

REVIEW



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Drug-induced liver injury related to gene therapy: A new challenge to be managed

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Abstract

Gene therapy is being successfully developed for the treatment of several genetic disorders. Various methods of gene transfer have been developed to enable the production of the deficient enzyme or protein. One of the most important is adeno-associated virus vectors, which have been shown to be viable for use in in vivo gene therapy. Several gene therapies have already been approved. They are also promising for acquired diseases. Important examples include gene therapy for haemophilia A and B, X-linked myotubular myopathy, spinal muscular atrophy and several liver diseases such as Crigler-Najjar disease, alpha-1 antitrypsin deficiency and Fabry disease. However, the introduction of a foreign compound into hepatocytes leads to hepatic reactions with heterogeneous phenotypic expression and a wide spectrum of severity, ranging from mild transaminase elevation to acute liver failure. Several mechanisms appear to be involved in liver injury, including an immune response, but also direct toxicity depending on the method of gene transfer. As a result, the incidence, expression and severity of liver injury vary from indication to indication and from patient to patient. Patients treated for haemophilia A are more prone to transaminase elevation than those treated for haemophilia B. Corticosteroids are successfully used to correct liver reactions but also to prevent degradation of the transferred gene and loss of therapeutic activity. The aim of this review is to describe the risk of liver injury according to the indication for gene therapy and the short- and long-term management currently proposed to prevent or correct liver reactions in clinical practice.

KEYWORDS

adeno-associated virus, drug-induced liver injury, gene therapy, immuno-suppression, management

1 | INTRODUCTION

The goal of gene therapy (GT) is to treat or prevent disease by altering the genetic material of a patient's cells.^{1–3} Among genetic

disorders, haemophilia A and B, sickle cell anaemia and muscular dystrophies are the most relevant diseases for the use of GT, as well as inherited genetic eye diseases such as Leber congenital amaurosis and retinitis pigmentosa. These therapies are also

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being developed in other areas such as cystic fibrosis, homozygous hypercholesterolaemia, Wilson's disease... In acquired diseases, cancer and viral infections are the most advanced areas, as well as haematopoietic stem cell disorders, but other diseases such as myocardial infarction are also affected.^{1–11} Tables 1 and 2 summarise the main non-hepatic and hepatic indications for GTs. Several GTs have already been approved by the European Medicines Agency (EMA) and/or the Food and Drug Administration (FDA) (Table 3).^{4–6,12} They also hold promise for a wide range of other genetic and acquired diseases. In fact, as of 13 February 2024, approximately 15 phase 3 clinical trials using GT are ongoing (<https://clinicaltrials.gov>) in various areas, including both genetic and acquired diseases.¹³

The first basic principle of GT is to identify the target gene(s) responsible for the specific disease, followed by the introduction of a healthy copy of the therapeutic gene into the patient's cells.^{1–3} Several methods can be used to achieve this, including the injection of viral vectors carrying the therapeutic gene (in vivo transduction), or the direct delivery of the gene into the physiologically relevant target cell using specialised carriers (ex vivo transduction). Once the therapeutic gene (transgene) is inside the patient's cells, it enables the production of the normal protein to correct the defect.^{1–3}

Hepatic adverse events associated with the use of GT have been described and few fatal cases have been reported.¹⁴ To date, the mechanisms of such liver toxicity remain poorly defined. However, several potential mechanisms can be postulated:

- (i) Off-target effects: the therapeutic genes may sometimes be delivered to unintended liver cells, leading to unwanted effects in the liver cells.

Metabolic disorders	Methylmalonic acidemia, Wilson's disease, congenital adrenal hyperplasia, glycogen storage diseases, familial hypercholesterolemia, urea cycle disorder...
Haematological disorders	Haemophilia A and B, beta-thalassemia, sickle cell disease, Diamond-Blackfan anaemia...
Muscular diseases	Spinal muscular atrophy, Duchene disease, myotubular myopathies...
Eye disorders	Leber's congenital amaurosis, cone-rod dystrophy, inherited retinal dystrophies, Stargardt's disease...
Immune deficiency	Leukocyte adhesion deficiency, adenosine deaminase deficiency...
Lysosomal disorders	Gaucher disease, Fabry disease, mucopolysaccharidosis type I, II, III and IV, Pompe's disease...
Neurodegenerative diseases	Adrenoleucodystrophy, metachromatic leukodystrophy, Friedreich's ataxia, Rett syndrome, Huntington's disease, Angelman syndrome...
Skin disorders	Epidermolysis bullosa, autosomal recessive congenital ichthyosis...
Cancer	Leukaemia, melanoma, advanced glioma...
Infectious diseases	HIV
Respiratory disorder	Cystic fibrosis
Deglycosylation disorder	NGLY1 deficiency

Key points

- Gene therapy is an important new treatment for genetic and acquired disorders. Several gene therapy treatments are now approved.
- Gene therapy can cause liver injury with a very wide spectrum of phenotypic expression and severity, with transaminitis being the most common.
- The mechanisms of hepatotoxicity associated with gene therapy are heterogeneous (immune-mediated and direct toxicity) and are only partially understood.
- Liver injury may alter the expression of transgenes and thereby reduce the efficacy of gene therapy.
- Corticosteroids are widely used to control the hepatotoxicity of gene therapy and restore its efficacy, taking into account immune-mediated mechanisms and expert experience.

- (ii) Gene regulation issues: the introduced genes may in some cases be over-expressed, leading to excessive production of certain proteins that could be detrimental to liver function.
- (iii) Pre-existing liver disease: patients with pre-existing liver disease may be more susceptible to liver toxicity following GT due to impaired liver function.
- (iv) Immune response: the use of viral vectors involves the use of modified versions of viruses that are thought to be harmless to humans. However, these viral vectors can still induce an immune response that leads to inflammation in the liver, potentially causing damage to liver cells.

TABLE 1 Gene therapy: main indications outside liver diseases.^{1–3}

TABLE 2 Gene therapy for liver diseases.^{4–11}

Disease	Clinical trial
Crigler-Najjar type 1 syndrome	Phase ½ (published) ⁷
Alpha-1 antitrypsin deficiency	Phases ½ studies completed (NCT02168686, NCT03804021 and NCT00377416) ^{8–10}
Acute intermittent porphyria	Phase 1 study completed (NCT02082860) ¹¹
Type 3 progressive familial intra-hepatic cholestasis (PFIC3)	No data on humans; no ongoing study
Fabry disease	Phase 1/2 (terminated). (NCT04040049)
Type II Citrullinemia	No data on humans; no ongoing study
Galactosemia	No data on humans; no ongoing study
Glycogen storage disease type 1a	Phases 1/2 ongoing (NCT03517085 and NCT05095727)

In this review, we will discuss liver-related adverse events of gene therapy and their mechanisms, prevention, treatment and management. The long-term hepatotoxicity effects which are largely unknown will be not discussed.

2 | GENERAL CONCEPT OF GENE THERAPY

Gene therapy (GT) is the modification of genetic material to treat or cure disease by inserting, deleting or modifying DNA sequences. It includes gene repair to correct mutations, gene supplementation to provide functional gene copies, and gene silencing to reduce the expression of harmful proteins.^{1–5,15} Gene silencing therapy involves the targeted downregulation of specific genes through mechanisms, such as RNA interference (RNAi), and offers a promising approach to treating genetic disorders by preventing the production of harmful proteins.^{1–5}

In ex vivo GT, cells are harvested, transduced with therapeutic genes via vectors and returned to the patient. Conversely, in vivo GT involves the direct delivery of vectors or genetic material to the patient using non-viral and viral vectors, such as cationic polymers, lipid nanoparticles (LNPs), adeno-associated viruses (AAVs) and lentiviruses (Figure 1).^{1–3,15}

AAV vectors are non-enveloped, single-stranded DNA vectors characterised by their episomal nature and serotype-dependent immunity. They are known for their low immunogenicity and high liver transduction efficiency. Lentiviruses, with a larger packaging capacity of up to 10kb, are enveloped, integrative vectors that can induce acute inflammatory responses and show high liver transduction, but low pre-existing immunity. Their in vivo dynamics are still under investigation.^{1–3,15}

Non-viral vectors have gained attention for liver-targeted gene delivery. Cationic polymers form nanoparticles with nucleic acids for cellular transport through electrostatic interactions. LNPs, which resemble cellular membranes, offer a promising nucleic acid delivery method due to their biocompatibility, biodegradability, low toxicity and immunogenicity, coupled with structural adaptability and scalable synthesis.^{1–3,15}

This therapeutic diversity highlights the complex interplay between vector selection, delivery method and immune response in advancing the use of GT, which requires a comprehensive understanding for clinical application. In some cases, the hepatocyte is not the site of production of the dysfunctional factor (e.g. in spinal muscular atrophy), so we can assume that GT may alter healthy hepatocytes in these cases.^{1–4}

3 | CHARACTERISTICS AND INCIDENCE OF LIVER DAMAGE FROM GENE THERAPY

3.1 | Characteristics of liver injury

Liver toxicity associated with gene therapy is characterised by elevated liver parameter values, usually those of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Drug-induced liver toxicity is frequently classified according to the pattern of elevation of liver enzymes based on the first set of laboratory tests available in relation to the clinical event.^{16,17} This pattern is defined by the increase of ALT or alkaline phosphatase (ALP) alone above a specific threshold or by the ratio of serum ALT to ALP levels (R value = $[\text{ALT}/\text{upper limit of normal (ULN)}]/[\text{ALP}/\text{ULN}]$) and can be categorised as hepatocellular ($\text{ALT} \geq 5$ -fold above ULN or $R > 5$), mixed ($R > 2$ to < 5), or cholestatic ($\text{ALP} \geq 2$ -fold above ULN or $R < 2$).^{16,17} The pattern of liver toxicity induced by gene therapy is heterogeneous; it may be hepatocellular, mixed or cholestatic. Liver toxicity depends on the type of gene therapy, the dose and baseline liver status.

Evaluating the severity of clinical liver toxicity is of primary importance as it will guide patient management. The method used to grade toxicity, usually applied during all clinical trials of gene therapy is the Common Terminology Criteria for Adverse Events (CTCAE) developed by the National Cancer Institute. However, this classification may not always be adequate to evaluate liver toxicity as it may overestimate the severity of the disease. This is particularly true when it is compared to the drug-induced liver injury network (DILIN) classification. For example, under the CTCAE classification, an elevation of transaminases to more than 20x the upper limit of normal without a rise in bilirubin levels or an impairment of

TABLE 3 Approved cellular and gene therapy products by the FDA and/or EMA in December 2023.^{12,13}

Commercial name	Molecule	Indications	FDA (1) and/or EMA (2) approved
Abecma®	Idecabtagene vicleucel	Multiple myeloma	1 and 2
Adstiladrin®	Nadofaragene firadenovec-vncg	High-risk non-muscle invasive bladder cancer (NMIBC) that is unresponsive to BCG	1 and 2
AlloCord®	Human cord blood haematopoietic progenitor cell injection	In unrelated donor haematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for haematopoietic and immunological reconstitution in patients with disorders affecting the haematopoietic system that are inherited, acquired, or result from myeloablative therapy	1
Breyanzi®	Lisocabtagene maraleucel	Large B-cell lymphoma (LBCL)	1 and 2
Carvykti®	Ciltacabtagene autoleucel	CAR T-cell therapy for relapsed or refractory multiple myeloma	1 and 2
Casgevy®	Exagamglogene autotemcel	Sickle cell disease (SCD) with recurrent vaso-occlusive crises	1 and 2
Clevecord®	Haematopoietic progenitor cell, cord blood injection	In unrelated donor haematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for haematopoietic and immunologic reconstitution in patients with disorders affecting the haematopoietic system that are inherited, acquired, or result from myeloablative therapy	1
Ducord®	Umbilical cord blood-derived haematopoietic progenitor cells	In unrelated donor haematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for haematopoietic and immunologic reconstitution in patients with disorders affecting the haematopoietic system that are inherited, acquired, or result from myeloablative therapy	1
Elevidys®	Delandistrogene moxeparvovec	Duchenne muscular dystrophy (DMD) with a confirmed mutation in the <i>DMD</i> gene	1 and 2
Gintuit®	Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen	Topical (non-submerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions	1
Hemgenix®	Etranacogene dezaparvovec	Adults with severe and moderately severe haemophilia B	1 and 2
Libmeldy®	Stem cells, (CD34 ⁺ cells), derived from the patient's own bone marrow or blood, that have been modified to contain a copy of the gene to make ARSA and can divide to produce other sorts of blood cells.	For children with metachromatic leukodystrophy	2
Provenge®	Sipuleucel-T	For asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer	1 and 2
Rethymic®	Allogeneic processed thymus tissue	For immune reconstitution in paediatric patients with congenital athymia	1
Roctavian®	Valoctocogène roxaparvovec	For severe haemophilia A (congenital factor VIII deficiency with factor VIII activity <1 IU/dL) without pre-existing antibodies to adeno-associated virus serotype 5 detected by an FDA-approved test	1 and 2
Skysona®	Elivaldogene autotemcel	To slow the progression of neurologic dysfunction in boys 4–17 years of age with early, active cerebral adrenoleukodystrophy	1
Stratagraft®	Dermis and fully-stratified, biologically functional epidermis generated from a pathogen-free, long-lived human keratinocyte progenitor cell line	For thermal burns containing intact dermal elements for which surgical intervention is clinically indicated (deep partial-thickness burns)	1

TABLE 3 (Continued)

Commercial name	Molecule	Indications	FDA (1) and/or EMA (2) approved
Tecartus®	Brexucabtagene autoleucel	In relapsed or refractory B-cell precursor acute lymphoblastic leukaemia and/or in relapsed/refractory mantle cell lymphoma	1 and 2
Upstaza®	Eladocagene exuparovec	In adults and children aged 18 months and older with severe aromatic L-amino acid decarboxylase deficiency	2
Vujuvek®	Beremagene geperpavec	For wounds in patients with dystrophic epidermolysis bullosa with mutation(s) in the <i>collagen type VII alpha 1 chain (COL7A1)</i> gene	1
Yescarta®	Axicabtagene ciloleucel	For large B-cell lymphoma that is refractory to first-line chemoimmunotherapy or that relapses within 12 months of first-line chemoimmunotherapy	1 and 2
Zynteglo®	Bétibéglogène autotemcel	For patients with B-thalassemia who require regular red blood cell transfusions	1
Zolgesma®	Onasemnogene abeparovec	For paediatric patients less than 2 years of age with spinal muscular atrophy with biallelic mutations in the <i>survival motor neuron 1 (SMN1)</i> gene	1 and 2

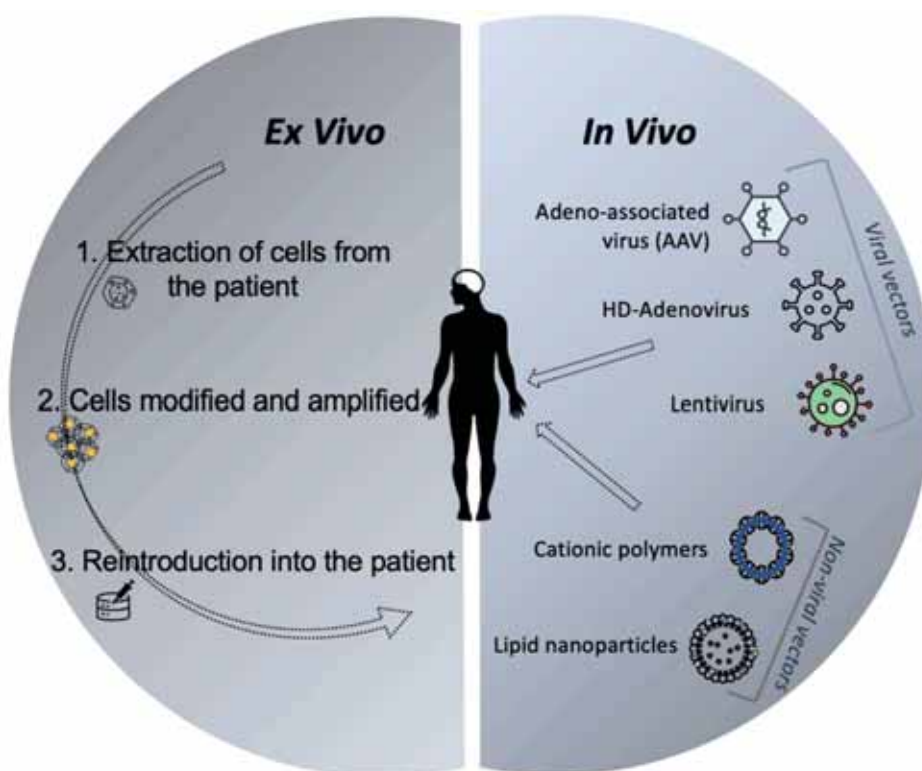


FIGURE 1 General concepts for gene therapy. In ex vivo GT, cells are harvested, transduced with therapeutic genes via vectors, and returned to the patient. Conversely, in vivo GT involves direct vector or genetic material delivery to the subject, utilising non-viral and viral vectors, such as cationic polymers, lipid nanoparticles (LNPs), adeno-associated viruses (AAV), and lentiviruses.

coagulation is considered as grade 4 toxicity, which corresponds to a life-threatening event. From a hepatological standpoint, acute hepatitis is considered severe if the INR is ≥ 1.5 , and fulminant if impaired coagulation is accompanied by hepatic encephalopathy.^{16,17}

In clinical trials, an elevation in ALT greater than three times the upper limit of normal in association with an elevation in total

bilirubin greater than two times the upper limit of normal in the absence of significant cholestasis defines a group of patients at high risk of developing liver failure (Hy's law rule).¹⁷

Spinal muscular atrophy (SMA) is a genetic disease characterised by a mutation in the *SMN1* gene that leads to the loss of motor neurons and impaired signalling between the brain and skeletal

muscles.¹⁸ It is usually diagnosed in infancy or early childhood and is characterised by weakness and wasting of the voluntary muscles. In 2017, Mendell JR & al published the first results of 15 patients with SMA1 treated with a single dose of intravenous onasemnogene abeparvovec, an adeno-associated virus serotype 9 that carries SMN complementary DNA encoding the missing SMN protein.¹⁹ In this study, 4 patients developed elevated serum aminotransferase levels without other liver enzyme abnormalities.¹⁹ The first case of liver toxicity occurred approximately 3 weeks after injection (grade 4 according to the CTCAE) and led to a protocol change in which all the next 14 patients received oral prednisolone at a dose of 1 mg per kilogram per day for approximately 30 days, starting 24 h before gene vector administration.¹⁹ Despite this prophylactic strategy, three additional patients developed GT-induced liver injury, one of whom required additional prednisolone to reduce elevated serum ALT and AST levels.¹⁹

Since its approval by the FDA in May 2019, more than 335 children have been treated with onasemnogene commercially or through a managed access program.²⁰ In 2021, a large cohort of 100 SMA patients from 5 clinical trials was analysed.²⁰ Ninety-nine out of 100 patients received prophylactic prednisolone (1 mg/kg/day prednisolone or equivalent 24 h prior to treatment) for a mean duration of 83 days (range: 33–229). Ninety per cent of patients had at least some degree of ALT and/or AST elevation during therapy without concomitant bilirubin elevation.²⁰ No biological cholestasis was detected. According to CTCAE classifications: 9% of cases were grade 2, 6% grade 3 and 5% grade 4. Peaks in liver enzyme elevations were described after the first week and at the end of the first-month post-injection at the time of tapering and discontinuation of systemic corticosteroid therapy. All elevations in serum aminotransferase concentrations normalised by the end of the study observation periods. Of note, in March 2019, the prophylactic regimen was adjusted due to a report of acute liver failure as follows: 2 mg/kg/day of prednisolone from 24 h prior to infusion until day 3 and then reduction to 1 mg/kg/day for 30 days with tapering. Interestingly, more than half of the patients had abnormal pre-treatment ALT and/or AST and/or bilirubin levels, suggesting pre-existing liver disease such as hepatic steatosis, which has been found in more than one-third of autopsies of children with SMA.²¹

Feldman et al. reported the results of liver biopsies performed in two patients who developed subacute liver failure between 3 and 8 weeks after onasemnogeneic abeparvovec administration, despite receiving corticosteroids before and after the infusion.²² In the first patient, pathological analysis showed massive ballooning degeneration with zone 3 hepatocyte loss, extensive inflammation in the periportal areas composed mainly of CD8⁺ T cells, a marked biliary reaction and moderate periportal and marked central venous fibrosis. Liver biopsy was repeated 2 months after liver injury and showed complete resolution of inflammation, while liver fibrosis was still present to a similar extent as previously reported.²² In the second patient, liver biopsy analysis showed mild interface hepatitis and lobular infiltration composed mainly of CD8⁺ T cells, proliferated bile ducts and mild portal fibrosis with occasional thin fibrous septa extending into the lobule with mild lobular pericellular fibrosis. In

both cases, intravenous methylprednisolone (2 mg/kg/day) was initiated, followed by oral prednisolone, resulting in normalisation of liver enzymes.²² The description of liver fibrosis in these two cases and the persistence of this fibrosis in one of the two patients raises the question not only of the existence of a pre-existing liver disease, the origin of which remains hypothetical in SMA patients, but also of the role of this pre-existing liver damage in the mechanisms of GT-related liver injury.

3.2 | X-linked myotubular myopathy

X-linked myotubular myopathy (XLMM) is a rare congenital myopathy caused by pathogenic variants in the MTM1 gene. Eighty per cent of affected boys have severe disease with profound skeletal muscle weakness, respiratory weakness and dysphagia at birth.^{23,24} Between August 2017 and June 2021, the safety and efficacy of resamirigene bilparvovec, an adeno-associated viral vector serotype 8 delivering human MTM1, was evaluated in 26 boys younger than 5 years with XLMM requiring mechanical ventilation (ASPIRO trial).²⁵ Of the 26 boys, 4 patients died as a result of severe treatment-related cholestatic liver injury with decompensation at the time of death, possibly due to pre-existing unrecognised cholestatic liver disease (intermittent hyperbilirubinaemia and/or cholestatic hepatitis). Elevations in total and direct bilirubin levels starting 1–4 weeks after dosing were observed in all 4 patients despite prophylactic prednisolone administration, progressing to liver dysfunction with ascites, and did not respond to immunosuppression (prophylactic prednisolone), including intensive multi-agent therapy (high-dose methylprednisolone, anakinra, tocilizumab, ruxolitinib). Non-fatal serious hepatobiliary adverse events also occurred in five other participants, three of whom were known to have pre-existing liver disease based on liver ultrasound or blood test abnormalities. Liver blood test abnormalities were characterised by elevated ALT and AST levels with high bilirubin levels in all but one patient. A liver biopsy was performed on one patient, which showed intrahepatic cholestasis.^{25,26}

Following the ASPIRO trial and the description of pre-existing chronic cholestatic liver disease in XLMM patients, two case series have been published highlighting that hepato-biliary injury was common in these patients, including jaundice, pruritus, serum bile acid and transaminases elevation, as well as, in some cases, cholestatic episodes following infection or vaccination. The main histological findings were peliosis or giant cell hepatitis and cirrhosis associated with severe intrahepatic cholestasis. As in SMA, this data reporting chronic liver disease in up to 24% of XLMM patients raise the question of the impact of chronic liver damage on the occurrence of GT liver toxicity.^{27,28}

3.3 | Haemophilia

Haemophilia A and B are rare congenital, recessive, X-linked disorders caused by a deficiency of coagulation factors VIII (FVIII) and IX

(FIX), respectively. The severity of the disease depends on the reduction in FVIII or FIX levels, which is determined by the type of causative mutation in the genes encoding the factors.^{29–32} The hallmark clinical feature, particularly in untreated severe forms, is bleeding (spontaneous or following trauma) in large joints such as the ankles, knees and elbows, which can lead to the development of arthropathy. Intracranial bleeding and bleeding into internal organs can be life-threatening. For several decades, haemophilia patients have been treated with FVIII and FIX concentrates by haematologists working in specialised comprehensive haemophilia care centres. In the last 15 years, haemophilia treatment has improved tremendously and new treatment options have been developed.^{29–32} To date, two gene therapies have been approved by the FDA and EMA for the treatment of haemophilia patients following two phase 3 clinical trials: valoctocogene roxaparvovec for haemophilia A (GENEr8-1 trial)³³ and etranacogene dezaparvovec for haemophilia B (HOPE-B trial).³⁴

The open-label, single-group, multicentre GENEr8-1 study evaluated a single infusion of valoctocogene roxaparvovec (AAV5-hFVIII-SQ) in 134 adult males with severe haemophilia A (FVIII ≤ 1 IU/dL) (33). The primary endpoint was the change from baseline in factor VIII activity. Elevations in ALT levels were reported in 115 (85.8%) of the patients, with a median time to first ALT elevation after infusion of 8 weeks and a median duration of elevation of 15 days.³³ According to the study protocol, all patients with ALT $\geq 1.5 \times$ ULN received immunosuppressive treatment. The majority of patients received oral prednisone or prednisolone at a dose of 60 mg per day. Two patients required intravenous methylprednisolone. Twenty-nine per cent of patients received other immunosuppressants (other forms of steroids such as budesonide or tacrolimus, or mycophenolate) if corticosteroids were contraindicated or if there was an inadequate response. Ninety-six per cent of hepatic adverse events resolved. Twelve grade 3 CTCAE liver injury events occurred: 9 events within 26 weeks of infusion and 3 events between weeks 26 and 36. All cases were treated with corticosteroids and resolved. No grade 4 was reported and no liver biopsy was performed in this study. Importantly, patients with pre-existing cirrhosis were excluded from this study.³³ No new safety signals were observed at 2 years after infusion.³⁴ A phase 3 trial evaluating the efficacy and safety of valoctocogene roxaparvovec with prophylactic corticosteroids in patients with haemophilia A is ongoing (GENEr8-3; [ClinicalTrials.gov](https://clinicaltrials.gov) number, NCT04323098).³³ To date, 22 patients with haemophilia A have been enrolled in this study. All patients received a single intravenous infusion of valoctocogene roxaparvovec in combination with a 19-week prophylactic corticosteroid regimen starting on the day of the gene therapy infusion. The available data report that an elevated ALT or AST level was observed in 90.9% and 18.2% of patients, respectively. The timing and severity of liver injury and the need for liver biopsy or therapeutic doses of corticosteroids are not yet known.³³

Also in haemophilia B, the HOPE-B study was conducted to evaluate the role of a single intravenous dose of the etranacogene dezaparvovec (AAV5-PdFIX) in 54 adult males with severe or moderate disease (FIX $\leq 2\%$).³⁵ Eleven participants (20%) had elevated ALT

levels at a median time from infusion of 5.1 weeks. Only one patient had grade 3 ALT elevation. Of the 11 patients, 9 received corticosteroids for a median of 79.8 ± 26.6 days (range, 51–130) before discontinuation in all patients.³³ No other immunosuppressive agents were used to control and resolve ALT elevations. As with haemophilia A, all cirrhotic patients were excluded from this study.³⁵

3.4 | Others

Several other GTs are in the pipeline for the treatment of various pathological conditions such as retinal disease, aromatic L-amino acid decarboxylase deficiency, Crigler-Najjar syndrome or Duchenne muscular dystrophy.^{5,7,12,13,36–40} There is also some data on GT-related hepatotoxicity in these conditions.

In Crigler-Najjar syndrome, characterised by deficiency of the enzyme uridine diphosphoglucuronate glucuronosyltransferase 1A, 5 patients were included in a phase 1–2 study to evaluate the safety and efficacy of a single intravenous infusion of an adeno-associated virus serotype 8 vector encoding UGT1A1. Four patients had elevated ALT levels above the upper limit of normal and were treated with a course of corticosteroids.⁷

Duchenne muscular dystrophy is an X-linked degenerative neuromuscular disease characterised by the absence or near absence of functional dystrophin protein.³⁶ The safety of a single intravenous infusion of delandistrogen moxeparvovec (recombinant adeno-associated virus serotype rh74) was evaluated in 4 patients in an open-label, phase 1/2a, non-randomised controlled trial. Importantly, prednisone (1 mg/kg/day) was administered to all patients from 1 day before to 30 days after treatment. Three of the four patients experienced elevated liver enzymes. Based on the available data, the severity was characterised as mild to moderate. All enzyme elevations resolved.³⁷ In contrast, a cardiac complication led to death in a 27-year-old patient with Duchenne muscular dystrophy treated with recombinant AAV serotype 9 containing dSaCas9. This young patient developed acute respiratory distress syndrome and cardiac arrest 6 days after transgene treatment, leading to death 2 days later.³⁸

No hepatic adverse events have been reported in retinal disease or aromatic L-amino acid decarboxylase deficiency treated by subretinal or putamen injection of AAV2-associated GT.^{39,40}

Table 4 summarises the available data on hepatic adverse events associated with GT.

4 | MECHANISMS OF GENE THERAPY-INDUCED LIVER TOXICITY

The clinical and biological profiles of liver toxicity differ depending on the AAV therapy administered. Furthermore, a high degree of heterogeneity has been observed in terms of presentation in clinical trials compared to animal studies and between different species.⁴¹ AAV therapy has been associated with both direct and immune-mediated liver injury.

TABLE 4 Current available data on liver injury associated with gene therapy.

Treatment	Disease	Hepatic Adverse Events	Severity (CTCAE)/ Timing of Liver Tests Abnormalities	Immunosuppressive Treatment	Histological Findings	References
Onasemnogene abeparvovec	Spinal muscular atrophy	Elevation of transaminases	-Mendell JR & al: grade 4 in 50% of the patients. Timing: $\pm$ 3 weeks post-injection.—Chand D & al: grade 2 in 9% of the patients, grade 3 in 6%, grade 4 in 5%. Timing: after the first week post-injection and at the end of the first month post-injection at the time of tapering and discontinuation of systemic corticosteroid therapy	-Mendell JR & al: prophylactic oral prednisolone 1 mg/kg/ day for approximately 30 days, starting 24 h before gene vector administration -Chand D & al: prophylactic prednisolone 1 mg/kg/day starting 24 h prior to treatment. Revision of the prophylactic regimen in March 2019: 2 mg/ kg/day of prednisolone from 24 h prior to infusion until day 3 and then reduction to 1 mg/kg/day for 30 days with tapering	Results of liver biopsies in two patients: -Patient 1: massive ballooning degeneration with drop-out of hepatocytes in zone 3, extensive inflammation in the periportal areas mainly composed of CD8 ⁺ T cells, marked bile ductular reaction and moderate periportal and marked central vein fibrosis -Patient 2: mild interface hepatitis and lobular infiltration composed mainly of CD8 ⁺ T cells, proliferated bile ducts and mild portal fibrosis with occasional thin fibrous septa extending into the lobule with mild lobular pericellular fibrosis. Other data available: -Assessment in 15 patients Phase 1/2: mostly sinusoidal infiltrates. No notable fibrosis and no evidence of dysplasia, necrosis or architectural distortion. Mild steatosis in 4 patients.—One patient developed hepatocellular carcinoma 1 year after receiving etranacogene dezaparvovec. This was considered to be related to individual susceptibility (previous chronic HCV with recent viral suppression, age)	[19,21,28]
Resamirigene bilparvovec	X-linked myotubular myopathy	Cholestatic liver injury	ASPIRO trial: 9/26 patients. 4 deaths with hepatic decompensation at the time of death. Timing: elevations in total and direct bilirubin levels starting 1–4 weeks after dosing	ASPIRO trial: prophylactic prednisolone (1 mg/kg/day) starting 1 day before infusion for 4 to 8 weeks with an 4–8 weeks taper	No data available	[24]
Valoctocogene roxaparvovec	Haemophilia A	Elevation of transaminases	-GENER8-1 trial: 115/134 (85.8%) of the patients. Timing: median time to first ALT elevation of 8 weeks after infusion -Grade 3 in 12/115 (10.4%) patients: 9 events within 26 weeks of infusion and 3 events between weeks 26 and 36	GENER8-1 trial: all patients with ALT $\geq$ 1.5x ULN received immunosuppressive treatment: oral prednisone or prednisolone at a dose of 60 mg/day	No data available	[33]

TABLE 4 (Continued)

Treatment	Disease	Hepatic Adverse Events	Severity (CTCAE)/ Timing of Liver Tests Abnormalities	Immunosuppressive Treatment	Histological Findings	References
Etranacogene dezaparvovec	Haemophilia B	Elevation of transaminases	HOPE-B trial: 11/54 (20.4%) of the patients. Timing: median time to first ALT elevation of 5.1 weeks from infusion. One grade 3. No grade 4	HOPE-B trial: 9 patients received oral corticosteroids. No other immunosuppressive agents were used	No data available	[1]
Adeno-associated virus serotype 8 vector encoding UGT1A1	Crigler-Najjar syndrome	Elevation of transaminases	Phase 1/2 study: 4/5 patients. Data not available	Sirolimus (2 mg/day) was started 1 week before infusion through week 12 after administration. On the day before vector administration, patients received also 100 mg of methylprednisolone and started a course of daily oral prednisone (40 mg), which was tapered beginning at week 3 until week 8	No data available	[7]
Delandistrogene moxeparvovec	Duchenne muscular dystrophy	Elevation of transaminases and GGT	Phase 1/2a study: % patients. Severity: mild in 1 patient and moderate in three patients	Prednisone (1 mg/kg/day) 1 day before to 30 days after treatment	No data available	[37]

The immune-mediated liver injury described in AAV trials may result from both innate and acquired immune responses to the vector capsid, as observed in haemophilia clinical trials (Figure 2), or to the transgene if it is highly immunogenic. Several studies have been published on the role of innate immune recognition in animals, but little is known in humans. On the other hand, the adaptive immune response has been recognised in systemic gene delivery.

The recombinant capsid is a close mimic of a viral capsid and an immune response is expected if the patient has been exposed to the virus from which the vector is derived.

An example of CD8⁺ T cell responses has been described in clinical trials of AAV-GT for haemophilia B.⁴² AAV used to treat patients with haemophilia B has been associated with an increase in transaminases and a decrease in factor IX several weeks after vector administration. Peripheral blood mononuclear cells (PBMCs) were collected at baseline and at the time of vector infusion, demonstrating an immune response to the capsid but not to the transgene product (factor IX).⁴³

The immune-mediated mechanism has been described in the context of AAV therapy in SMA patients. In this population, the magnitude of anti-capsid T-cell responses was shown to be related to the dose of vector infused (Figure 3).⁴⁴

A large proportion of SMA patients developed transient transaminase elevations 1 and 4 weeks after initiation of therapy, despite corticosteroid prophylaxis.^{19,20} In two patients, liver injury was severe

with elevation of bilirubin 2–7 weeks after initiation of therapy, but both patients responded to corticosteroid treatment. Liver histology showed the presence of periportal CD8⁺ T cells associated with a polymorphic inflammatory infiltration (neutrophils, eosinophils and plasma cells), hepatocyte degeneration and ductal reaction. Overall, the histology was consistent with immune-mediated damage.²²

Liver toxicity associated with transgene overexpression has been reported in rodents and non-rodents for short hairpin RNA overexpression⁴⁵ and for overexpression of frataxin protein, which is involved in intracellular respiration and iron metabolism.⁴⁶

Another example of an immune response to a transgene, although not related to liver toxicity, was reported in children treated for Duchenne muscular dystrophy, in whom an AAV vector encoding a functional truncated version of the dystrophin protein was administered intramuscularly. Analysis of PBMCs showed the presence of transgene-specific CD4⁺ and CD8⁺ T cells in 4 out of 6 subjects.⁴⁷ Furthermore, in patients treated for α 1-antitrypsin deficiency who received intramuscular gene transfer, anti-capsid T cells were detected in the majority of patients. However, in some patients there was a decrease in transgene product and in one subject T-cell reactivity was directed against α 1-antitrypsin protein.⁸

A direct mechanism of liver toxicity has also been reported for AAV therapy. This type of toxicity has been described in patients treated for XLMM.^{25,26} Of note, XLMM is associated with pre-existing liver

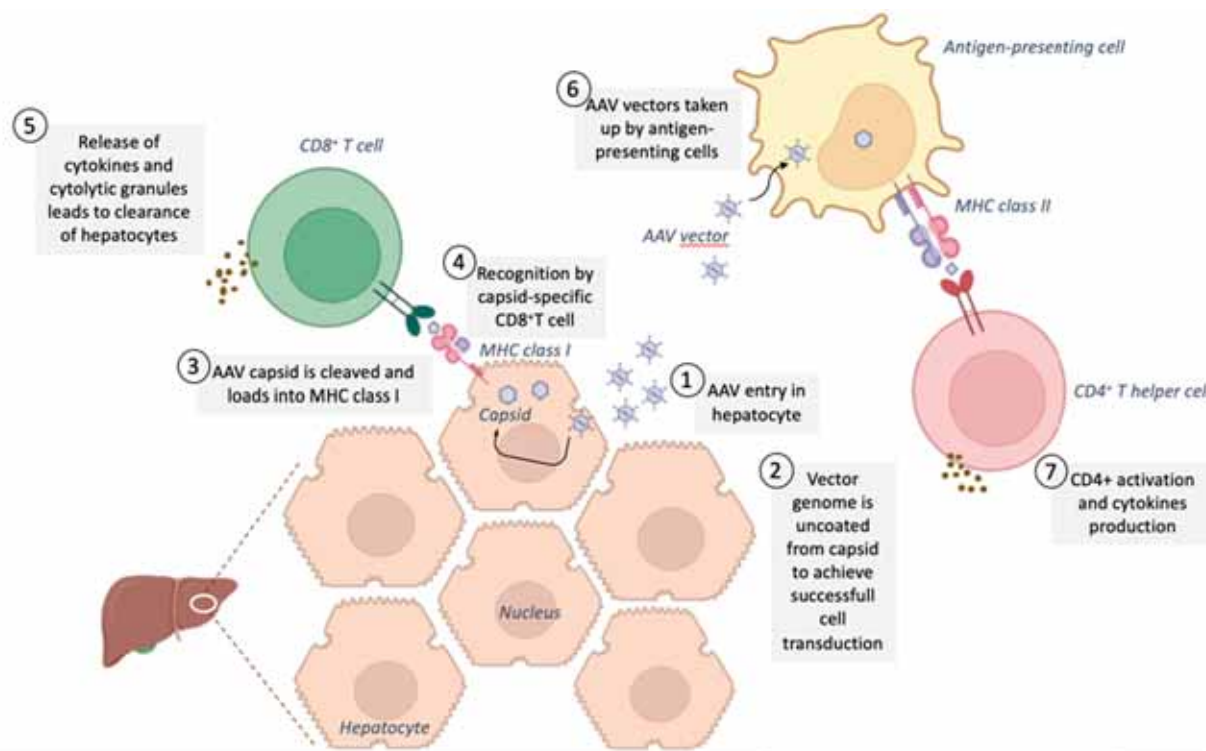


FIGURE 2 Proposed mechanism behind the loss of factor IX expression and transient hepatic enzyme elevation observed during the AAV2 gene therapy trial for haemophilia B. After AAV entry into hepatocytes (1), the vector genome is uncoated from the capsid to achieve successful cell transduction (2). Peptides derived from proteasomal processing of the capsid are loaded onto MHC class I molecules (3), which can lead to clearance of transduced cells by CD8⁺ T cells (4, 5). In addition, AAV vectors are also taken up by antigen-presenting cells (6), leading to presentation to and activation of CD4⁺ T helper cells (7). These proposed mechanisms highlight the immune complexities associated with AAV vector-based therapies.⁴²

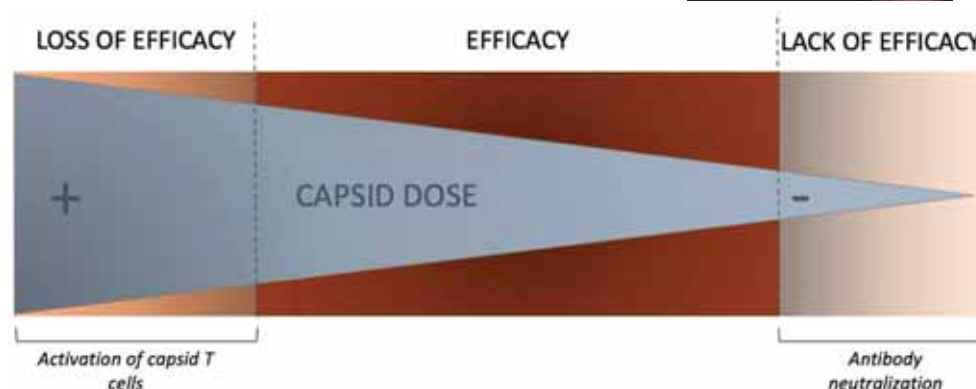


FIGURE 3 Relation between the magnitude of the T-cell responses directed against the capsid to the dose of the vector infused as illustrated in AAV therapy in SMA patients.⁴⁴ T-cell responses directed to the AAV-1 capsid are dose-dependent, as shown in a clinical trial for adeno-associated virus serotype 1 (AAV-1)-mediated gene transfer to muscle for lipoprotein lipase (LPL) deficiency. Activation of capsid-specific CD4⁺ and CD8⁺ T cells was observed. Detectable T-cell responses to capsid with dose-dependent kinetics of appearance was observed. Subjects with detectable T-cell responses to capsid also had higher anti-AAV-1 IgG3 antibody titre (46).

disease, which may increase the toxic effect of AAV therapy. In the study, severe cholestatic liver failure was reported 37–142 days after initiation of therapy, but the first liver test abnormalities were reported 1–4 weeks after treatment. The immune-mediated mechanism was ruled out as no immune cell infiltration was seen on histology. The sponsor hypothesised that high exposure to the vector genome leads to disruption of hepatocellular metabolism.^{27,48}

Another example of direct toxicity was reported in patients treated for haemophilia A. The transgene used, BDD factor VIII, is prone to misfolding and can accumulate in the endoplasmic reticulum of hepatocytes, leading to intracellular stress and apoptosis.⁴⁹ Increased cellular stress was not associated with elevated ALT in pre-clinical studies, questioning cellular stress as a potential mechanism of liver toxicity. However, the number of patients included in this study is small and this hypothesis should be considered.⁵⁰

In conclusion, there is great heterogeneity in the mechanisms and phenotypes of liver injury associated with GT. The biological profile of hepatotoxicity can be both cytolytic and cholestatic and there are no specific biological markers to aid in diagnosis. In order to understand the mechanism of liver injury and to characterise the liver damage, biopsy may be discussed. In contrast to AAV-gene therapy, there are no well-documented observations of liver injury associated with other types of gene therapy based on other viral vectors, such as lentivirus, or non-viral vectors, such as virus-like nanoparticles (VLP), liposome (Lipo) and exosomes.^{51,52}

5 | MANAGEMENT OF GENE THERAPY

5.1 | General concepts

Gene therapy is a completely new therapeutic approach with many promises but also many uncertainties. Practical experience with GT is extremely limited worldwide. There are no clinical experts in this

field, and experience is based only on participation in clinical trials. Furthermore, it is important to remember that the potential benefits of GT should be balanced against the risk of introducing GT-related side effects and the benefits of other existing therapeutic options.^{1–3,36,53–58} In fact, in some diseases, such as haemophilia or Fabry disease, the deficiency can be compensated by regular administration of the missing functional protein.^{1–3} Replacement therapy can be more or less restrictive and have a greater or lesser impact on quality of life. In other diseases, there is no substitutive treatment to replace the deficient enzymes or receptors, and GT is the only therapeutic option.^{1–3} Interestingly, the risk of liver injury is not the same in different models of GT. It seems to be particularly increased in GT for haemophilia A compared to GT for haemophilia B.^{31–35,50,53,54} All these parameters should be taken into account when discussing GT as a therapeutic option for a specific disease and when organising its management.

A common consequence of GT is the induction of an immune response against the target organ, particularly the liver. While corticosteroid therapy is the backbone of treatment, the degree of response and efficacy varies according to the mechanisms leading to the hepatic response and transaminase elevation. Furthermore, the criteria that should trigger this treatment, as well as when and how to stop it, vary between GT indications or even within a given indication. Similarly, there is no consensus on the place of second-line immunosuppressive therapy.

Nevertheless, several guidelines for the management of GT, particularly in haemophilia and some other indications based on AAV, have recently been presented.^{32,36,50,53–59}

In all cases, it is clear that multidisciplinary management is required to ensure the most appropriate decision in the indication and follow-up of GT. The frequency of liver injury highlights the usefulness of collaboration with hepatologists. Therapeutic education of the patient and follow-up by a team specialised in this field also seems important to optimise the safety of GT. Patient

associations can also help patients. The development of educational programmes for patients and healthcare providers, including more specific training on issues such as the use of AAVs, whose use in haemophilia patients is becoming increasingly common, is a good example. The World Federation of Haemophilia (WFH) is working on a gene therapy registry. The purpose of the registry is to provide a comprehensive view of the conditions under which gene therapy is administered.³²

There are general and common criteria for the organisation of GT, the pre-treatment work-up and the selection of patients across the different indications to ensure patient safety. Similarly, there are largely common parameters for monitoring the tolerance of GT and the indication for immunotherapy in the event of side effects after GT has been administered.^{32,36,53-59} The details of these criteria are presented in Table 5.

5.2 | Initial pre-treatment assessment

Treatment must be initiated and carried out in specialised hospital centres under the supervision of a doctor experienced in the management of the disease. Because of the risk of an immune system reaction, it is required that the patient has a stable general state of health (hydration, nutrition, absence of infection...) before the perfusion of GT. Overall, it is important to have a complete clinical and biological pre-treatment work-up and to ensure that the patient has a good understanding of the requirements for a successful GT and the necessary regular follow-up. The patient's immunisation schedule should be reviewed before and after GT perfusion, including corticosteroid administration. A complete assessment of the liver, especially of previous liver history such as HCV or HBV infection, is mandatory. An important issue in this specific population is the detection and evaluation of potential hepatic fibrosis or even cirrhosis in order to prevent hepatic complications after GT administration. However, due to a lack of data, the current recommendations do not provide details on how to address this issue, as data in the literature are too scarce or not available. However, this assessment may at least include liver imaging, liver stiffness measurement or liver biopsy. Particularly in patients with previous HCV infection, liver imaging has been shown to have a high predictive value for detecting advanced liver damage after HCV clearance.⁶⁰⁻⁶²

5.3 | Follow-up

The main goals of follow-up are to rapidly detect the onset of side effects, especially liver damage and loss of transgene expression, before they become irreversible. Elevation of liver enzymes should lead to rapid control and trigger a specific aetiological work-up to determine the causality of GT. Once GT has been administered, there is consensus on the need for very regular monitoring of liver

TABLE 5 Management of the risk of liver injury with gene therapy.^{32,36,50,53-59}

Common parameters for selection of patients for gene therapy, exclusion criteria, follow-up parameters

Initial assessment of the patient candidate for gene therapy

Identification of the patient

Some patients may be identified by their physician as "good" candidates for gene therapy

Patients' wishes should be taken into account

Patient discussion and information

Initial pre-treatment work-up

Clinical assessment

Medical history including chronic liver disease, history of viral hepatitis, HIV

-Current treatments or regular use of drugs, herbal medicines, dietary supplements, illegal substances that may cause liver damage, and even mild changes in liver enzymes, especially transaminases

- Surgery (especially of the bile ducts and digestive tract)
- Lifestyle, including physical activity, diet, alcohol and tobacco consumption
- Vaccination schedule
- Physical examination to detect/assess recent or chronic liver injury

Biological work-up

- Detection of antibodies to the gene therapy vector using a validated assay
- Assessment of liver status: ALT, AST, alkaline phosphatase, GGT, total and direct bilirubin, albuminemia, prothrombin time, international normalised ratio (INR)
- HBV, HCV, HIV serologies. In addition, patients with a history of HCV infection should be tested for serum HCV RNA, and patients with HBV infection should be tested for serum HbsAg, anti-Hbs antibodies, HBV DNA, anti-HVD antibodies (in carriers of HbsAg)
- Renal function: serum creatinine, e-GFR
- Blood cell counts

Exclusion criteria for gene therapy

- Detection of antibodies against the gene therapy vector
- Poorly controlled HIV, HCV, HBV infection
- Severe liver disease (mostly cirrhosis) In addition to clinical and biological criteria, assessment may include liver imaging, hepatomegaly, liver biopsy, as advised by the treating physician
- Exposure to hepatotoxic drugs or other compounds such as herbal medicine, dietary supplements, illegal drugs
- Patient reluctance to comply with regular medical follow-up
- Patient reluctance to limit alcohol consumption, especially during the first 6 months

Follow-up criteria after gene therapy

- Regular clinical monitoring to detect the occurrence of adverse events and the efficacy of the therapy
- Periodic biological monitoring including
- ALT, AST, alkaline phosphatase, gamma glutamyl transferase, total and direct bilirubin, albuminemia, prothrombin time, international normalised ratio (INR)

TABLE 5 (Continued)

- Renal function: serum creatinine, e-GFR
- Blood cell counts
- Specifically for the liver, current guidelines recommend that transaminases be monitored weekly during the first 6 months of gene therapy, regardless of immunosuppressive therapy, and then monthly for 2 years (48–51)
- The frequency of monitoring of liver function tests should be adjusted according to corticosteroid therapy (50–53)

tests, particularly transaminases, every week for the first 6 months and then every month for up to 2 years. Additional liver tests, including not only transaminases but also bilirubin, INR, prothrombin time, are required if immunomodulatory treatment is indicated. Current guidelines also recommend regular viral monitoring in patients with a history of HBV infection, including those who are HBsAg negative and anti-HBc positive, because of the risk of HBV reactivation during corticosteroid therapy.

5.4 | Management of immunosuppressive therapy

There is a consensus that immunosuppressive therapy should be initiated when liver test abnormalities occur, in particular transaminase elevations, even if these are rather limited, as opposed to other situations such as drug-induced liver injury (DILI).^{16,17} In fact, in DILI, only significant acute liver injury with ALT above 10 times the upper limit of normal and often with jaundice leads to immunosuppressive treatment.^{16,17} In GT, the purpose of immunosuppressive treatment is mostly to protect the vector-bearing liver cells and thereby preserve the efficacy of the treatment. Therefore, immunotherapy is initiated at low transaminase elevations, sometimes even when transaminases remain below the upper limit of normal.²⁷ There is no well-defined ALT elevation cut-off that should trigger immunotherapy. Recommendations vary from one indication of GT to another, but also within the same indication such as haemophilia.^{32,36,50,53–59}

In general, first-line therapy consists of oral prednisolone (1 mg/kg/day) or its equivalent. Depending on the clinical course, adjustment of the immunomodulatory treatment should be considered by increasing the dose of corticosteroids, prolonging the duration of therapy, or tapering the doses. If corticosteroid therapy appears inadequate or is poorly tolerated, other immunosuppressive treatments may be considered, including budesonide, mycophenolate mofetil or tacrolimus.^{32,36,50,53–59} The initiation of immunotherapy leads to intensified monitoring of liver tests but also of viral parameters in patients with a history of HBV infection, including those who are HBsAg negative and anti-HBc positive, because of the risk of HBV reactivation during corticosteroid therapy.^{32,36,50,53–59} Similarly, it has been recommended that patients with serum anti-HCV antibodies should be tested for HCV RNA.⁵⁴

5.5 | Specificities of management by indication

Among the different indications for GT, SMA and haemophilia have generated a particular amount of experience and suggestions for management.

5.5.1 | Spinal muscular atrophy

The most common side effects of onasemnogenetic abeparvovec are increased liver enzymes, low platelet counts (thrombocytopenia), increased troponin levels, fever and vomiting.⁵⁵ In addition to the usual tests mentioned above, the biochemical work-up includes troponin I.⁵⁵ Prophylactic immunomodulatory treatment is recommended to reduce the immune response.⁵⁵ It is recommended to start immunosuppressive treatment 24 h prior to onasemnogene abeparvovec infusions according to the calendar below to reduce the risk of liver injury and other immunologically modulated side effects. In the case of transaminase elevation, it has been recommended not to taper corticosteroids until ALT/AST levels are still below 2 times the upper limit of normal.⁵⁵ At the end of this period, if the patient is clinically well with no significant adverse effects, follow-up liver tests can be reduced to every 2 weeks.⁵⁵

5.5.2 | Haemophilia management

The European Association for Haemophilia and Allied Disorders and the European Haemophilia Consortium have recently described a new healthcare setting for the management of patients receiving GT. This “hub-and-spoke” model aims to ensure the safe administration and monitoring of GT by expert comprehensive haemophilia care centres in close collaboration with nearby haemophilia treatment centres where the patient is followed up.^{32,50,53,56,57,59} Any adverse events should be managed by the expert centres in collaboration with the spoke centre to provide the most up-to-date treatment and management options to patients receiving GT. All adverse events and efficacy data should be reported to a central registry for life.³²

5.5.3 | Specific tests in the pre-treatment work-up of eligible patients

- Detection and titration of neutralising anti-AAV antibodies (variable depending on the type of AAV).
- FVIII or FIX levels.

5.5.4 | Specific exclusion criteria for gene therapy

- Age <18 years.
- Mild haemophilia B and mild/moderate haemophilia A.

- (iii) Presence of anti-FVIII or anti-FIX inhibitors.
- (iv) Allergy to FIX-containing drugs in haemophilia B.
- (v) Neutralising anti-AAV anti-capsid antibodies according to GT type.

5.5.5 | Follow-up after gene therapy

- (i) In the short and medium term: biological follow-up including blood counts, assessment of FVIII or FIX, and liver tests. The frequency of monitoring should be adapted to the risk of hepatotoxicity of each treatment.
- (ii) For valoctocogene roxaparvovec, biological monitoring is recommended every week for the first 6 months, regardless of additional immunosuppressive treatment, and then every 2 to 4 weeks from months 6 to 12.
- (iii) In the case of etranacogene dezaparvovec, which is indicated for severe haemophilia B, biological monitoring may be performed at a lower frequency due to a reduced risk of hepatotoxicity.
- (iv) In all cases, more regular monitoring of liver function is indicated in patients on immunosuppressive therapy.

The reduction in corticosteroid dose should be adapted to the individual decrease in transaminases and factor VIII/IX levels. In the case of transaminase elevation $>5N$, a methylprednisolone bolus (7.5–15 mg/kg) for 1–3 days may be considered (53). In case of lack of efficacy (corticoreistance/cortico-dependence) or poor tolerance, second-line immunosuppressive treatment should be considered, but there is no consensus on the place of second-line immunosuppressive agents. Similarly, preemptive therapy has been proposed, but its place needs to be carefully assessed.

6 | IMPORTANT UNRESOLVED QUESTIONS THAT NEED TO BE ADDRESSED IN THE MANAGEMENT OF LIVER INJURY ASSOCIATED WITH GT

6.1 | To what extent should liver disease be extensively characterised before a patient receives GT?

It is not uncommon for haemophilic patients to have baseline liver fibrosis due to previous viral hepatitis B or C, HIV and fatty liver disease. In addition to clinical examination and biochemical parameters, various methods can be suggested. Liver imaging, including ultrasound and MRI, is helpful in detecting advanced liver disease and cirrhosis by showing irregular nodular surface, caudal lobe hypertrophy, and signs of portal venous hypertension.^{61,62}

Particularly in patients with previous HCV infection, liver imaging has been shown to have a high predictive value for detecting advanced liver damage after HCV clearance.^{61,62}

Elastometry is currently used to assess liver fibrosis and cirrhosis, particularly in patients with HCV and HVB infection. It may also

be useful in patients with a history of alcoholic liver injury and fatty liver disease. However, the availability of Fibroscan varies widely between countries and MRI elastometry is not readily available.

Liver biopsy is the most powerful test to assess the presence and staging of liver fibrosis but has the disadvantage of exposing the patient to complications such as pain, bleeding, bladder perforation and pneumothorax. Therefore, its use should only be considered when non-invasive methods of assessment are not available or are not sufficiently accurate.

6.2 | How should pre-existing liver disease and/or the potential risk of developing severe GT liver injury be considered when weighing the risks and benefits of GT for a patient?

This depends on two main factors, the availability of other options with their level of efficacy, tolerability, practical aspects and impact on quality of life versus the risk of developing acute liver injury, which varies according to the model of GT. As mentioned above, the risk of liver injury is higher with gene therapy for haemophilia A than with GT for haemophilia B. However, it is not easy to apply general rules. Therefore, the benefits and risks should be weighed very carefully and the decision can be tailored to the individual.^{50,53,54,56,57}

6.3 | How should long-term liver monitoring be carried out, especially to detect fibrosis?

It has been suggested that regular assessment should be carried out using a non-invasive method such as elastometry with the limitation in the availability raised in point 1.^{50,61,62} Biological markers of liver fibrosis such as FIB-4 are far from being universally accepted.⁵⁴

6.4 | How often should transaminases be monitored?

In patients undergoing AAV-GT, current guidelines recommend weekly monitoring during the first 6 months of GT and monthly thereafter for 2 years. However, some scientific societies have recommended even more frequent monitoring, especially within the first 3 months of therapy.⁵⁰

6.5 | What is the level of transaminase elevation that becomes significant in deciding to treat with corticosteroids?

It can be assumed that the risk of liver damage is not the same for the different compounds proposed. For example, valoctocogene

roxaparvovec, used in haemophilia A, appears to be more hepatotoxic than etranacogene dezaparvovec, indicated in severe haemophilia B. Furthermore, there is no international consensus for the same compound in the same indication. Indeed, even in haemophilia A treated with valoctocogene roxaparvovec, there is no consensus on the use of immunosuppressive treatment (ALT level, dose and duration of corticosteroids). One suggestion has been to initiate corticosteroid therapy if ALT increases by more than 30–50% of the lowest pre-treatment value, even if the value remains within the general normal range after an infectious cause has been ruled out.⁵⁰ If the increase in ALT is less than <30% of the pretreatment value, a new control should be performed within the next few days.⁵⁰

6.6 | What is the benefit of prophylactic corticosteroid therapy at the start of gene therapy?

There are currently studies evaluating prophylactic immunosuppressive therapy in haemophilia A patients (e.g. GENE8-3, NCT04323098). The feasibility and safety of extended ALT monitoring intervals in patients receiving GT with low rates of drug-induced hepatitis and less severe cases, without compromising long-term efficacy, needs to be evaluated in future studies. In a recent study investigating the safety and efficacy of AAV8 GT in Crigler-Najjar syndrome, patients were given preemptive steroid therapy as follows: an initial intravenous dose of 100mg methylprednisolone followed by an oral dose of 40mg prednisone, tapered from week 3 and discontinued at week 8.⁷ Preliminary results are encouraging for this population and a preemptive steroid protocol may be an interesting option that needs further evaluation in GT associated with a high risk of associated hepatitis or in patients in whom GT is targeting an underlying liver disease.⁷

6.7 | What is the place of other immunosuppressive agents such as budesonide, mycophenolate mofetil, and tacrolimus?

If it is generally accepted that patients with an inadequate response or serious adverse events to corticosteroids require the use of alternative immunosuppressive agents, studies are needed to define the most appropriate agents and the management of their administration.

6.8 | What is the role of liver biopsy in case of liver injury occurs during GT?

Liver biopsy can be discussed to better assess the interest of immunotherapy, as it occurs in the management of immune checkpoint inhibitor-related hepatotoxicity.¹⁶

6.9 | Is there an interest in detecting HEV infection in patients who are candidates for GT?

To date, it is not generally recommended, partly because the risk of HEV contamination varies in different geographical areas. The prevalence of patients with anti-HEV antibodies is very high in Asia, Africa and South America, common in Western and Eastern Europe and appears to be low in Northern Europe and North America. Acute infection is usually asymptomatic and resolves spontaneously. However, chronic infection can develop in immunocompromised patients. This raises the question of the detection of HEV at baseline in patients who are susceptible to receive immunosuppressive agents, but also of a regular control to detect contamination by periodically testing anti-HEV antibodies and HCV RNA in patients with positive serology.⁵⁴

7 | CONCLUSIONS

Gene therapy offers an enormous field of new therapeutic approaches in many genetic disorders and is promising in some acquired diseases. However, it may be associated with the occurrence of liver injury with heterogeneous clinical and biochemical expression and variable frequency and severity depending on the method of gene transfer and therapeutic indication. While there are common general rules regarding the inclusion and exclusion criteria for patients undergoing GT and the need for careful post-treatment follow-up, there are many issues for which there is no consensus regarding management to prevent or treat hepatic reactions. New studies are needed to better define the criteria for triggering corticosteroids, the place of prophylactic treatment, and that of other immunosuppressive agents, in order to preserve the therapeutic effect of GT with the lowest level of drug-induced adverse events.

AUTHOR CONTRIBUTIONS

Dominique Larrey: Conception of the manuscript, analysis and discussion of the data, writing of original draft (review and editing). Benedicte Delire: Conception of the manuscript, analysis and discussion of the data, writing of original draft (review and editing). Dominique Larrey and Benedicte Delire equal contribution. Lucy Meunier: Conception of the manuscript, contribution to the writing of a part of the draft, review and editing. Eleonora De Martin: Conception of the manuscript, contribution to writing of a part of the draft, review and editing. Amel Zahhaf: analysis of data. Yves Horsmans: Conception of the manuscript, contribution to the writing of a part of the draft, review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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