.Pipe S. Dose Confirmation Trial of AAV5-hFIXco-Padua. https://ClinicalTrials.gov/show/NCT03489291; 2022.
19.Reiss U. Dose-Escalation Study Of A Self Complementary Adeno-Associated Viral Vector For Gene Transfer in Hemophilia B. https://ClinicalTrials.gov/show/NCT00979238; 2022.
20.Chowdary P. A Factor IX Gene Therapy Study (FIX-GT). https://ClinicalTrials.gov/show/NCT03369444; 2021.

21.Long Term Follow-up (LTFU) of Subjects Who Received SB-318, SB-913, or SB-FIX. https://ClinicalTrials.gov/show/NCT04628871; 2022.
22.A Long-Term Follow-Up Study of Haemophilia B Patients Who Have Undergone Gene Therapy. https://ClinicalTrials.gov/show/NCT03641703; 2020.
23.Open-Label Single Ascending Dose of Adeno-associated Virus Serotype 8 Factor IX Gene Therapy in Adults With Hemophilia B. https://ClinicalTrials.gov/show/NCT01687608; 2022.
24.Safety and Dose Finding Study of DTX101 (AAVrh10FIX) in Adults With Moderate/Severe to Severe Hemophilia B. https://ClinicalTrials.gov/show/NCT02618915; 2018.
25.Safety of a New Type of Treatment Called Gene Transfer for the Treatment of Severe Hemophilia B. https://ClinicalTrials.gov/show/NCT00076557; 2007.
26.Trial of AAV5-hFIX in Severe or Moderately Severe Hemophilia B. https://ClinicalTrials.gov/show/NCT02396342; 2022.
27.Gene Therapy Study in Severe Haemophilia A Patients (270-201). https://ClinicalTrials.gov/show/NCT02576795; 2022.
28.A Gene Transfer Study for Hemophilia A. https://ClinicalTrials.gov/show/NCT03003533; 2022.
29.Single-Arm Study To Evaluate The Efficacy and Safety of ValoctocogeneRoxaparvovec in Hemophilia A Patients (BMN 270-301) (BMN 270-301). https://www.clinicaltrials.gov/ct2/show/NCT03370913; 2017.
30.A Study of BAX 888 in Male Adults With Severe Hemophilia A. https://ClinicalTrials.gov/show/NCT03370172; 2021.
31.A Study of Recombinant AAV2/6 Human Factor 8 Gene Therapy SB-525 (PF-07055480) in Subjects With Severe Hemophilia A. https://ClinicalTrials.gov/show/NCT03061201; 2022.
32.Study to Test the Safety and How Well Patients With Severe Hemophilia A Respond to Treatment With BAY 2599023 (DTX 201), a Drug Therapy That Delivers a Healthy Version of the Defective Factor VIII Gene Into the Nucleus of Liver Cells Using an Altered, Non-infectious Virus (AAV) as a "Shuttle". https://ClinicalTrials.gov/show/NCT03588299; 2022.
33.Clinical Study of DTX301 AAV- Mediated Gene Transfer for Ornithine Transcarbamylase(OTC) Deficiency. https://ClinicalTrials.gov/show/NCT05345171; 2022.
34.Batshaw M. Phase I Pilot Study of Liver-Directed Gene Therapy for Partial Ornithine Transcarbamylase Deficiency. https://ClinicalTrials.gov/show/NCT00004386; 2005.
35.Raper S. Phase I Study of Adenoviral Vector Mediated Gene Transfer for Ornithine Transcarbamylase in Adults With Partial Ornithine Transcarbamylase Deficiency. https://ClinicalTrials.gov/show/NCT00004498; 2015.
36.Safety and Dose-Finding Study of DTX301 (scAAV8OTC) in Adults With Late-Onset OTC Deficiency. https://ClinicalTrials.gov/show/NCT02991144; 2022.
37.AAV Gene Therapy Study for Subjects With PKU. https://ClinicalTrials.gov/show/NCT04480567; 2021.
38.Bodamer O. Gene Therapy Clinical Study in Adult PKU. https://ClinicalTrials.gov/show/NCT03952156; 2021.
39.Ruiz J.; Prieto JS, R. Phase I Gene Therapy Clinical Trial Using the Vector rAAV2/5-PBGD for the Treatment of Acute Intermittent Porphyria. https://ClinicalTrials.gov/show/NCT02082860; 2014.
40.Gruskin D. Gene Therapy With hLB-001 in Pediatric Patients With Severe Methylmalonic Acidemia. https://ClinicalTrials.gov/show/NCT04581785; 2022.

41.A Gene Therapy Study for Homozygous Familial Hypercholesterolemia (HoFH). https://ClinicalTrials.gov/show/NCT02651675; 2021.
42.Dose-Ranging Study of ST-920, an AAV2/6 Human Alpha Galactosidase A Gene Therapy in Subjects With Fabry Disease. https://ClinicalTrials.gov/show/NCT04046224; 2022.
43.A Fabry Disease Gene Therapy Study. https://ClinicalTrials.gov/show/NCT04040049; 2021.
44.Schiffmann R. An Open-label, Phase 1/2 Trial of Gene Therapy 4D-310 in Adult Males With Fabry Disease. https://ClinicalTrials.gov/show/NCT04519749; 2022.
45.Ascending Dose Study of Genome Editing by the Zinc Finger Nuclease (ZFN) Therapeutic SB-318 in Subjects With MPS I. https://ClinicalTrials.gov/show/NCT02702115; 2021.
46.Ascending Dose Study of Genome Editing by the Zinc Finger Nuclease (ZFN) Therapeutic SB-913 in Subjects With MPS II. https://ClinicalTrials.gov/show/NCT03041324; 2022.
47.Brunetti-Pierri N. Gene Therapy in Patients With Mucopolysaccharidosis Disease. https://ClinicalTrials.gov/show/NCT03173521; 2021.
48. Clinical Study of UX701 AAV-Mediated Gene Transfer for the Treatment of Wilson Disease. https://ClinicalTrials.gov/show/NCT04884815; 2022.
49. Philippe L. Gene Therapy for Severe Crigler Najjar Syndrome. https://ClinicalTrials.gov/show/NCT03466463; 2021.
50. Prasad S. Gene Transfer Clinical Study in Crigler-Najjar Syndrome. https://ClinicalTrials.gov/show/NCT03223194; 2022.
51. Murillo O, Luqui DM, Gazquez C, et al. Long-term metabolic correction of Wilson's disease in a murine model by gene therapy. J Hepatol. 2016 Feb;64(2):419-426.
52. Murillo O, Moreno D, Gazquez C, et al. Liver Expression of a MiniATP7B Gene Results in Long-Term Restoration of Copper Homeostasis in a Wilson Disease Model in Mice. Hepatology. 2019 Jul;70(1):108-126.
53.Kaiser RA, Mao SA, Glorioso J, et al. Lentiviral Vector-mediated Gene Therapy of Hepatocytes Ex Vivo for Autologous Transplantation in Swine. J Vis Exp. 2018 Nov 4(141).
54.Nicolas CT, VanLith CJ, Hickey RD, et al. In vivo lentiviral vector gene therapy to cure hereditary tyrosinemia type 1 and prevent development of precancerous and cancerous lesions. Nat Commun. 2022 Aug 25;13(1):5012.
55.Merle U, Encke J, Tuma S, et al. Lentiviral gene transfer ameliorates disease progression in Long-Evans cinnamon rats: an animal model for Wilson disease. Scand J Gastroenterol. 2006 Aug;41(8):974-82.
56.Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013 Jan;14(1):49-55.
57.Vo PLH, Ronda C, Klompe SE, et al. CRISPR RNA-guided integrases for high-efficiency, multiplexed bacterial genome engineering. Nat Biotechnol. 2021 Apr;39(4):480-489.
58.Dasgupta I, Flotte TR, Keeler AM. CRISPR/Cas-Dependent and Nuclease-Free In Vivo Therapeutic Gene Editing. Hum Gene Ther. 2021 Mar;32(5-6):275-293.
59.Kim MS, Kini AG. Engineering and Application of Zinc Finger Proteins and TALEs for Biomedical Research. Mol Cells. 2017 Aug;40(8):533-541.
60.Moore M, Klug A, Choo Y. Improved DNA binding specificity from polyzinc finger peptides by using strings of two-finger units. Proc Natl Acad Sci U S A. 2001 Feb 13;98(4):1437-41.
61.Laoharawee K, DeKelver RC, Podetz-Pedersen KM, et al. Dose-Dependent Prevention of Metabolic and Neurologic Disease in Murine MPS II by ZFN-Mediated In Vivo Genome Editing. Mol Ther. 2018 Apr 4;26(4):1127-1136.

62.Ou L, DeKelver RC, Rohde M, et al. ZFN-Mediated In Vivo Genome Editing Corrects Murine Hurler Syndrome. Mol Ther. 2019 Jan 2;27(1):178-187.
63.Boch J, Bonas U. Xanthomonas AvrBs3 family-type III effectors: discovery and function. Annu Rev Phytopathol. 2010;48:419-36.
64.Bloom K, Ely A, Mussolino C, et al. Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. Mol Ther. 2013 Oct;21(10):1889-97.
65.Lv W, Li T, Wang S, et al. The Application of the CRISPR/Cas9 System in the Treatment of Hepatitis B Liver Cancer. Technol Cancer Res Treat. 2021 Jan-Dec;20:15330338211045206.
66.Mnyandu N, Limani SW, Arbuthnot P, et al. Advances in designing Adeno-associated viral vectors for development of anti-HBV gene therapeutics. Virol J. 2021 Dec 13;18(1):247.
67.Grimm D, Wang L, Lee JS, et al. Argonaute proteins are key determinants of RNAi efficacy, toxicity, and persistence in the adult mouse liver. J Clin Invest. 2010 Sep;120(9):3106-19.
68.Stone D, Long KR, Loprieno MA, et al. CRISPR-Cas9 gene editing of hepatitis B virus in chronically infected humanized mice. Mol Ther Methods Clin Dev. 2021 Mar 12;20:258-275.
69.Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NTLA-2001 in Patients With Hereditary Transthyretin Amyloidosis With Polyneuropathy (ATTRv-PN) and Patients With Transthyretin Amyloidosis-Related Cardiomyopathy (ATTR-CM). https://ClinicalTrials.gov/show/NCT04601051; 2021.
70.Barzel A, Paulk NK, Shi Y, et al. Promoterless gene targeting without nucleases ameliorates haemophilia B in mice. Nature. 2015 Jan 15;517(7534):360-4.
71.De Caneva A, Porro F, Bortolussi G, et al. Coupling AAV-mediated promoterless gene targeting to SaCas9 nuclease to efficiently correct liver metabolic diseases. JCI Insight. 2019 Jun 18;5.
72.Watts JK, Corey DR. Silencing disease genes in the laboratory and the clinic. J Pathol. 2012 Jan;226(2):365-79.
73.Dana H, Chalbatani GM, Mahmoodzadeh H, et al. Molecular Mechanisms and Biological Functions of siRNA. Int J Biomed Sci. 2017 Jun;13(2):48-57.
74.Rozema DB, Lewis DL, Wakefield DH, et al. Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. Proc Natl Acad Sci U S A. 2007 Aug 7;104(32):12982-7.
75.ENVISION: A Study to Evaluate the Efficacy and Safety of Givosiran (ALN-AS1) in Patients With Acute Hepatic Porphyrias (AHP). https://ClinicalTrials.gov/show/NCT03338816; 2022.
76.Simon A. A Phase 1 Study of Givosiran (ALN-AS1) in Patients With Acute Intermittent Porphyria (AIP). https://ClinicalTrials.gov/show/NCT02452372; 2018.
77.A Study to Evaluate Lumasiran in Children and Adults With Primary Hyperoxaluria Type 1. https://ClinicalTrials.gov/show/NCT03681184; 2022.
78.Debacker AJ, Voutila J, Catley M, et al. Delivery of Oligonucleotides to the Liver with GalNAc: From Research to Registered Therapeutic Drug. Mol Ther. 2020 Aug 5;28(8):1759-1771.
79.Huang Y. Preclinical and Clinical Advances of GalNAc-Decorated Nucleic Acid Therapeutics. Mol Ther Nucleic Acids. 2017 Mar 17;6:116-132.
80.Safety, Tolerability, Pharmacokinetics and Antiviral Activity of IONIS-HBVRx in Treatment-Naïve Patients With Chronic HBV Infection. https://ClinicalTrials.gov/show/NCT02981602; 2021.
81.Bowman T. Study of Safety and Tolerability of DCR HBVS. https://clinicaltrials.gov/ct2/show/NCT03772249; 2022.

82.A Study of Inclisiran in Participants With Homozygous Familial Hypercholesterolemia (HoFH). https://ClinicalTrials.gov/show/NCT03851705; 2022.
83.Ray KK, Wright RS, Kallend D, et al. Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol. N Engl J Med. 2020 Apr 16;382(16):1507-1519.
84.Study of VIR-2218 in Healthy Subjects and Patients With Chronic Hepatitis B. https://ClinicalTrials.gov/show/NCT03672188; 2021.
85.El Dika I, Lim HY, Yong WP, 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 Jun;24(6):747-e218.
86.Di Fusco D, Dinallo V, Marafini I, et al. Antisense Oligonucleotide: Basic Concepts and Therapeutic Application in Inflammatory Bowel Disease. Front Pharmacol. 2019;10:305.
87.Dhuri K, Bechtold C, Quijano E, et al. Antisense Oligonucleotides: An Emerging Area in Drug Discovery and Development. J Clin Med. 2020 Jun 26;9(6).
88.Wong E, Goldberg T. Mipomersen (kynamro): a novel antisense oligonucleotide inhibitor for the management of homozygous familial hypercholesterolemia. P T. 2014 Feb;39(2):119-22.
89.A Study to Assess the Long-Term Safety and Efficacy of Eplontersen (Formerly Known as ION-682884, IONIS-TTR-LRx and AKCEA-TTR-LRx) in Patients With Hereditary Transthyretin-Mediated Amyloid Polyneuropathy. https://ClinicalTrials.gov/show/NCT05071300; 2022.
90.Kariko K, Buckstein M, Ni H, et al. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005 Aug;23(2):165-75.
91.An D, Schneller JL, Frassetto A, et al. Systemic Messenger RNA Therapy as a Treatment for Methylmalonic Acidemia. Cell Rep. 2017 Dec 19;21(12):3548-3558.
92.Gallego-Colon E, Daum A, Yosefy C. Statins and PCSK9 inhibitors: A new lipid-lowering therapy. Eur J Pharmacol. 2020 Jul 5;878:173114.
93.Kujovich JL. Coagulopathy in liver disease: a balancing act. Hematology Am Soc Hematol Educ Program. 2015;2015:243-9.
94.Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A. N Engl J Med. 2020 Jan 2;382(1):29-40.
95.Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-Year Outcomes of ValoctocogeneRoxaparvovec Therapy for Hemophilia A. N Engl J Med. 2023 Feb 23;388(8):694-705.
96.Pipe SW, Leebeek FWG, Recht M, et al. Gene Therapy with EtranacogeneDezaparvovec for Hemophilia B. N Engl J Med. 2023 Feb 23;388(8):706-718.
97.D'Avola D, Lopez-Franco E, Sangro B, et al. Phase I open label liver-directed gene therapy clinical trial for acute intermittent porphyria. J Hepatol. 2016 Oct;65(4):776-783.
98.Calcedo R, Wilson JM. Humoral Immune Response to AAV. Front Immunol. 2013 Oct 18;4:341.
99.Harmatz P, Lau HA, Heldermon C, et al. EMPOWERS: A phase 1/2 clinical trial of SB-318 ZFN-mediated in vivo human genome editing for treatment of MPS I (Hurler syndrome). Molecular genetics and metabolism. 2019;126(2):S68-S68.
100.Muenzer J, Prada CE, Burton B, et al. CHAMPIONS: A phase 1/2 clinical trial with dose escalation of SB-913 ZFN-mediated in vivo human genome editing for treatment of MPS II (Hunter syndrome). Molecular genetics and metabolism. 2019;126(2):S104-S104.

101.Ou L, Przybilla MJ, Ahlat O, et al. A Highly Efficacious PS Gene Editing System Corrects Metabolic and Neurological Complications of Mucopolysaccharidosis Type I. Mol Ther. 2020 Jun 3;28(6):1442-1454.
102.Strnad P, Mandorfer M, Choudhury G, et al. Fazirsiran for Liver Disease Associated with Alpha1-Antitrypsin Deficiency. N Engl J Med. 2022 Aug 11;387(6):514-524.
103.A Study Evaluating Treatment Regimens Containing Vebicorvir (ABI-H0731) in Participants With Chronic Hepatitis B Infection. https://clinicaltrials.gov/ct2/show/NCT04820686; 2021.
104.A Study to Assess Safety, Tolerability, PK and PD of AZD2693 in Non-alcoholic Steatohepatitis Patients. https://ClinicalTrials.gov/show/NCT04483947; 2022.
105.A Study to Assess the Safety, Efficacy, and Pharmacokinetics of Multiple Doses of ION224. https://ClinicalTrials.gov/show/NCT04932512; 2022.
106.McLaren DG, Han S, Murphy BA, et al. DGAT2 Inhibition Alters Aspects of Triglyceride Metabolism in Rodents but Not in Non-human Primates. Cell Metab. 2018 Jun 5;27(6):1236-1248 e6.
107.Aiyegbusi OL, Macpherson K, Elston L, et al. Patient and public perspectives on cell and gene therapies: a systematic review. Nat Commun. 2020 Dec 8;11(1):6265.
108. Study of Fazirsiran (TAK-999, ARO-AAT) in Patients With Alpha-1 Antitrypsin Deficiency Associated Liver Disease (AATD). https://clinicaltrials.gov/study/NCT03946449; 2019.
109. A Study of ARC-AAT in Healthy Volunteer Subjects and Patients With Alpha-1 Antitrypsin Deficiency (AATD). https://clinicaltrials.gov/study/NCT02363946; 2015.
110. T. Bowman. A Study of Belcesiran in Patients With AATLD (ESTRELLA). https://clinicaltrials.gov/study/NCT04764448; 2021.
111. P. Haslett. A Study of an Investigational Drug, ALN-AAT, in Healthy Adult Subjects and Patients With ZZ Type Alpha-1 Antitrypsin Deficiency Liver Disease. https://www.clinicaltrials.gov/study/NCT02503683; 2015.

112. T. Bowman. Study of DCR-A1AT in Healthy Adult Volunteers. https://clinicaltrials.gov/study/NCT04174118;2019.
113. S. Huang. A Study of ALN-HBV in Healthy Adult Volunteers and Non-cirrhotic Patients With Chronic Hepatitis B Virus (HBV) Infection. https://www.clinicaltrials.gov/study/NCT02826018; 2016.
114. Study of ARO-HBV in Normal Adult Volunteers and Patients With Hepatitis B Virus (HBV). https://clinicaltrials.gov/study/NCT03365947; 2018.
115. J. Wilson. Phase I Study of Ex Vivo Liver-Directed Gene Therapy for Familial Hypercholesterolemia. https://clinicaltrials.gov/study/NCT00004809; 1992.
116. R Kauffman. A Phase 1 Study of an Investigational Drug, ALN-PCSSC, in Subjects With Elevated Low Density Lipoprotein Cholesterol (LDL-C). https://clinicaltrials.gov/study/NCT02314442; 2014.
117. Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IONIS ANGPTL3-LRx in Healthy Volunteers With Elevated Triglycerides and Participants With Familial Hypercholesterolemia. https://clinicaltrials.gov/study/NCT02709850; 2015.
118. A Study of mRNA-3745 in Adult and Pediatric Participants With Glycogen Storage Disease Type 1a (GSD1a). https://clinicaltrials.gov/study/NCT05095727; 2022.
119. Clinical Sciences & Operations, Sanofi. A Study of Fitusiran in Severe Hemophilia A and B Patients Previously Receiving Factor or Bypassing Agent Prophylaxis (ATLAS-PPX). https://clinicaltrials.gov/study/NCT03549871; 2018.
120. Clinical Sciences & Operations, Sanofi. Long-term Safety and Efficacy Study of Fitusiran in Patients With Hemophilia A or B, With or Without Inhibitory Antibodies to Factor VIII or IX (ATLAS-OLE). https://www.clinicaltrials.gov/study/NCT03754790; 2019.
121. Clinical Sciences & Operations, MD,Sanofi. A Study of Fitusiran (ALN-AT3SC) in Severe Hemophilia A and B Patients Without Inhibitors. https://clinicaltrials.gov/study/NCT03417245; 2018.
122. Clinical Sciences & Operations, MD,Sanofi. A Study of Fitusiran (ALN-AT3SC) in Severe Hemophilia A and B Patients With Inhibitors (ATLAS-INH). https://clinicaltrials.gov/study/NCT03417102; 2018.
123. M. Kowalski. Safety, Pharmacokinetics and Preliminary Anti-Tumor Activity of Intravenous TKM-080301 in Subjects With Advanced Hepatocellular Carcinoma. https://clinicaltrials.gov/study/NCT02191878; 2014.
124. M. Shi. HAIC Plus H101 vs HAIC Alone for Unresectable HCC at BCLC A-B. https://clinicaltrials.gov/study/NCT03780049; 2018.
125. Open Label Study of mRNA-3704 in Patients With Isolated Methylmalonic Acidemia. https://clinicaltrials.gov/study/NCT03810690; 2019.
126. A Study to Assess Safety, Pharmacokinetics, and Pharmacodynamics of mRNA-3705 in Participants With Isolated Methylmalonic Acidemia. https://clinicaltrials.gov/study/NCT04899310; 2021.
127. An Extension Study to Evaluate the Long-Term Safety and Clinical Activity of mRNA-3705 in Participants Previously Enrolled in the mRNA-3705-P101 Study. https://www.clinicaltrials.gov/study/NCT05295433; 2022.
128. Safety, Tolerability and PK/PD Evaluation of Intravenous Administration of MRT5201 in Patients With OTC Deficiency (STEP-OTC). https://clinicaltrials.gov/study/NCT03767270; 2019.

129. S. Shergill. Nedosiran in Pediatric Patients From Birth to 11 Years of Age With PH and Relatively Intact Renal Function (PHYOX8). https://clinicaltrials.gov/study/NCT05001269; 2022.
130. Open-Label Study of mRNA-3927 in Participants With Propionic Acidemia. https://clinicaltrials.gov/study/NCT04159103; 2021.
131. Evaluate the Safety and Tolerability, as Well as the Pharmacokinetic and Pharmacodynamic Profiles of Single and Multiple Doses of Eplontersen Administered Subcutaneously to Healthy Volunteers and Patients With Hereditary Transthyretin-Mediated Amyloidosis (hATTR). https://clinicaltrials.gov/study/NCT03728634; 2018.
132. Medical Director, Alnylam Pharmaceuticals. HELIOS-A: A Study of Vutrisiran (ALN-TTRSC02) in Patients With Hereditary Transthyretin Amyloidosis (hATTR Amyloidosis). https://clinicaltrials.gov/study/NCT03759379; 2019.
133. Medical Director, Alnylam Pharmaceuticals. HELIOS-B: A Study to Evaluate Vutrisiran in Patients With Transthyretin Amyloidosis With Cardiomyopathy. https://clinicaltrials.gov/study/NCT04153149; 2019.
134. Bjursell M, Porritt MJ, Ericson E, Taheri-Ghahfarokhi A, Clausen M, Magnusson L, Admyre T, Nitsch R, Mayr L, Aasehaug L, Seeliger F, Maresca M, Bohlooly-Y M, Wiseman J. Therapeutic Genome Editing With CRISPR/Cas9 in a Humanized Mouse Model Ameliorates α1-antitrypsin Deficiency Phenotype. EBioMedicine. 2018 Mar;29:104-111. doi: 10.1016/j.ebiom.2018.02.015. Epub 2018 Feb 19. PMID: 29500128; PMCID: PMC5925576.

135. Li H, Haurigot V, Doyon Y, Li T, Wong SY, Bhagwat AS, Malani N, Anguela XM, Sharma R, Ivanciu L, Murphy SL, Finn JD, Khazi FR, Zhou S, Paschon DE, Rebar EJ, Bushman FD, Gregory PD, Holmes MC, High KA. In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature. 2011 Jun 26;475(7355):217-21. doi: 10.1038/nature10177. PMID: 21706032; PMCID: PMC3152293.

136. Schneller JL, Lee CM, Venturoni LE, Chandler RJ, Li A, Myung S, Cradick TJ, Hurley AE, Lagor WR, Bao G, Venditti CP. In vivo genome editing at the albumin locus to treat methylmalonic acidemia. Mol Ther Methods Clin Dev. 2021 Nov 11;23:619-632. doi: 10.1016/j.omtm.2021.11.004. PMID: 34901307; PMCID: PMC8634044.

137. Sharma R, Anguela XM, Doyon Y, Wechsler T, DeKelver RC, Sproul S, Paschon DE, Miller JC, Davidson RJ, Shivak D, Zhou S, Rieders J, Gregory PD, Holmes MC, Rebar EJ, High KA. In vivo genome editing of the albumin locus as a platform for protein replacement therapy. Blood. 2015 Oct 8;126(15):1777-84. doi: 10.1182/blood-2014-12-615492. Epub 2015 Aug 21. PMID: 26297739; PMCID: PMC4600017.

138. Singh K, Evens H, Nair N, Rincón MY, Sarcar S, Samara-Kuko E, Chuah MK, VandenDriessche T. Efficient In Vivo Liver-Directed Gene Editing Using CRISPR/Cas9. Mol Ther. 2018 May 2;26(5):1241-1254. doi: 10.1016/j.ymthe.2018.02.023. Epub 2018 Mar 6. PMID: 29599079; PMCID: PMC5993986.

139. Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, Koteliansky V, Sharp PA, Jacks T, Anderson DG. Genome editing with Cas9 in adult mice corrects a disease mutation and

phenotype. Nat Biotechnol. 2014 Jun;32(6):551-3. doi: 10.1038/nbt.2884. Epub 2014 Mar 30. Erratum in: Nat Biotechnol. 2014 Sep;32(9):952. PMID: 24681508; PMCID: PMC4157757.

140. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, Park A, Yang J, Suresh S, Bizhanova A, Gupta A, Bolukbasi MF, Walsh S, Bogorad RL, Gao G, Weng Z, Dong Y, Koteliansky V, Wolfe SA, Langer R, Xue W, Anderson DG. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol. 2016 Mar;34(3):328-33. doi: 10.1038/nbt.3471. Epub 2016 Feb 1. PMID: 26829318; PMCID: PMC5423356.

141. Rathbone T, Ates I, Stuart C, Parker T, Cottle RN. Electroporation-mediated Delivery of Cas9 Ribonucleoproteins and mRNA into Freshly Isolated Primary Mouse Hepatocytes. J Vis Exp. 2022 Jun 2;(184). doi: 10.3791/63828. PMID: 35723482.

142. Song CQ, Jiang T, Richter M, Rhym LH, Koblan LW, Zafra MP, Schatoff EM, Doman JL, Cao Y, Dow LE, Zhu LJ, Anderson DG, Liu DR, Yin H, Xue W. Adenine base editing in an adult mouse model of tyrosinaemia. Nat Biomed Eng. 2020 Jan;4(1):125-130. doi: 10.1038/s41551-019-0357-8. Epub 2019 Feb 25. PMID: 31740768; PMCID: PMC6986236.

143. Wen J, Cao T, Wu J, Chen Y, Zhi S, Huang Y, Zhen P, Wu G, Aagaard L, Zhong J, Liang P, Huang J. Single AAV-mediated CRISPR-Nme2Cas9 efficiently reduces mutant hTTR expression in a transgenic mouse model of transthyretin amyloidosis. Mol Ther. 2022 Jan 5;30(1):164-174. doi: 10.1016/j.ymthe.2021.05.010. Epub 2021 May 14. PMID: 33992807; PMCID: PMC8753293.