Molecular Therapy
Editorial

# Liver Gene Therapy: Reliable and Durable?

The liver is an important target for gene therapy for metabolic diseases and systemic delivery of therapeutic proteins. The vast majority of our knowledge on clinical gene therapy directed to the liver comes from treatment of hemophilia A and B with adeno-associated viral (AAV) vectors. This approach relies on hepatic tropism of the viral capsid following intravenous infusion of the vector. Interestingly, early clinical data suggest that there appear to be differences in the reliability and durability of liver-derived therapeutic expression between hemophilia A (deficiency in factor VIII [FVIII]) and hemophilia B (factor IX [FIX], deficiency). The field eagerly awaits more complete reporting and longer-term outcomes of these clinical studies, which should provide insight for the treatment of other diseases.

An earlier academic trial using an AAV8 vector with a hepatocyte-specific FIX expression cassette (with potentially immune stimulatory CpG motifs edited out) resulted in stable expression of ~5% of normal levels for at least 8 years, consistent with large animal studies. Using an engineered AAV capsid (with similarity to AAV8) and a transgene expressing the naturally occurring FIX-Padua variant that exhibits substantially increased specific activity, Spark Therapeutics raised coagulation activity in patients with severe hemophilia B from <2% to ~30%. This was accomplished with fairly low vector doses, and levels have been stable for at least 3 years. UniQure has now reported similar levels of correction using an AAV5 vector that also expresses FIX-Padua. In their original phase I/II trial, expression of conventional FIX has been stable for >3 years.

While FIX is an enzyme, FVIII serves as its crucial co-factor in the coagulation cascade. Unlike FIX, FVIII is not naturally expressed in hepatocytes but rather in sinusoidal endothelial cells of the liver. FVIII has been traditionally more challenging to express at therapeutic levels in gene therapy trials. Even the coding sequence of the shorter (but fully biologically active) B domain-deleted version is at the packaging limit of AAV, necessitating use of small promoter elements. Moreover, FVIII is more difficult for cells to secrete. Hence, large vector doses have been employed to reach therapeutic levels. Nonetheless, Biomarin has been able to achieve average FVIII levels in the normal range (50%–150%) in hemophilia A patients using AAV5 dosed at $6 \times 10^{13}$ vector genomes/kg. Levels fluctuated then stabilized during the first year after gene transfer but subsequently decreased from the end of year one levels by ~50% by the end of the third year, perhaps reaching a plateau. These numbers were obtained by a one-stage clotting assay. Interestingly, these levels were only ~30% of normal when using a two-stage "chromogenic" assay (which measures enzymatic cleavage of FX by the FVIII/IX complex). Following further analysis, Biomarin decided to report levels by chromogenic assay results, which their scientists believed to more accurately correlate with how FVIII activity units are defined. However, scientists at Spark, who also found a similar assay discrepancy in their FVIII clinical trial, found that some chromogenic assay kits, which utilize reagents based on human proteins, yield results that are quite similar to those obtained with clot-based measurements, thus sparking a continued discussion about which assay more accurately reflects actual FVIII activity in gene therapy patients. Data are just now starting to emerge from two more phase I/II clinical trials on hepatic AAV-FVIII gene transfer (Spark, using LK03, which is similar to AAV3; and Sangamo, using AAV6). Again, relatively high vector doses were given. Furthermore, when comparing different dose cohorts, dose responses show a steep threshold effect. This raises the question of a threshold for a minimal number of viral genomes per cell, seen earlier in a number of animal studies with various transgenes, above which substantially more gene expression is obtained. There appears to also be more variability than in the FIX trials, which achieved somewhat more consistent results in patients that lacked pre-existing neutralizing antibodies to the vector.

Now that various treatment modalities are available for patients with hemophilia, including a bispecific antibody that mimics FVIII but requires less frequent injections, the bar for efficacy expected from gene therapy has been raised. Gene therapy vectors remain the only medications that can provide a lasting cure and free a hemophilic patient from the need for more or less frequent injections of protein products. However, substantial variability or limited durability could make gene therapy less attractive as a treatment option and also more difficult to accept by health care providers. Certainly, these parameters will be carefully evaluated by the regulatory agencies. Although patients with mild hemophilia (>5% of normal) rarely have a spontaneous bleed, additional factor is needed in case of trauma or for surgical procedures, for example. How much FVIII activity a gene therapy should provide is therefore the subject of an ongoing debate in the community. Greater than 30% is certainly a desired target, while levels in the normal range would be ideal. Should expression fall over time or should gene therapy with AAV vectors (which do not efficiently integrate unless combined with gene editing tools) be considered for pediatric patients with rapidly growing livers, the question of the ability to re-administer becomes more pressing.

Obviously, longer follow-up from the different trials is needed to conclude whether a decline in FVIII activity is a more general phenomenon. There are multiple differences between the various vectors, including vector constructs (promoters, CpG contents and so on), capsid/serotype, and production system (transfection of mammalian cells versus baculoviral infection of insect cells). These could impact efficacy, variability, and immunogenicity. Differences between FVIII and FIX gene transfer could be related to the transgene products themselves, as overexpression of FVIII has been implicated in cellular stress responses. At the vector doses tested by Biomarin, mild elevations in liver transaminases have appeared regardless of glucocorticoid prophylaxis or not. Glucocorticoids may limit inflammation and counter $CD8^+$ T cell responses against AAV capsid, although these hypotheses have not been formally proven since liver biopsies post vector infusion have not been performed. Interestingly,

glucocorticoids have also been described in the literature as alleviating cellular stress caused by protein misfolding. One could also envision that the resulting capsid and vector genome load per transduced cell at very high doses could result in cellular stress or instability of at least a portion of the vector genomes. Vector doses $>10^{13}$/kg were also reported for UniQure's FIX trial. Both this trial and the Biomarin FVIII trial utilized AAV5 capsids and baculoviral production systems. At first glance, the FVIII appears the main culprit in causing the more variable outcomes and questions about durability. However, interpretation is not straightforward as vector doses were still not identical, vector titers were not determined side-by-side, and efficiency of vector infectivity may not be identical.

In conclusion, despite successes, there are still important known unknowns in liver-directed gene therapy. Fortunately, a multitude of ongoing clinical trials will generate a treasure trove of results that should help the field identify obstacles and point to solutions so that more patients can experience a lasting cure of their genetic disease.

Roland W. Herzog[1] and Glenn F. Pierce[2,3]

[1]Wells Center for Pediatric Research, Indiana University, Indianapolis, IN 46202, USA; [2]World Federation of Hemophilia, Montreal, Quebec, Canada; [3]Ambys Medicines, South San Francisco, CA, USA

**Correspondence:** Roland W. Herzog, Wells Center for Pediatric Research, Indiana University, Indianapolis, IN 46202, USA.
**E-mail:** rwherzog@iu.edu

https://doi.org/10.1016/j.ymthe.2019.10.005