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WFH State-of-the-art paper 2020: In vivo lentiviral vector gene therapy for haemophilia

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Abstract

Over the last decade, the development of new treatments for haemophilia has progressed at a very rapid pace. Despite all the promising advances in protein products, the prospect offered by gene therapy of a single potentially lifelong treatment remains attractive for people with haemophilia. Transfer to the liver of coagulation factor VIII (FVIII) or factor IX (FIX) transgenes has indeed the potential to stably restore the dysfunctional coagulation process. Recombinant adeno-associated virus (AAV)-derived vectors are widely employed for liver-directed gene therapy, given their very good efficacy and safety profile, shown in several preclinical and clinical studies. However, there are some limitations associated with AAV vectors, such as their predominantly episomal nature in the nucleus of target cells and the widespread pre-existing immunity against the parental virus in humans. By contrast, HIV-derived lentiviral vectors (LV) integrate into the target cell chromatin and are maintained as the cells duplicate their genome, a potential advantage for establishing long-term expression especially in paediatric patients, in which the liver undergoes substantial growth. Systemic administration of LV allowed stable multi-year transgene expression in the liver of mice and dogs. More recently, improved phagocytosis-shielded LV were generated, which, following intravenous administration to non-human primates, showed selective targeting of liver and spleen and enhanced hepatocyte gene transfer, achieving up to supra-normal activity of both human FVIII and FIX transgenes. These studies support further preclinical assessment and clinical evaluation of in vivo liver-directed LV gene therapy for haemophilia.

KEYWORDS

gene therapy, lentiviral vectors

Over the last decade, the development of new treatments for haemophilia has progressed at a very rapid pace. In addition to conventional recombinant clotting factors, largely adopted for prophylaxis in high-income countries, extended half-life products more recently allowed reducing the prophylactic regimens down to once weekly or even biweekly (for haemophilia B) dosing and more products are in clinical development.[1] An activated-factor VIII (FVIII) mimetic antibody has recently entered the market for subcutaneous administration for prophylaxis in both inhibitor-negative and inhibitor-positive individuals with haemophilia A.[2] Other non-factor therapy drugs, such as inhibitors of the antithrombin pathway, are in clinical development. Despite all these promising advances in protein products,

the prospect offered by gene therapy of a single potentially lifelong treatment remains attractive for people with haemophilia.[3]

Transfer to the liver of an expression cassette encoding clotting FVIII or factor IX (FIX) has indeed the potential to constantly and stably restore the dysfunctional coagulation process. Recombinant adeno-associated virus (AAV)-derived vectors are widely employed for liver-directed gene therapy, given their very good efficacy and safety profile, as shown in several preclinical and clinical studies. AAV vector-based liver gene therapy for both haemophilia A and B has reached phase III clinical testing and is today among the most successful examples of gene therapy, as a result of more than two decades of research and preclinical and clinical development.[4] It has been shown that a single intravenous (iv) administration of AAV vectors provides for multi-year therapeutically relevant FVIII and FIX activity and clinical benefit in adults with haemophilia A or B.[5-7] Despite this success, there are some important limitations associated with AAV vectors, such as their predominantly episomal nature in the nucleus of target cells and the widespread pre-existing immunity against the parental virus in humans. The former feature challenges AAV vector application to paediatric patients, since hepatocyte proliferation during liver growth would lead to dilution and eventual loss of the vector episome from the nucleus, while the immune response to AAV components after the first administration currently precludes effective re-administration. Pre-existing anti-AAV immunity currently precludes access to AAV vector-based liver gene therapy to 20%-30% of individuals.[8] Moreover, in contrast to the stable FVIII activity shown in dogs, it has been recently reported a progressive decrease in FVIII transgene activity in AAV-treated subjects with haemophilia A, the causes of which are not completely understood.[7] The reasons for this outcome might be consequent to the challenges posed by delivering and stably maintaining a vector genome reaching up to its packaging limit. For the above-mentioned reasons, the development of additional vector platforms and strategies is beneficial for broader and more robust application of liver-directed gene therapy.

Whereas hepatocyte-based AAV vector gene transfer is the most advanced gene therapy for haemophilia, several other strategies have been proposed, based on different vector platforms, cell types (such as hematopoietic cells and liver sinusoidal endothelial cells) or gene-editing approaches, that are currently all at a preclinical stage.[9,10] Here, we will focus on hepatocyte-based lentiviral vector (LV) gene transfer. HIV-derived (LV) are replication-defective hybrid enveloped viral particles made by a minimal set of capsid and enzymatic proteins of the parental virus, a surface protein of an unrelated virus (referred to as pseudotype) and a recombinant viral genome, comprising the *cis* acting viral genome elements necessary for gene transfer and a transgene expression cassette of choice[11] (Figure 1). The vesicular stomatitis virus surface glycoprotein (VSV.G) is most often used to pseudotype LV, as it confers high stability and wide tropism mediated by the low-density lipoprotein receptor (LDL-R).[12] Upon iv administration, both the filtering action of liver sinusoids and the engagement of LDL-R on hepatocytes by VSV.G on LV particles provide for substantial liver tropism. LV integrate into the target cell chromatin and are maintained as the cells duplicate their genome, a potential advantage for establishing long-term expression especially in paediatric patients, in which the liver undergoes substantial growth. LV comfortably accommodate the FVIII transgene, with a packaging size of about 10 kb. Furthermore, the low prevalence of HIV (estimated at 0.8% of adults worldwide (www.who.int/gho/hiv) and the absence of specific anti-VSV.G immunity in humans, make LV attractive gene delivery vehicles for in vivo liver-directed gene therapy.[13,14]

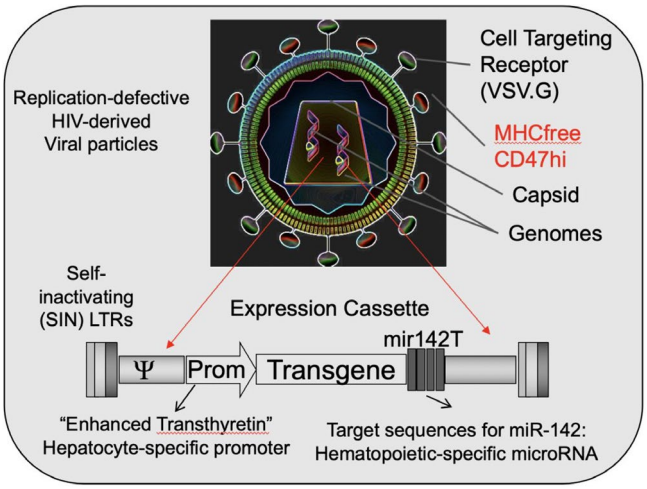

FIGURE 1 Schematic representation of third-generation LV designed for liver-directed gene therapy[23,25,26]

Lentiviral vector (LV) are emerging as powerful and versatile delivery vehicles in gene therapy and have recently reached the market with two cell-based ex vivo gene therapy products: one based on autologous T cells containing chimeric T-cell receptors against CD19, approved for the treatment of acute lymphoblastic leukaemia, and another one based on autologous hematopoietic stem/progenitor cells (HSPC) approved for patients affected by beta thalassemia.[15,16] LV-based HSPC gene therapy for several immune-haematologic and lysosomal storage diseases is also in advanced clinical testing.[17] Despite being crucial to sustain stable transgene expression in the hematopoietic progeny of the transduced cells, integration of the LV into the genome of target cells raises concern of long-term genotoxicity. A substantial amount of research on vector design and its testing in stringent models has helped to alleviate this concern. Extensive studies in preclinical models and in human patients treated with HSPC gene therapy for different types of disease have shown that LV integrate genomewide with a reproducible pattern, reflecting intrinsic biases at the time of transduction and not the results of selection due to a gain of function. Longitudinal tracking of vector integration site (IS) in treated patients has shown stable polyclonal hematopoietic reconstitution by the transduced HSPC and no evidence for skewing in genomic distribution or specific gene classes by comparing vector IS in pre- and post-transplant samples.[18-21] Moreover, no evidence of insertional oncogenesis has been reported in hundreds of patients treated by LV-transduced

HSPC or T cells in several clinical trials, with a follow-up of >10 years for the earliest treated patients, each receiving approximately 10^7 vector integrations/kg for HSPC.[17,22] These findings are reassuring, given the sensitivity of HSPC to oncogenic transformation, which is likely to be higher than hepatocytes, and support the claim that LV design, integration sites selection, and polyclonal reconstitution all contribute to alleviate such risk, compared to earlier-generation vectors.[13] Furthermore, the potential for unintended hepatic tumorigenicity of the LV platform is considered low based on a study of liver-targeted systemically delivered LV in tumour-prone mice that showed no evidence for induction of hepatocellular carcinomas.[23] Notwithstanding these reassuring data, the intrinsic risk of genotoxicity should continue to be modelled, reduced as feasible and appropriate risk-benefit assessments conducted.

Lentiviral vector (LV) intended for liver-directed gene therapy are designed to stringently target transgene expression to hepatocytes through transcriptional and microRNA-mediated regulation. Systemic administration of such LV provided stable multi-year transgene expression in the liver of mice and dogs.[23,24] More recently, the vector surface has been modified by changing the protein composition of the producer cell plasma membrane to reduce immunogenicity of LV particles. First, it was performed genetic disruption of the β-2 microglobulin (B2M) gene, a required component for the assembly and trafficking of all class-I major histocompatibility complexes (MHC-I) to the plasma membrane in LV producer cells.[25] The resulting B2M-negative cells were devoid of surface-exposed MHC-I and produced MHC-free LV (see Figure 1). These LV retained their infectivity on all tested cells in vitro and efficiently transduced the mouse liver upon iv administration. These MHC-free LV showed significantly reduced immunogenicity in a T-cell activation assay performed on human primary T cells co-cultured with autologous monocytes exposed to LV, from several healthy donors, suggesting that conventional MHC-bearing LV may trigger allogeneic immune responses.[25] Secondly, LV with increased levels of the professional phagocytosis inhibitor CD47 on the surface were generated (CD47hi LV; see Figure 1). These LV showed substantially decreased uptake by human macrophages in vitro. The role of CD47 in LV biodistribution upon iv administration was evaluated in the non-obese diabetic (NOD) mouse model whose SIRPα (the CD47 receptor) is known to have high affinity for the human CD47. In this setting, CD47 proved to be a key player in reducing phagocytosis of LV by Kupffer's cells, decreasing the inflammatory cytokine response following their administration and increasing hepatocyte transduction and FIX output. MHC-free or MHC-free/CD47hi LV encoding the hyper-functional R338L human FIX were then administered to 6 non-human primates (NHP, 3 for each LV version). Administration via peripheral vein was well tolerated, without significant elevation of serum aminotransferases or body temperature and only caused a transient self-limiting leukopenia. Human-specific FIX activity reached up to 300% of normal and was nearly threefold higher in the CD47hi-LV compared to LV-treated animals and showing a much more favourable LV dose-response than observed in mice and dogs. Upon necropsy, vector copies in the liver of treated animals accounted for 80%-90% of all the retrieved LV copies, showing selective targeting and efficient gene transfer to the liver by LV in NHP.[26] To apply this strategy to haemophilia A, LV with human B-domain deleted FVIII have been generated. To improve FVIII expression, FVIII cDNA was codon-optimized (coFVIII), and a DNA fragment encoding a non-structured, 144-aa XTEN polypeptide, known to increase the half-life and expression level of the payload protein, was incorporated into the B-domain region of FVIII (coFVIIIXTEN).[1,27] Persistent FVIII expression was observed post-LV treatment of newborn haemophilia A mice throughout the 6-month study period despite a greater than 10-fold increase in body weight of the treated animals, with coFVIII and coFVIIIXTEN conferring several fold improvement on plasma FVIII levels. Administration of coFVIII- or coFVIIIXTEN-expressing MHC-free/CD47hi LV to NHP resulted in FVIII levels in the therapeutic range up to 100% in treated animals, under immune suppression. Whereas translating in vivo LV gene therapy to humans certainly poses challenges such as manufacturing at sufficient quantity and quality, overall the data described here support further development of LV as a systemic gene delivery vehicle to complement the reach of AAV vectors and address some of the outstanding challenges in gene therapy for haemophilia.

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DISCLOSURES

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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