Hepatology Snapshot:

# Gene therapies targeting the liver

JOURNAL OF HEPATOLOGY

Andrei Claudiu Cozmescu[1,2], John Counsell[1,3,4], Paul Gissen[1,2,*]

[1]NIHR Great Ormond Street Hospital Biomedical Research Centre, University College London, London, UK
[2]MRC Laboratory for Molecular Cell Biology, University College London, London, UK
[3]Gene Transfer Technology Group, EGA Institute for Women's Health, University College London, London WC1E 6HX, UK
[4]Dubowitz Neuromuscular Centre, Molecular Neurosciences Section, Developmental Neurosciences Programme, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
*Corresponding author. Address: NIHR Great Ormond Street Hospital Biomedical Research Centre, University College London, 30 Guilford Street, London, WC1N 1EH, UK; Tel.: +44 (0) 20 7242 9789, fax +44 (0) 20 7905 2000.
E-mail address: p.gissen@ucl.ac.uk (P. Gissen).

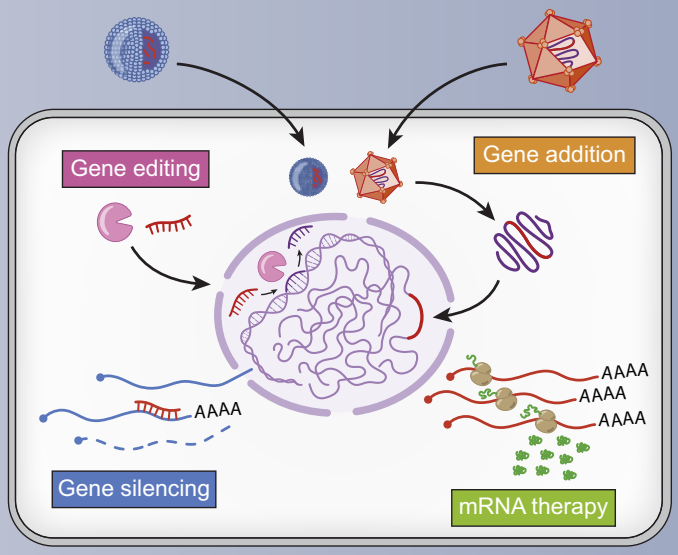
## Liver-directed gene therapy

Monogenic disorders due to defect in gene expressed in liver cells

Liver viral infections

Multifactorial disorder treated by targeting a single gene

## Gene therapy strategies




### *In vivo* administration

Gene therapy vectors

Human:
- Peripheral vein
- Intraportal

Mouse model:
- Superficial temporal vein
- Tail vein
- Intrahepatic

### Delivery

**Viral**

Engineered virus carrying therapeutic genes to a target tissue:
- Adenovirus
- Adeno-associated virus
- Lentivirus

**Non-viral**

1. Naked nucleic acid
2. Vector based

Transported via chemical or biological structure:
- Lipid nanoparticle
- Exosome
- PEI coated particle

### Gene addition

**Introduction of a wild type form of a mutated gene into an affected tissue using viral vectors**
- Can offer a permanent cure (depending on disease and vector type)
- Efficient expression of transgene driven from engineered promoters (thyroxine binding globulin and hepatic control region-human α-1 antitrypsin promoters)

### Adenovirus

- Non-enveloped, dsDNA vector
- Can package up to 37 kb transgene
- Generally used in oncology
- Efficient liver transduction
- Elicits strong immune responses

### Lentivirus

- Enveloped, ssRNA vector
- Can package up to 10 kb of transgene
- Allows long term gene expression (transgene integration into host genome)
- Low pre-existent immunity in humans
- Possible genotoxicity limited by good vector design

### Adeno-associated virus (AAV)

- Non-enveloped, ssDNA vector
- Can package up to 4.7 kb of transgene
- Remains mostly episomal in the cells
- Episomal DNA concentration decreases with liver growth
- High liver transduction (Serotypes 8 and 9 in animal models, LK03 in humans)

### Gene editing

**Targeted DNA cutting and editing using nucleases (ZNFs, TALENs, Cas9)**
- Editing tools can be delivered using viral (AAVs, integration deficient lentiviruses) or non-viral vectors.
- Permits *in situ* correction of mutations
- Can produce gene knockouts
- Possible off-target effects

### mRNA therapy

**Regulation of protein stability and translation using chemically modified RNAs**
- Generally delivered by non-viral vectors
- Allows dose control and transient expression of proteins
- Requires repeated administration

### Gene silencing

**Degradation of target mRNAs using siRNAs and ASOs**
- Can be delivered naked, with non-viral vectors or expressed from viral vectors
- Chemical modifications improve hepatocyte transfection and reduce off-target effects
- Requires re-administration when delivered using non-viral strategies

Examples of current treatments, strategies and stage of development. Selected therapies target hepatocytes *in vivo* to produce cytosolic or secreted proteins or to knockdown specific genes.

(Updated 01/04/2020)

| Technology<br>*Disease/condition* | Liver affected | Drug | Treatment effect: Cytoplasmic protein production | Treatment effect: Production of secreted protein | Treatment effect: Gene expression silencing | Stages of development: Preclinical | Phase I | Phase II | Phase III | Approved |
|---|---|---|---|---|---|---|---|---|---|---|
| **Adeno-associated virus derived vector** | | | | | | | | | | |
| *Haemophilia A* | | SPK-8011 | | + | | | | | | |
| *Ornithine transcarbamylase deficiency* | + | DTX301 | + | | | | | | | |
| *Diabetes and obesity* | | RT-200/210 | | + | | | | | | |
| **Adenoviral vector** | | | | | | | | | | |
| *Hepatocellular carcinoma and liver metastasis* | + | Ad-p53 | + | | | | | | | |
| *Hepatocellular carcinoma* | + | H101 | + | | | | | | | |
| **Lentiviral vector** | | | | | | | | | | |
| *Haemophilia B* | | SIN.ET.FIX | | + | | | | | | |
| **Gene editing** | | | | | | | | | | |
| *Mucopolysaccharidosis type I* | + | SB-318 | | + | | | | | | |
| *Mucopolysaccharidosis type II* | + | SB-913 | | + | | | | | | |
| **mRNA therapy** | | | | | | | | | | |
| *Methylmalonic acidemia* | + | mRNA-3704 | + | | | | | | | |
| *Alpha-1-antitrypsin deficiency* | + | MRT5201 | + | | | | | | | |
| *Autoimmune hepatitis* | | mRNA-6981 | + | | | | | | | |
| **Gene silencing** | | | | | | | | | | |
| *Primary hyperoxaluria type 1* | | Lumasiran | | | + | | | | | |
| *Hypercholesterolemia* | | Inclisiran | | | + | | | | | |
| *Chronic hepatitis B virus infection* | + | ALN-HBV02 | | | + | | | | | |
| *Liver cancers* | + | TKM-080301 | | | + | | | | | |

*Keywords: Gene therapy; Monogenic defects; Adeno Associated-Viruses; Lentiviruses; Adenoviruses; Episomes; Lipid nanoparticles; Exosomes; Polyethylenimine; Messenger RNAs; Antisense oligonucleotides; Zinc-Finger Nucleases; CRISPR/Cas9*

*Received 15 May 2020; received in revised form 4 August 2020; accepted 4 August 2020.*

The liver is a key organ in the human body involved in a variety of functions that influence other organs. Among other roles, it is essential for digestion, metabolism, detoxification, immunity and blood clotting. Hepatocytes constitute the bulk of cells in the liver parenchyma and are affected by the majority of monogenic liver inherited disorders, viral infections and malignancies.

Whilst conventional therapies can alleviate symptoms of some liver disorders, very few curative treatments currently exist. Recently, several gene therapy strategies have arisen as attractive treatment options for monogenic disorders or multifactorial disorders with specific gene targets.[1]

This snapshot illustrates the delivery methods, as well as the advantages and disadvantages of some of these technologies. It also offers examples of the development stage of gene therapy products for selected disorders.

The best-known gene therapy strategy is gene addition which allows the phenotypic correction of a disorder by providing a wild-type form of a mutated gene to affected tissues. Viral vectors such as adeno-associated viruses (AAVs), adenoviruses and lentiviruses have been used as carriers for these genes. Depending on the disease and vector type, gene addition can achieve life-long benefits. AAV-derived vectors are non-enveloped, single-stranded DNA viruses that can package up to 4.7 kb of a transgene.[2] AAVs efficiently transduce hepatocytes, have low immunogenicity and are mostly preserved as episomes inside cells, which might limit their efficacy in dividing cell systems, such as the growing liver.[2]

Adenoviruses are non-enveloped, double-stranded DNA viruses capable of carrying around 37 kb of a transgene and transducing liver cells with high efficiency. They can elicit strong immune effects and are generally used in oncolytic therapy.[3]

Lentiviral vectors are single-stranded RNA viruses that can efficiently accommodate 10 kb of a transgene.[4] They integrate their genetic material into the host genome, allowing long-term expression of the therapeutic gene. Depending on design, they present a low risk of genotoxicity, as they integrate into intronic regions of actively transcribed genes.[5] To date, lentiviral vectors have been approved for *ex vivo* gene therapy,[6] but there is interest in using lentiviral vectors for *in vivo* liver-directed treatments.

Non-viral delivery is an alternative to the viral vector transport systems. For this, engineered particles such as lipid nanoparticles, exosomes or polyethylenimine -coated particles can be produced to encapsulate several therapeutic compounds including mRNAs for mRNA therapy, or small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) for gene-silencing therapies.[7,8] The cellular uptake of nanoparticles is reduced by this method and the treatment effect is short-lasting, requiring frequent re-administration. Alternatively, siRNAs and ASOs can be delivered directly into the circulation, with certain chemical modifications enabling efficient hepatocyte transfection.[8]

Another gene therapy strategy is genetic correction using platforms such as Zinc-Finger Nucleases or CRISPR/Cas9 complexes. These systems can be delivered using both viral and non-viral strategies and allow *in situ* correction of mutations. However, their *in vivo* use is limited by possible off-target effects, which can lead to carcinogenesis.[9]

The table included in the snapshot was up to date on 01.04.2020 and contains examples of treatments employing some of the aforementioned technologies.[10] The genetic treatments included in the table are delivered *in vivo*.

## Financial support

JRC is funded by a Wellcome Innovator Award (210774/2/18/Z) and London Advanced Therapies Collaborate to Innovate Award. All research at Great Ormond Street Hospital NHS Foundation Trust and UCL Great Ormond Street Institute of Child Health is made possible by the NIHR Great Ormond Street Hospital Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. This work was supported by MRC funding to the MRC LMCB University Unit at UCL, award code MC_U12266B.

## Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

PG conceived the idea and contributed to writing and editing, ACC wrote manuscript and designed the figure, JC contributed to writing and editing.

## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.08.003.

## References

[1] Baruteau J, Waddington SN, Alexander IE, Gissen P. Gene therapy for monogenic liver diseases: clinical successes, current challenges and future prospects. J Inherit Metab Dis 2017;40:497–517.
[2] Ginocchio VM, Ferla R, Auricchio A, Brunetti-Pierri N. Current status on clinical development of adeno-associated virus-mediated liver-directed gene therapy for inborn errors of metabolism. Hum Gene Ther 2019;30:1204–1210.
[3] Abudoureyimu M, Lai Y, Tian C, Wang T, Wang R, Chu X. Oncolytic adenovirus-a nova for gene-targeted oncolytic viral therapy in HCC. Front Oncol 2019;9:1182.
[4] Counsell JR, Asgarian Z, Meng J, Ferrer V, Vink CA, Howe SJ, et al. Lentiviral vectors can be used for full-length dystrophin gene therapy. Sci Rep 2017;7:44775.
[5] Cantore A, Ranzani M, Bartholomae CC, Volpin M, Valle PD, Sanvito F, et al. Liver-directed lentiviral gene therapy in a dog model of hemophilia B. Sci Transl Med 2015;7:277ra28.
[6] Schuessler-Lenz M, Enzmann H, Vamvakas S. Regulators' advice can make a difference: European Medicines Agency approval of Zynteglo for Beta Thalassemia. Clin Pharmacol Ther 2020;107:492–494.
[7] Berraondo P, Martini PGV, Avila MA, Fontanellas A. Messenger RNA therapy for rare genetic metabolic diseases. Gut 2019;68:1323–1330.
[8] Setten RL, Rossi JJ, Han S. The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov 2019;18(June):421–446.
[9] Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther 2020;5:1.
[10] Clinical Trials. Home - Clinical Trials. 2020. Available at: http://www.clinicaltrials.com/. Accessed April 1, 2020.