Gene Therapy: Practical Aspects of Implementation

Steven W. Pipe[1], K. Rajender Reddy[2] and Pratima Chowdary[3]

1. Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, MI, USA
2. Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA
3. Katharine Dormandy Haemophilia and Thrombosis Centre, Royal Free Hospital, London, United Kingdom

Corresponding author:

Steven W. Pipe, MD

D4207 MPB
1500 E Medical Center Drive
Ann Arbor, MI 48109-5718

Ph: 734-232-9335
Fax: 734-615-0464
ummdswp@med.umich.edu

Word Count: Abstract 240, Body 4472

Keywords: gene therapy, liver health, immunosuppression, infrastructure, haemophilia treatment center

Running title: Practical aspects of implementation of gene therapy

Abstract

The first wave of gene therapies for haemophilia submitted for regulatory review utilize a liver-directed approach in which a functional gene copy of factor VIII (FVIII) or factor IX (FIX) is packaged inside a recombinant adeno-associated viral vector (rAAV). Following a single treatment event, these particles are taken up into liver cells, where the rAAV uncoats and delivers the DNA to the nucleus of the cell, where genetic elements that accompany the gene allow for efficient expression and secretion of FVIII or FIX protein into the plasma. An immune response to the vector capsid has been manifest by elevations in common liver enzymes that must be diligently followed post-infusion for weeks and months afterward and if signs of toxicity appear, will trigger a course of

immunosuppression. Despite this, the studies have shown that this works in the great majority of individuals and the immunosuppression course is either avoided or short-lived for many. Optimal outcomes in the haemophilia population will be dependent on proper screening assessment and maintenance of liver health prior to consideration of gene therapy, close short-term follow up and implementation of immunomodulatory strategies to identify and manage liver toxicity and preserve durable transgene expression. This review proposes best practices to assist clinical teams with overcoming the challenges this platform of therapy poses to the traditional clinical care models and infrastructure within the haemophilia treatment centers (HTCs) who will be coordinating the patient's journey through this potentially transformative therapy.

Liver health, screening and short-term follow-up

Several hepatic considerations are relevant in the context of gene therapy as not uncommonly, such patients may have underlying liver disease at baseline or have hepatic abnormalities while undergoing therapy with the attendant long term risk of hepatocellular carcinoma (HCC), a potential concern in patients undergoing gene therapy. The common hepatic conditions that are encountered in the general population and in gene therapy candidates include non-alcoholic fatty liver disease (NAFLD), chronic viral B and C hepatitis, alcoholic liver disease, and autoimmune hepatitis. As such, it is important to have a fundamental knowledge of the commonly encountered liver diseases, in general and in those undergoing gene therapy. There are several ubiquitously applicable non-invasive serologic tests and biomarker panels, and liver biopsy, available to diagnose a specific liver disease and assess severity of liver disease and thus risk stratify patients while being assessed for gene therapy. The assessment of the presence of liver disease, and its severity, can be done through a combination of serologic tests and non-invasive biomarkers, and tools that assess liver stiffness. The non-invasive markers for assessing severity of liver disease are helpful, have ease of use, and well accepted by patients and as such can facilitate monitoring, as well, of liver health following gene

therapy. There has been a diminishing role for liver biopsy, although hepatic inflammation is best characterized by hepatic histopathology.

Assessment of hepatic biochemical tests

Serum alanine aminotransferase activity (referred to as ALT) is a liver enzyme activity measurement that is commonly used to evaluate liver health and assess liver disease[1]. ALT is measured through widely available and low-cost blood tests. Many patients with subtle ALT elevations often are asymptomatic, making this measurement valuable for detection of subclinical liver disease[2]. There are several factors which can affect ALT measurements, including sex, BMI, triglyceride levels, total cholesterol, alcohol consumption, smoking status, and age[2-4]. It can be generalized that patients with greater elevations in serum ALT levels have more severe hepatic inflammation. However, one limitation of measuring serum ALT levels is that there is only a weak correlation between serum ALT levels and degree of hepatic fibrosis[1].

Rises in serum ALT levels can indicate hepatocellular injury, even if the patient is asymptomatic[1], such as in NAFLD, chronic HCV or HBV infection, alcoholic liver disease, drug-induced liver injury and autoimmune hepatitis[5]. The degree to which serum ALT levels rise from baseline is a helpful indicator of the severity of liver injury. Beyond its utility in detecting liver disease, serum ALT levels are a helpful indicator of overall patient health and mortality. Studies conducted globally show that serum ALT elevations in both men and women are correlated with higher liver-specific mortality and cardiovascular mortality, amongst other causes of mortality[6,7].

Taken together, measuring serum ALT levels is a useful screening test for liver diseases and several causes of mortality. This measurement is not diagnostic, but could rather be used to separate low-risk from high-risk patients before following up with further investigations. Other hepatic biochemical tests that can be used to assess liver disease are AST, alkaline phosphatase, total

bilirubin, albumin, prothrombin time and GGT levels. Total bilirubin to a large extent, albumin, and prothrombin time are specifically used to assess synthetic function (see Table 1).

Non-Invasive Liver Disease Assessment (NILDA)

Imaging Techniques (Table 2)

Several imaging techniques have evolved over time and are being more readily used in the assessment of the degree of hepatic fibrosis. These include US-based elastography (Transient elastography (TE, FibroScan, Echosens, Paris, FR), point shear wave elastography (pSWE), also known as acoustic radiation force impulse imaging (ARFI), (2-D) shear-wave elastography (2D-SWE), and Magnetic resonance elastography (MRE)[8]. Standard gray scale ultrasound, and other routine imaging methods such as computed tomography [CT] and magnetic resonance imaging [MRI]), that have been in use for several years, cannot reliably estimate the degree of liver fibrosis, but they can be helpful in well-established cirrhosis and particularly in those with portal hypertension[9].

Both histologically and with non-invasive modalities, hepatic fibrosis is staged from F0 (no fibrosis) to F4 (cirrhosis), while there are intermediate stages of F2 and F3 (bridging fibrosis). Hepatic steatosis is a clinical entity that is commonly encountered and is graded 0-3 histologically and with non-invasive modalities, based on hepatocyte content of fat: S0 (0-4%), S1 (5-33%), S2 (34-66%), and S3 (>66%) steatosis. Most often used imaging techniques for assessment of fat include Controlled Attenuated Parameter (CAP) and MRI-PDFF[10,11]. CAP is a type of transient elastography which measures hepatic steatosis using the FibroScan probe at a specific frequency. While CAP is an US-based imaging technique, MRI-PDFF is an MRI-based technique which measures proton density fat fraction, and allows hepatic steatosis to be quantified[11].

The principal of US-based elastography involves the tracking of the speed of propagation of a mild amplitude low-frequency (50 Hz) elastic wave that is produced by a mechanical vibrator and which

travels through the skin and intercostal space into the liver. The wave speed correlates with liver stiffness and is expressed in kPa. It is generally accepted that a stiffness of > 15 kPa as determined by TE is indicative of cirrhosis. The cut-offs for liver stiffness values vary for the various tools and the ranges for intermediate stages of fibrosis vary by etiology. Further, there are limitations to these techniques, such as failure to obtain a proper assessment in those with high BMI, and in those with concomitant severe hepatic inflammation, cholestasis, and hepatic congestion. Acoustic radiation force imaging (ARFI) techniques assess liver stiffness based on tissue displacement from acoustic compression pulses. Magnetic resonance elastography (MRE) is similar to US-based techniques where the assessment of liver stiffness is made based on the speed of propagation of the mechanical shear waves generated by an acoustic driver device placed over the right upper quadrant. A major advantage of MRE is that it allows for more complete assessment relative to other elastography methods as it covers almost the entire liver[8]. While the failure of liver stiffness assessment rates is low with MRE, it may not be readily available, has higher costs, and presents challenges in logistically setting it up, thus not practical if frequent monitoring of liver health is needed[12-14].

Blood-based Biomarkers (Table 3)

The principles of blood-based assessment of hepatic fibrosis are based on the complex and dynamic interplay of extracellular matrix synthesis and their degradation often due to inflammation and cytokine release. Such tests include some of the routinely used blood tests such as AST and ALT, clinical variables, and in some instances, include complex markers that reflect direct measurements of collagen synthesis or degradation. The most frequently used blood-based biomarker panels are APRI, FIB-4, ELF, Fibro test, and NFS. FibroTest is a test with broad utility, which provides a quantitative score to assess liver damage for patients with a range of liver diseases[15-17]. The Fibrosis-4 Index (FIB-4) and AST to Platelet Ratio Index (APRI) score are both used to estimate the degree of fibrosis, based on robust data, often in patients with HBV and HCV. FIB-4 score is calculated using the patient's age, platelet count, ALT and AST levels. APRI score is calculated using the patient's AST

level and platelet count. Both of these scores can easily be determined using an online calculator[18]. NAFLD Fibrosis Score (NFS) is also a specific test, used to assess patients with NAFLD for fibrosis[19]. One or more of these tests are readily available and are being increasingly used to assess the severity of liver disease. They have a high degree of sensitivity and specificity in assessing degree of fibrosis, although there is variability based on factors such as etiology of liver disease, and presence of inflammation. The major advantage is that they are non-invasive, widely accepted, and can be used to monitor the degree of hepatic fibrosis but not to assess severity of inflammation either at baseline or in response to therapy[20].

Liver Biopsy

Liver biopsy has been in use as a diagnostic tool since the late 1800's[21]. With the advent of several serologic and non-serologic non-invasive tools for diagnosing and staging severity of liver disease, liver biopsy is being used less frequently. Further, there are limitations such as risks associated with the procedure, suboptimal patient acceptance, sampling error for assessment of fibrosis, and inter-observer variability in interpretation. However, it remains the only reliable method of precisely characterizing hepatic inflammation.

In patients undergoing gene therapy, there might be the evolution of several immunologic perturbations or non-immunological reasons leading to hepatic biochemical test abnormalities[22]. Clinical and hepatic biochemical test assessment is reasonable, but the diagnosis may not be definitive and the extent of the inflammatory process cannot be gauged without a liver biopsy. While, in general, interventions using immunosuppression, in those with hepatic biochemical tests abnormalities while undergoing rAAV-gene therapy has been the practice, it would be reasonable to consider a paradigm shift to considering a liver biopsy (can be done with acceptable bleeding risk in patients with hemophilia via a transjugular approach), particularly in those cases that have

significant hepatic biochemical test abnormalities, are recalcitrant to immunosuppression or require prolonged immunosuppressive treatment. Such a strategy would also help us understand more specifically the type and cause for the hepatic abnormalities.

In summary, the evaluation of liver health and disease status can be assessed through a variety of conventional hepatic biochemical tests, non-invasive imaging guided techniques and biomarker panels. Liver biopsy is seldom used to diagnose and stage liver disease severity while it remains the only modality of reliably assessing hepatic inflammation.

Infrastructure required for gene therapy implementation

Multidisciplinary haemophilia experts have established four universal principles for the introduction of gene therapy: 1) The Person with haemophilia (PWH) should be at the center of decision-making, 2) All PWH should have equal opportunity to access gene therapy, 3) Safe introduction of commercial gene therapy with lifelong follow-up is paramount to ensuring long-term success and 4) The integrated comprehensive care model currently employed for the treatment of haemophilia improves outcomes and is best placed to support the introduction and long-term follow up of gene therapy[23]. Accordingly, the haemophilia treatment centers (HTCs) will need to be involved throughout the patient's journey, leveraging the existing expertise and relationship with PWH under their care.

HTCs should be directing their efforts toward establishing lines of education, collaboration and communication that will be essential in order to be prepared for clinical delivery of gene therapy while striving for continued excellence in patient outcomes. The pathway to preparedness for

implementation of gene therapy within the HTCs begins with education of patients and multidisciplinary staff, including communication of the safety and efficacy observed from pre-clinical studies, long-term outcomes from phase I/II clinical trials and early data from phase III pivotal trials[24]. Given recent progress of gene therapy for both haemophilia A and B, manufacturers will likely be seeking regulatory approval within the next year[25]. This will need to be followed by a viable pathway for access including criteria for authorization and reimbursement. In the mean time, attention can be directed to preparations for integration of gene therapy into the clinical care work flows within the HTCs. The joint publication from the European Association for Haemophilia and Allied Disorders (EAHAD) and the European Haemophilia Consortium has outlined a proposed 'hub and spoke' model of integrated care that could be implemented with modifications within any country with the expectation that the care models would be dynamic and adaptable as more is learned regarding safety and efficacy of this treatment modality and with better understanding of the hurdles that must be overcome at individual sites[22]. Potential division of responsibilities among one or more centers within this hub and spoke model include a *Supervisory/Coordinating Center* with responsibility for all aspects of gene therapy care (consenting, dosing, follow up and data reporting), *Dosing Center* responsible for the receipt, preparation and administration of the gene therapy product and a *Referral/Follow Up Center*, responsible for identification and screening of eligible patients and involved in specific aspects of follow up care under guidance of the Coordinating Center[23].

Both the 'hub and spoke' and division of responsibility models have developed organically among the HTCs within the clinical trial programs to date. Practical implementation of gene therapy within the HTCs will involve expanding these models of care and transitioning from the clinical trial infrastructure to the clinical care infrastructure. The regional haemophilia network in the USA is supported by the Health Resources and Services Administration and organized into 8 regional

networks that comprise 149 HTCs[26]. Each region has a regional core center that collaborates with national, regional and local partners. Presently, there are approximately 36 HTCs within the network that have gene therapy clinical trial experience, the majority modeling as Coordinating Centers, though about 25% of these sites have never dosed a patient within the phase III clinical trials, related to various institutional and infrastructural barriers. These sites have remained a part of the collaborative model described above as Referral Centers to identify patients, coordinating infusion at an identified Dosing Center and then resuming care as a Follow Up center following their infusion. Importantly, there is at least one Coordinating center within each of the 8 USA regions of care, often with several HTCs in proximity that have or can serve as Referral/Follow Up centers.

We can envision that the implementation of gene therapy nationally will happen first within these specialized and experienced HTCs. However, this will involve moving from a *clinical trial* infrastructure to gene therapy delivery as part of the *clinical care* infrastructure. Within the clinical trials, these HTCs have been primarily utilizing their investigational pharmacies, clinical research centers and dedicated research nurses and coordinators. In transitioning to gene therapy as part of clinical care, this will now involve their clinical pharmacies, clinical nurses and coordinators – most of whom do not yet have any gene therapy experience. In the first phase of implementation, the priorities should be on education of the staff in these areas, addressing any evolution of the care models that will be needed to coordinate this new work flow and establishing standard operating procedures (SOPs) for clinical care pathways (Figure 1). In a second phase of expanded HTC engagement, we can anticipate sharing of best practices and SOPs, full implementation of care coordination (across clearly identified Coordinating, Dosing and Referral/Follow Centers) and continued evolution of care models.

There are unresolved challenges with the practical implementation of this infrastructure:

Reimbursement/funds flow models – There are financial responsibilities associated with gene therapy product acquisition, storage, reconstitution, administration and then patient monitoring as part of follow up. With clinical care potentially distributed across more than one HTC, how will each of the HTCs be reimbursed for their role in a patient's clinical gene therapy? The gene therapy manufacturers and private/public payers bear significant responsibility to ensure that the HTCs will be compensated adequately for their contribution to gene therapy delivery whether serving as a Referral/Follow Up, Dosing and/or Coordinating Center.

Coordination of care between HTCs – there is still limited experience with patients moving fluidly for services between HTCs. This can be related to geographies, health coverage limitations, and established trust with their home HTC. In keeping with the four universal principles[23], access to gene therapy for a PWH should not be limited by their geography or home HTC's experience with gene therapy to date. Thus, we should be looking to models of care that allow for shared care across sites without sacrificing communication and data collection.

Institutional approvals and local infrastructure needs – for each gene therapy product, each site will need to secure the appropriate Infection Control Committee review and approval, assess the needed infrastructure within their clinical pharmacies to support product receipt, storage, handling, preparation for infusion and identify the suitable site to administer the product and conduct the appropriate peri-infusion monitoring.

Personnel/staffing - leaving the supports of the clinical trial infrastructure and shifting to the heavy demands of the clinical care infrastructure will require targeted education, new divisions of responsibility for the clinical care staff and possibly new personnel (eg. a dedicated care coordinator) to assist in the navigation of the PWH across the entirety of their gene therapy treatment journey)

Long-term Data Collection – As a new and evolving therapy, lifelong follow up will be critical to reporting the safety and efficacy of gene therapy and guiding subsequent innovations. A communication from the ISTH has identified a core data set on safety, efficacy and durability of gene therapy[27] that has been incorporated into national gene therapy registries and the World Federation of Hemophilia Gene Therapy Registry (WFH GTR)[28]. These will be prospective, observational and longitudinal with the expectation that this data will be collected through the existing relationship of the PWH and their HTC. Thus, regardless of whether an HTC will make preparations for dosing of patients at their center, they can expect to be involved in some aspect of the long-term data collection. The American Thrombosis and Hemostasis Network (ATHN) partners with 146 HTCs across the USA, providing a national database for PWH with the goals of securing data, advancing knowledge and transforming care. This facilitates continuity of care, fosters collaboration, maintains confidentiality and conserves resources through a common infrastructure. PWH can move between the HTCs with a common unique identifier with shared data access across providers. They have established the Hemophilia Gene Therapy Outcomes Arm of ATHN Transcends, the national longitudinal, observational cohort study that evaluates the effectiveness and practice of all haemophilia therapies in the USA (NCT04398628). The study aims to enroll all people with hemophilia A or B who will receive a gene transfer product. Data will be collected from participants at the time of enrollment and at the following timepoints relative to vector infusion: 3 months, 6 months, 1 year, 18 months, 2 years, and annually thereafter. Participants will be followed longitudinally for at least 15 years after vector infusion. Safety will be measured

according to medical events in the European Haemophilia Safety Surveillance (EUHASS) protocol[29], as well as liver toxicity. A central lab (Versiti, Milwaukee, WI, USA), will provide results on factor level, inhibitor, and genetic testing. To advance global data collection, this arm of ATHN Transcends will provide data directly to the WFH GTR.

Gene therapy and management of immunosuppression

Successful gene therapy requires the safe and effective delivery of a functioning gene (transgene), resulting in protein expression at levels capable of ameliorating the disease phenotype, potentially for an individual's lifetime. The host immune response affects both the predictability and durability of transgene expression. The immune response includes both innate and adaptive immune responses and is targeted against the viral Vector, transgene and the transgene product.

The management of the immune response is crucial for both the long term expression of the transgene and for limiting the short term toxicity in the tissues targeted for gene transfer. In haemophilia gene therapy trials, where the target organ has been the liver, the immune response clinically presents as transaminitis with loss of expression of FVIII and FIX, and further, the response to immune management has been variable[30,31]. In other disorders, fatalities have been observed and are under investigation to understand factors contributing to death. Indeed the pre-clinical models have not mirrored the immune responses observed in clinical trials, and the increasing number of trials with rAAV across many disease areas can contribute to our understanding of this complex area[32,33].

Immune response to the Vector

Vector immunogenicity is determined by the interaction of rAAV with the host immune system[32]. In addition to the capsid proteins, the transgene and its products can also trigger an immune response.

The innate response to rAAV is mild and short-lived, with minimal clinical impact compared to adenoviruses making them attractive vectors for gene therapy [34].

The humoral or antibody response includes the development of antibodies, both IgM and all subclasses of IgG. The antibodies that develop can be neutralizing or non-neutralizing antibodies. The former binds rAAV and prevents rAAV transduction of the cells, thus impacting the efficiency of gene transfer [35]. The development of neutralizing antibodies following infection with wild type AAV or following administration of rAAV prevents further retreatment, suggesting that AAV-mediated gene therapy can potentially be once in a lifetime treatment. The impact of non-neutralizing antibodies is less well characterized.

Following administration of the rAAV, transaminitis with loss of protein expression has been associated with a marked rise in capsid specific T cells around eight weeks after vector infusion [30,36]. This requires the proliferation of capsid-specific T cells and the display of sufficient numbers of the peptide-MHC complexes on the surface of the hepatocyte as the magnitude of immune response seems to determine the clinical effect[36]. Importantly, steroids have been used to control transaminitis with stabilization of expression levels [30,36].

Both Vector dependent factors and host-dependent factors contribute to vector immunogenicity. Vector dependent factors include the serotype of the capsid, the dose of the Vector, the purity of the Vector, potentially the manufacturing platform, and codon sequences in the transgene that are potentially non-human [32,37]. The purity is related to the number of empty capsids and protein and DNA components. Host-dependent factors include age, pre-existing immunity, inflammation, and potentially the patient's immune genotype/phenotype, which determine the immune response to external stimuli [32].

Immunosuppressants used in gene therapy clinical trials

Several therapeutic interventions are being employed to overcome rAAV immunogenicity to improve the predictability and longevity of gene therapy [38,39]. The choice of therapeutic agents originates from their use in other autoimmune disorders or organ transplantation and trial and error. Immune management of autoimmune disorders typically includes escalating interventions starting with single drugs and progressing to multi-drug regimens based on response to treatment, assessed either clinically or by biomarkers. In contrast, in organ transplantation, the immunosuppressive regimens are designed to be effective immediately post-transplantation in the majority of the patients as the loss of an organ can be potentially fatal, particularly in liver transplantation. Further, the use of therapeutic drug monitoring facilitates titration of therapy, and an established body of evidence from organ transplantation is available for extrapolation.

Corticosteroids in the form of prednisone and prednisolone are the most commonly employed immune-modulatory agents. They demonstrate both anti-inflammatory and immunosuppressive properties with broad inhibitory effects on innate and adaptive cells by reducing the production of pro-inflammatory cytokines, chemokines, and T cells[39]. Other agents that affect both T and B cells responses and are used with rAAV therapies are sirolimus and mycophenolate mofetil. Sirolimus results in the generation of regulatory T cells (Treg) and suppression of cytotoxic T lymphocytes and helper T cell activation. At higher doses, it impairs B cell proliferation and differentiation. Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid, suppresses T and B cells proliferation. Indeed a combination therapy has been tested in pre-clinical models with no impact on the rAAV transduction [40].

Calcineurin inhibitors, cyclosporin and tacrolimus, are widely used in solid organ transplantation with an extensive safety profile. They inhibit T cell differentiation, survival, subsequent antibody production, and cytotoxic T lymphocyte activities via effector helper T cells. There is some suggestion that they might inhibit the proliferation of regulatory T cells, which might be detrimental to tolerance to the transgene. The other agent that has been used is rituximab, a monoclonal

antibody targeting CD20 positive pre-B and mature B cells, limiting antibody production and epitope presentation to helper T cells [41]. All of the mentioned agents have been used in various rAAV clinical trials, and several combination therapies are being trialled in pre-clinical models [39].

Immunosuppressive strategies in Gene Therapy

In addition to discussions about the choice of agent(s), there is an ongoing debate about the risks and benefits of a prophylactic versus reactive strategy and duration of treatment. A significant challenge of a reactive strategy is the need for close monitoring to identify transaminitis, as it can result in significant irreversible loss of expression over one to two weeks secondary to loss of transduced hepatocytes. Further, an immune response can be challenging to control promptly once a vigorous response has been mounted. Typically, the treatment is continued until normalization of liver function tests followed by slow taper of immunosuppression.

A prophylactic strategy aims to facilitate an optimal response to all gene therapy participants. If optimized, it can enable a more predictable response and decrease the need for close monitoring. This does come with the burden of additional adverse events related to the use of immunomodulatory agents. Steroids in the form of oral prednisolone or high doses of intravenous methylprednisolone have been the most commonly used immunomodulatory agents, with patients receiving them for variable periods and up to one year. Steroid-induced adverse events are related to both dose and duration of therapy. Common side effects include increased appetite, weight gain progressing to cushingoid appearance, skin changes and cognitive changes like poor sleep, anxiety and other mood disturbances. The severe side effects include the known association between extended duration of steroids from three months onwards and osteoporosis and osteoporotic fracture, which is of particular concern in the older adult with severe joint disease. Other long term side effects include adrenal suppression, increased risk of glaucoma and cataract formation, dyslipidemia, hyperglycemia and diabetes[42]. The potency of the capsid and observed incidence of transaminitis in a study may also determine the need for a prophylactic strategy. The other issue is

the optimal time to introduce prophylactic immunosuppression. Typically, in haemophilia trials, steroids have been introduced around two to four weeks post-infusion, before the onset of transaminitis. The duration of treatment tends to cover the weeks that coincide with peak transaminitis. In some non-haemophilia studies, pre-dosing immune-modulatory prophylaxis has been initiated. Indeed in one study, this resulted in the lack of development of neutralizing antibodies, despite the development of anti-capsid antibodies [41].

The other intriguing aspect of haemophilia gene therapy is the differential response seen between Factor IX and factor VIII gene therapy trials. In FIX gene therapy trials, recurrences have not been seen with preserved long-term expression following the initial control of transaminitis. In contrast, a steady loss of expression of FVIII has been noted and whether this has an immunological basis is unknown.

Several strategies have been suggested to overcome neutralizing antibodies as retreatment may be required in haemophilia and other gene therapies [36], with additional strategies in development to decrease the immunogenicity of rAAV through improved manufacturing and increased understanding of the host immune response [43,44].

Unknowns and research priorities

The use of immune-modulatory strategies is an evolving area in gene therapy, and long term follow-up studies are required to understand the role of prophylactic versus reactive strategy to immune response and the ideal choice of therapeutic agents. A significant limitation of the current trials is the lack of exploratory studies and consensus protocols for gathering information on rAAV immunogenicity. There is an urgent need for correlation studies with exploratory biomarkers that characterize the host immune response to the Vector used and some consensus recommendations for active investigation in this area.

Acknowlegment: KRR, SWP and PC each wrote the paper and contributed to revisions.

Disclosures:

KRR - Consultant: Spark Therapeutics, Mallinckrodt, GENFIT, Novo Nordisk
Grant/Research Support(Paid to the University of Pennsylvania): Mallinckrodt, BMS, Intercept, Exact Sciences, Grifols, Sequana, HCC-TARGET, NASH-TARGET, Gilead DSMB-Novartis

SWP – Consultant: Apcintex, ASC Therapeutics, Bayer, Biomarin, Catalyst Biosciences, CSL Behring, GenVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sangamo Therapeutics, Sanofi, Takeda, Spark Therapeutics, uniQure

PC - Advisory boards: Bayer, Boehringer Ingelheim, CSL Behring, Chugai, Freeline, NovoNordisk, Pfizer, Roche, Sanofi, Spark, Sobi and Takeda; Research funding: Bayer, CSL Behring, Freeline, Novo Nordisk, Pfizer, SOBI and Takeda

References

1. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC, Public Policy Committee of the American Association for the Study of Liver D. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology.* 2008;47(4):1363-1370.
2. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med.* 2002;137(1):1-10.
3. Piton A, Poynard T, Imbert-Bismut F, et al. Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. *Hepatology.* 1998;27(5):1213-1219.
4. Salvaggio A, Periti M, Miano L, Tavanelli M, Marzorati D. Body mass index and liver enzyme activity in serum. *Clin Chem.* 1991;37(5):720-723.
5. Mathiesen UL, Franzen LE, Fryden A, Foberg U, Bodemar G. The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. *Scand J Gastroenterol.* 1999;34(1):85-91.
6. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. *BMJ.* 2004;328(7446):983.
7. Lee TH, Kim WR, Benson JT, Therneau TM, Melton LJ, 3rd. Serum aminotransferase activity and mortality risk in a United States community. *Hepatology.* 2008;47(3):880-887.
8. Kennedy P, Wagner M, Castera L, et al. Quantitative Elastography Methods in Liver Disease: Current Evidence and Future Directions. *Radiology.* 2018;286(3):738-763.
9. Yen YH, Kuo FY, Chen CH, et al. Ultrasound is highly specific in diagnosing compensated cirrhosis in chronic hepatitis C patients in real world clinical practice. *Medicine (Baltimore).* 2019;98(27):e16270.
10. Festi D, Schiumerini R, Marzi L, et al. Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther.* 2013;37(4):392-400.

11. Stern C, Castera L. Non-invasive diagnosis of hepatic steatosis. *Hepatol Int.* 2017;11(1):70-78.
12. Cassinotto C, Boursier J, de Ledinghen V, et al. Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. *Hepatology.* 2016;63(6):1817-1827.
13. Cassinotto C, Lapuyade B, Guiu B, et al. Agreement Between 2-Dimensional Shear Wave and Transient Elastography Values for Diagnosis of Advanced Chronic Liver Disease. *Clin Gastroenterol Hepatol.* 2020;18(13):2971-2979 e2973.
14. Singh S, Venkatesh SK, Wang Z, et al. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. *Clin Gastroenterol Hepatol.* 2015;13(3):440-451 e446.
15. Houot M, Ngo Y, Munteanu M, Marque S, Poynard T. Systematic review with meta-analysis: direct comparisons of biomarkers for the diagnosis of fibrosis in chronic hepatitis C and B. *Aliment Pharmacol Ther.* 2016;43(1):16-29.
16. Naveau S, Gaude G, Asnacios A, et al. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. *Hepatology.* 2009;49(1):97-105.
17. Ratziu V, Massard J, Charlotte F, et al. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol.* 2006;6:6.
18. Li J, Gordon SC, Rupp LB, et al. The validity of serum markers for fibrosis staging in chronic hepatitis B and C. *J Viral Hepat.* 2014;21(12):930-937.
19. Treeprasertsuk S, Bjornsson E, Enders F, Suwanwalaikorn S, Lindor KD. NAFLD fibrosis score: a prognostic predictor for mortality and liver complications among NAFLD patients. *World J Gastroenterol.* 2013;19(8):1219-1229.
20. Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: a systematic review. *Ann Intern Med.* 2013;158(11):807-820.
21. Frerichs FT. *Über den Diabetes.* Berlin: A. Hirschwald; 1884.
22. Miesbach W, Chowdary P, Coppens M, et al. Delivery of AAV-based gene therapy through haemophilia centres-A need for re-evaluation of infrastructure and comprehensive care: A Joint publication of EAHAD and EHC. *Haemophilia.* 2021;27(6):967-973.
23. Miesbach W, Pasi KJ, Pipe SW, et al. Evolution of haemophilia integrated care in the era of gene therapy: Treatment centre's readiness in United States and EU. *Haemophilia.* 2021;27(4):511-514.
24. Pipe SW. Delivering on the promise of gene therapy for haemophilia. *Haemophilia.* 2021;27 Suppl 3:114-121.
25. Pipe SW, Gonen-Yaacovi G, Segurado OG. Hemophilia A gene therapy: current and next-generation approaches. *Expert Opin Biol Ther.* 2022:1-17.
26. Valentino LA, Baker JR, Butler R, et al. Integrated Hemophilia Patient Care via a National Network of Care Centers in the United States: A Model for Rare Coagulation Disorders. *J Blood Med.* 2021;12:897-911.
27. Konkle B, Pierce G, Coffin D, et al. Core data set on safety, efficacy, and durability of hemophilia gene therapy for a global registry: Communication from the SSC of the ISTH. *J Thromb Haemost.* 2020;18(11):3074-3077.
28. Konkle BA, Coffin D, Pierce GF, et al. World Federation of Hemophilia Gene Therapy Registry. *Haemophilia.* 2020;26(4):563-564.

29. Makris M, Calizzani G, Fischer K, et al. EUHASS: The European Haemophilia Safety Surveillance system. *Thromb Res.* 2011;127 Suppl 2:S22-25.
30. Nathwani AC, Tuddenham EGD, Rangarajan S, et al. Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B. *New England Journal of Medicine.* 2011;365(25):2357-2365.
31. Mingozzi F, High KA. Overcoming the Host Immune Response to Adeno-Associated Virus Gene Delivery Vectors: The Race Between Clearance, Tolerance, Neutralization, and Escape. *Annual Review of Virology.* 2017;4(1):511-534.
32. Ronzitti G, Gross D-A, Mingozzi F. Human Immune Responses to Adeno-Associated Virus (AAV) Vectors. *Frontiers in Immunology.* 2020;11(670).
33. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov.* 2019;18(5):358-378.
34. Bessis N, GarciaCozar FJ, Boissier MC. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. *Gene Therapy.* 2004;11(1):S10-S17.
35. Calcedo R, Wilson J. Humoral Immune Response to AAV. *Frontiers in Immunology.* 2013;4(341).
36. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood.* 2013;122(1):23-36.
37. Pipe S, Leebeek FWG, Ferreira V, Sawyer EK, Pasi J. Clinical Considerations for Capsid Choice in the Development of Liver-Targeted AAV-Based Gene Transfer. *Mol Ther Methods Clin Dev.* 2019;15:170-178.
38. Monahan PE, Négrier C, Tarantino M, Valentino LA, Mingozzi F. Emerging Immunogenicity and Genotoxicity Considerations of Adeno-Associated Virus Vector Gene Therapy for Hemophilia. *Journal of Clinical Medicine.* 2021;10(11):2471.
39. Chu WS, Ng J. Immunomodulation in Administration of rAAV: Preclinical and Clinical Adjuvant Pharmacotherapies. *Frontiers in Immunology.* 2021;12(858).
40. Jiang H, Couto LB, Patarroyo-White S, et al. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. *Blood.* 2006;108(10):3321-3328.
41. Mueller C, Berry JD, McKenna-Yasek DM, et al. SOD1 Suppression with Adeno-Associated Virus and MicroRNA in Familial ALS. *New England Journal of Medicine.* 2020;383(2):151-158.
42. Liu D, Ahmet A, Ward L, et al. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy Asthma Clin Immunol.* 2013;9(1):30.
43. Chan YK, Wang SK, Chu CJ, et al. Engineering adeno-associated viral vectors to evade innate immune and inflammatory responses. *Science translational medicine.* 2021;13(580).
44. Meliani A, Boisgerault F, Hardet R, et al. Antigen-selective modulation of AAV immunogenicity with tolerogenic rapamycin nanoparticles enables successful vector re-administration. *Nature Communications.* 2018;9(1):4098.

Table 1: Hepatic Biochemical Tests

Chemistry	Interpretation
Bilirubin**	Overproduction, impaired conjugation Hepatocellular damage Cholestasis (both intra- and extra-hepatic)
ALT[1], AST[2]	Hepatocellular damage
ALP[3]	Cholestasis (infiltration, SOL[6])
GGT[4]	Cholestasis (infiltration, SOL[6])
Albumin*	Synthetic function
PT[5*]	Synthetic function

Legend

1. ALT: alanine aminotransferase
2. AST: aspartate aminotransferase
3. ALP: alkaline phosphatase
4. GGT: gamma-glutamyltransferase
5. PT: prothrombin time
6. SOL: Space occupying lesion
    a. * Marker of hepatic function
    b. ** Mostly a marker of hepatic function but can represent other conditions (e.g. hemolysis)

Table 2: Noninvasive Liver Disease Assessment (NILDA) Tools and Liver Biopsy

Type of Test	Strengths	Limitations
Image Technique Guided Tools (Transient elastography (TE), ARFI (pSWE), 2-D SWE, MR	• Easy to use • Minimal operator experience • High sensitivity and	Transient elastography and ultrasound elastography • High BMI may limit

elastography)	specificity, particularly for cirrhosis • Generally readily available • Can be used for monitoring hepatic fibrosis • Degree of steatosis can be assessed (CAP, MRI-PDFF) • MR elastography examines the entire liver	interpretation • Hepatic congestion may lead to false readings • Food intake associated with increased liver stiffness-patients need to fast for 2-3 hours prior to the procedure • Helpful in assessing and monitoring fibrosis but not inflammation • Inability to discriminate well between intermediate stage of fibrosis MRI elastography • Not readily available • Expensive and thus may not be practical for long term monitoring of fibrosis
Blood based biomarker panels	• Readily available • Can be done with online calculator (APRI, FIB-4) • Can be done commercially (Fibrotest, ELF, NFS) • Can be used for monitoring hepatic fibrosis	
Liver biopsy	• Has been the "gold" standard for the diagnosis and staging of liver disease while there has been diminishing role in the diagnosis (e.g HBV, HCV, alcoholic liver disease)	• Invasive with some risk albeit small • Suboptimal patient acceptance • Inadequate sample may lead to inaccurate diagnosis and staging of fibrosis • Not practical for long term monitoring of hepatic fibrosis

Table 3: Non-Invasive Liver Disease Assessment (NILDA) with Biomarker Panels

Biomarkers	How to Calculate	Comments
APRI[1]	AST level, Platelet count	Can calculate online
FIB-4[2]	Age, AST level, ALT level, Platelet count	Can calculate online
FibroTest[3]	Age, Sex, GGT level, Total bilirubin level, Alpha-2-macroglobulin level, Haptoglobin level, Apolipoprotein A1 level, ALT level (included for ActiTest)	Commercially available
ELF[4]	Hyaluronic acid, Procollagen III amino-terminal peptide, Tissue inhibitor of metalloproteinase 1	Commercially available
NFS[5]	Age, BMI, Diabetes disease status, AST level, ALT level, Platelet count, Albumin level	Commercially available

Legend

1. APRI: AST to Platelet Ratio Index
2. FIB-4: Fibrosis-4 Index
3. Known as FibroSure in the United States
4. ELF: Enhance Liver Fibrosis Test
5. NFS: NASH/NAFLD Fibrosis Score

Figure 1. Priority Areas for Hemophilia Treatment Center Preparedness for Implementation of Gene Therapy

- **Education of PWH and Staff**
    - Shared decision making that incorporates gene therapy among existing therapies
    - Potential benefits and risks
        - Fully informed from clinical trial data
- **Biologic**

    - Institutional preparedness for product handling and administration
        - infection control committee review
        - nursing handling and infusion
        - patient and staff precautions
    - Pharmacy preparedness
        - Product receipt, handling, storage
        - Reconstitution – thaw time and containment needs
- **Infrastructure and Staff**
    - Clinical pharmacist willing, able and trained for product handling
    - Trained skilled nursing for infusion
    - Physicians available during infusion
    - Safe area for infusion
        - Appropriate containment
        - Suitable to respond to infusion reactions
        - Plan for infusion modification if needed
            - Infusion rate change, supportive therapeutics
- **Pre-infusion screening**
    - Liver health
    - Neutralizing antibody assay (companion device central lab sendout)
    - Obtain baseline transaminase results at planned post-infusion monitoring site
    - Reimbursement approvals, authorization for drug acquisition
- **Day of Infusion Plan**
    - Coordination of product receipt, reconstitution, infusion and immediate post-infusion monitoring
    - Patient instructions on peri-infusion and post-infusion treatment plan
- **Post-infusion monitoring**
    - Schedule of assays – transaminases and factor levels
    - Establish immunomodulation plan and appropriate prescriptions
    - Communication plan between patient, lab and follow up center
- **Long-term monitoring**
    - Data collection plan for safety and efficacy endpoints
    - Data sharing plan – across HTCs, national and global registries