GENE THERAPY FOR HAEMOPHILIA: REVIEW

T. Arvind Prabhu[1] & S. Ivo Romauld[1*] & K. Rajakumari[1]

Department of Bio- Engineering, Vels Institute of Science, Technology and Advanced Studies,
Chennai, India

ABSTRACT: *Haemophilia is a chromosome-related haemorrhage, bleeding recessive disorder that occurs due to the insufficiency of Factor VII and Factor IX. There are two main kinds of Haemophilia – Haemophilia A and Haemophilia B. The insufficiency of Factor VII (F VII) causes Haemophilia A and the insufficiency of Factor IX leads to Haemophilia B. The other types of Haemophilia include Haemophilia C, which occurs due to the deficiency of Factor XI and Para Haemophilia which occur due to deficiency of Factor V. Advanced method of haemophilia treatment is done using gene-therapy involving the use of clotting factors. These clotting factors are either extracted from human blood or synthesized by recombinant methods. Though the current treatment methods of using purified clotting factor concentrate have improved the lives of Haemophilia patients to a greater extent, this method has some limitations, this includes physiological barriers, technical barriers. In order to overcome these barriers, alternate treatment methodologies have been adapted this includes treatment approaches by Gene- therapy. Treating the haemophilia patients with Gene therapy using therapeutic vectors such as adenosine associated vector (AAV) has shown a profound increase in prolonged and sustained expression of therapeutic FIX. Various alternate treatment methodologies that are been employed for treating Haemophilia, and gene therapy strategies with viral and non-viral delivery vectors have been elaborated in this review article.*

Key Words: *Haemophilia, Para Haemophilia, Clotting factor, Recombinant Gene therapy, Viral vectors.*

INTRODUCTION:

The insufficiency of the blood clotting Factor VII and Factor IX causes haemophilia A and B respectively. Both the haemophilia A and B are inheritable disorder and are considered as most common X – linked recessive chromosomal disorder. This genetic disorder impairs the blood clotting ability of the body by inhibiting the activity of the bleeding factors needed to stop bleeding.   As a result, haemophilia patients bleed long after injuries, significant compression of the blood vessel and skin abrasions and increase the risk of developing blood clots or during surgery. In a severe case of intracranial haemophilia occurrence, the bleeding can be fatal. Acquired haemophilia may cause severe chronic synovitis, crippling arthropathy, and physical disability. According to the World Federation of Haemophilia, this blood clotting factor insufficiency affects over 400,000 individuals worldwide[1]. Depending on the level of the clotting factor present in plasma, the haemophilia is categorized into mild, moderate and severe. In most cases, haemophilia is inheritable from parents through X- chromosome with a non-functional gene to the off-springs. By increasing the blood circulating bleeding factors level above the threshold of 1% of the normal count, the risk of mortality and morbidity will reduce to a great extent.
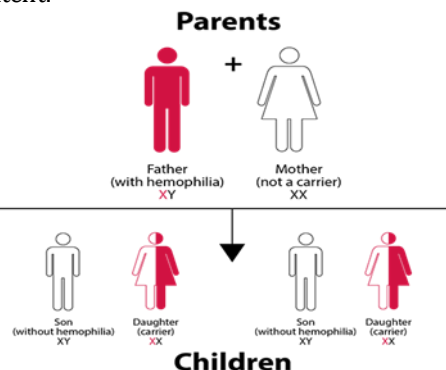

FIGURE 1: HAEMOPHILIA B INHERITANCE[2]

Recent advancement in technologies paved way for the development of different types of viral and non-viral delivery system targeting particular hepatocytes, hematopoietic stem cell, endothelial cells, skeletal muscles. The current method of treatment involves parentally administration of plasma-derived or

recombinant blood clotting concentrates as in Protein Substitution Therapy (PST). This treatment is found to prolong the life expectancy. Though this method of treatment is highly beneficial it has few limitations. The main drawback of this protein substitution therapy is that it does not produce a permanent cure, it just provides a temporary solution, the treated patients may still develop internal bleeding episodes, chronic joint damage. Second is that the therapeutic gene that is clotting factor employed in the treatment have a half-life and it is less stable. Third, parentally substitution therapy is prohibitively an expensive technique, limiting its accessibility to rural patients worldwide. Due to these drawbacks, much scientific interest is shifting towards the gene-based treatment approaches for haemophilia. The current review article focuses on the recent advancement and treatment strategies involved in the treatment of Haemophilia A and B.

OBJECTIVE OF GENE THERAPY:

The ultimate objective of gene therapy is to alternate the defective genetic constitutions in situ in order to achieve complete reversion of a disease phenotype for the lifetime of the patients. The current method of gene therapy relies on the strategies of gene insertion and addition rather than gene replacement (*Umov.et.al.*2005). This method of addition of foreign gene into the host relies on simplified approaches of using delivery mechanisms, vectors containing a modified, altered, corrected copy of the defective gene in order to correct the genetic disorder.

VARIOUS METHODS EMPLOYED IN GENE THERAPY FOR HAEMOPHILIA:

The effectiveness of gene therapy is highly dependent on the efficiency and the choice of vectors employed for gene transfer. The vector employed should be stable and should not degrade its genetic material in the extracellular environment of the target cell. Many studies on gene-based therapies to treat animal haemophilia has been conducted. Various methods and vectors that are currently employed in treating haemophilia are briefly explained below.

VECTORS USED FOR DELIVERY THERAPEUTIC GENE FOR HAEMOPHILIA TREATMENT:

1. ADENO- ASSOCIATED VIRAL VECTOR:

Vectors are biological vehicles used for the delivery of the therapeutic gene into the host organism. The organism that is derived from the adeno-associated virus is one of the promising vectors used for gene-based therapy of Haemophilia. Moreover, the induced expression of the therapeutic gene by gene therapy is long-term, in absence of the expression of the viral genes and are capable to transduce non-dividing cells in-vivo, including in liver and muscle. The Adenovirus-associated viral vector is characterized for their single-stranded genetic material Deoxyribonucleic acid (DNA) that are incapable of replication. The added advantage is that adeno-associated viral vectors are non-pathogenic in nature, and this favour the use of AAV as a biological vector for haemophilia treatment for persistent gene-expression profiling studies. The therapeutic gene remains stable extra chromosomally organized as higher molecular weight concatemers. This, in turn, reduces the risk of insertional mutagenesis and ensures the transduced cell not to expand after haemophilia gene therapy. However, this does not rule out the existence of oncogene events after gene transfer regulated by Adenovirus-associated vectors[1](DonsantA.Li Y. Science -2007). The major drawback of employing adeno associated viral vectors is that the vector particles only pack genetic material DNA of certain size (~4.7 Kb). These restrictions in the sequence size of transgene cassette accommodation in the functional vector particle possess a major hindrance for therapeutic F VIII insertion. Various distinct stereotypes of Adenovirus-associate vector are been available [2r] (Gao G: viral-2004) but initially, haemophilia gene-therapy employed the use of vectors derived from adenovirus associated viral vector stereotype 2 which is the most prevalent. Adeno associated viral vector-based gene therapy is considered to be a promising and near future technology, as clinical success has already been achieved in patients with congenital blindness[3](Bainbridge; Med-2008).

2.MUSCLE DIRECTED GENE TRANSFER:

Adenovirus-associated viral vector-mediated haemophilia B gene-therapy was first targeted at skeletal muscles[4](High KA-2011). Which does not directly express the clotting factors- F VIII or FIX. Muscle cells are capable of undergoing post-translational modification of protein polypeptides to generate the functional transgene product. However, modification like proteolytic cleavage and glycosylation are not as effective a*s in h*epatocytes[5] (Hertzog et al). Static expression of the therapeutic clotting factor in muscle-directed gene-therapy has been demonstrated in haemophilia B dogs. The mutant trans-gene present in Factor IX gene as great impact on influencing the risk of inhibitor formation. This Mutation can be hindered by reducing the vector does per site and to avoid multiple localized intramuscular injection. Adeno associated viral FIX vector is directed into skeletal muscle via an intra-vascular pathway. Muscle- directed gene delivery of this adenoviral vector in haemophilia in dogs in resulted via an intravascular pathway. Muscle- directed gene delivery of this adeno associated viral vector in haemophilia in dogs resulted in increasing the Factor IX

activity up to eight to nine hold with phenotype correction. Muscle targeted adeno associated viral vector gene therapy is considered to be promising near future strategy to delivery therapeutic gene in haemophilia B patients[6].

3.HEPATOCYTE -DIRECTED GENE THERAPY:

An alternate attractive approach for adeno-associated viral vector gene therapy in treating haemophilia is liver mediated gene transfer. Various preclinical experimental studies have been conducted with Murine and Canine haemophilia models or Non- human primates. The result of these experimental studies revival that these liver-mediated gene therapy using adeno associated viral vector are capable to produce steady and persistent therapeutic expression leading to partial or complete phenotype correction[7-13]. Further, the transducing liver cells induce the regulatory Thymus cells, which in turn induces resistance to the FIX antigen[14]. This method of gene therapy test performed in null mutated haemophilia B dogs resulted in a complete correction without any sort of inhibitory antibodies development[15].Further modified therapy using Codon- optimized FIX, has more advantages; stronger promotor, an enhancer element, double-stranded adeno- associated viral vector (SCAAVS)[16-19]. Recent studies conducted on mice reported that the use of various stereotypes of adenoviral vector namely AAV8 and AAV9 in hepatocyte-directed gene therapy has more efficiency than theuse of Lentiviral vectors and has been found to reduce the risk of inflammation[20.] Delivery by using Adenoviral associated viral vector for Factor IX by hepatic artery catheterization resulted in maximum transient therapeutic FIX of 12% normal[21]. Regrettably, Adenoviral associate vector capsid – antigens were presented to thymus lymphocytes by transduced hepatocytes[22-23]. Thymus cell-mediated rejection of transduced hepatic liver cell lines was prevented by incorporation of motif that prevents proteasome-mediated processing[25]. This concept was confirmed independently by Martino et.al[26]

4.LENTIVIRAL VECTOR:

Lentiviral vectors are biological vehicles derived from primates (human immunodeficiency virus, simian immunodeficiency virus) and non-mammalian primate lentiviruses (Feline immunodeficiency virus, Equine infectious anemia virus). These viral vector compounds consist of trans-gene of interest along with cis-acting element that are essential for steady-state tranduction[29]. Early studies conducted on animals suggested that the lentiviral vectors are toxic to liver hepatocytes and showed that the hepatoxicity of the lentiviral vector was restricted and transient [30-33]. Integration of cis-acting sequence from the human immunodeficiency virus polymerase 1 gene (Central purine tract) into the biological delivery system- lentiviral vector is facilitated by intranuclear transport of the lentiviral preintegration complex[30,33-35]. The full potential of a lentiviral vector for haemophilia gene therapy can be achieved by further improving the vector design, and by augmentation of specificity and efficiency of gene transfer into liver cells – hepatocytes.

5.STEM CELL BASED- GENE TRANSFER:

The stem cells can be used for in-vivo genetic alterations, retroviral vectors and lentiviral vector can be employed for the administration of gene ex-vivo into stem cells[34-35]. Human stem cells are pluripotent in nature, they are capable of self- regeneration and to differentiate into any kind of cells. These unique properties of stem cells make it as a target cell for gene therapy in haemophilia. Stem cells are pluripotent in nature and can be modified genetically to improve its potential. Moreover, it is feasible to trigger immune hypo-responsiveness against the gene therapy product. Thereby targeting stem cell, it is possible to synthesize blood clotting factors in the circulation. After advancement in methodologies, transplantation of bone marrow cells transduced by Factor VIII containing gamma- retroviral into haemophilia A mice resulted in prolonged phenotypic expression correction of the disorder[36, 37]. Prolonged expression of Factor VIII at therapeutic levels was observed in mice by using lentiviral transduced human stem cell with a hybridoma human–porcine Factor VIII transgene[38]. Haemophilia can be physio-chemically rectified by using lentiviral with transducing Hematopoietic Stem Cells containing Factor VIII or Factor IX specifically directed to platelets[39,40]. The challenges of human stem cell transplantation are that patients have to pre-biologically treated with conditioning regimens, these preconditioning regimens have some adverse effects and can cause server-side effects. Besides using human stem cells, epithelial progenitors can also be employed as targeting region for gene therapy, this usage further improves its therapeutic efficiency to a considerable extent[44]. But it still remains a challenge to generate endothelial progenitor that are able to engraft efficiency and persistence. This limitation can be overcome by potential development of endotheliotropic lentiviral vectors provided that high – titer vector can be manufactured[45].

APPROACH CONSIDERATIONS:

Comprehensive haemophilia care centre should be ideally provided to treat haemophilia patients. The treatment of haemophilia involves treatment, protection, management of bleeding episodes, prophylaxis of

Factor VIII (F VIII) inhibitors and restoration of haemophilia inflammation of synovial membrane. The method of using factor replaced product and other medications including pain killer medication is typically required. Depending on the bleeding event, the patients are treated with prophylaxis or intermittent, on-demand therapy. It has been reported that early preventive therapy conducted on six randomized controlled trials, has considerably reduced the total bleeds; resulting in complete joint dystrophy and improved quality of life compared to that of on-demand treatment. In developed countries, with accesses to genetically modified recombinant products; pre and post-treatment are primary and the therapy is started as early as at the age of one in haemophilia infants, and continuous up to adolescence period. These are the early approaches of treatment of haemophilia eliminates the overall use of blood clotting factors and in great extent reduces fatality. Treatment of patients with inhibitors of factor VIII is difficult. High doses of factor VIII are used to overcome the low-titer inhibitors (concentration below five Bethesda unit)- bleeding disorder. Another method of approach involves treatment of haemophilia patients by injection of radioactive isotope into the joints to ablate the synovium. The method of using radioisotopes as a therapeutic indication is called radio synovectomy. This method of radio synovectomy can be employed to reduce internal bleeding, slow progression of cartilage and bone damage, and prevent arthropathy.

Unresponsive cases may require arthroscopic synovectomy or arthroplasty. In severe case of Haemophilia patients, increasing evidence shows that haemophilia is associated with low- mineral density in bones. In such severe cases of haemophilia continuous examination, assessment and treatment of fracture risk are highly essential. Proper physical exercise, accidental fall prevention strategies and optimization of calcium content, vitamin D consumption are suggested, along with prophylactic factor replacement therapy in severe haemophilia. In patients with a severe haemophilia; gene therapy may reduce the symptoms to those that a mild or moderate person with haemophilia might have[26]. In 2017 a gene therapy trial on nine people with haemophilia A reported that high doses did better than low doses[27-28]. Giangrande advocates an approach in which gene therapy is targeted for conditions for which there is currently no effective treatment, like muscular dystrophy and cystic fibrosis. This method therapies are not currently accepted for the treatment for haemophilia due to logical and ethical issues.

FUTURE PERSPECTIVES OF GENE THERAPY FOR HAEMOPHILIA:

There has been a tremendous progression in gene-based therapy during the last decade. The realization of the potential of the immune response to the therapeutic trans-genes in addition to the biological vectors is now well appreciated. This realization of potential is not only essential for treating haemophilia but also for other monogenetic disorders. Thereby by using the combined treatment involving novel vectors and mitochondrial antisense Ribonucleic Acid technology, it is possible to modulate the immune response both to the transgene and vector epitopes bode in near future. It still remains a challenge to reverse ongoing immune response, the understanding of specific B and Thymus cell recognition and regulation by Thymus regulatory cells suggest that this hurdle can be overcome the immune response.

CONCLUSION:

There has been a consistent improvement in employing gene therapy to treat haemophilia affected animals. Novel approaches for haemophilia A in mice include induction of blood clotting Factor VIII in blood cells or platelets derived from ex-vivo transduced hematopoietic stem cells, or in-vivo transfer of transposable elements of DNA (that can change its position within the genome) expressing Factor VIII into endothelial cells or hepatocytes. Improvement in large animal sample include the demonstration that infant administration of a retroviral vector expressing canine Factor VIII completely corrected[45]. Haemophilia A in dogs, the expression of Factor IX of 28-fold is obtained by using single-stranded adeno-associated viral vector. In humans, one haemophilia B patient achieved 10 % of normal activity after liver-directed gene therapy with a single-stranded Adeno–associated virus vector expressing human Factor IX.

SUMMARY

Though the gene therapy has been accomplishable and promising for treating patients with haemophilia B, the expression of the induced therapeutic gene was less stable due to an immune response produced the body. Revoking and neutralizing immune response against the therapeutic gene is the next major obstacle for accomplishing prolonged stable expression of therapeutic drug by gene therapy. Advancement and development in different combination fields, strategies may likely to bring a permanent cure for haemophilia one step closer to reality.

ACKNOWLEDGEMENT

REFERENCES:

1. Donsante A, Miller DG, Li Y, Vogler C, Brunt EM, Russell DW, Sands MS. AAV vector integration sites in mouse hepatocellular carcinoma. Science 2007; 317: 477.
2. Gao G, Vandenberghe LH, Alvira MR, Lu Y, Calcedo R, Zhou X, Wilson JM. Clades of adeno-associated viruses are widely disseminated in human tissues. J Virol 2004; 78: 6381–8.
3. Ainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, Petersen-Jones S, Bhattacharya SS, Thrasher AJ, Fitzke FW, Carter BJ, Rubin GS, Moore AT, Ali RR. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med 2008; 358: 2231–9.
4. High KA. Gene therapy for haemophilia: a long and winding road. J ThrombHaemost 2011; 9(Suppl. 1): 2–11.
5. Herzog RW, Yang EY, Couto LB, Hagstrom JN, Elwell D, Fields PA, Burton M, Bellinger DA, Read MS, Brinkhous KM, Podsakoff GM, Nichols TC, Kurtzman GJ, High KA. Long-term correction of canine haemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. Nat Med 1999; 5: 56–63.
6. Finn JD, Nichols TC, Svoronos N, Merricks EP, Bellenger DA, Zhou S, Simioni P, High KA, Arruda VR. The efficacy and the risk of immunogenicity of FIX Padua (R338L) in haemophilia B dogs treated by AAV muscle gene therapy. Blood 2012; 120: 4521–3.
7. Arruda VR, Stedman HH, Haurigot V, Buchlis G, Baila S, Favaro P, Chen Y, Franck HG, Zhou S, Wright JF, Couto LB, Jiang H, Pierce GF, Bellinger DA, Mingozzi F, Nichols TC,High KA. Peripheral transvenular delivery of adeno-associated viral vectors to skeletal muscle as a novel therapy for haemophilia B. Blood 2010; 115: 4678–88.
8. Mount JD, Herzog RW, Tillson DM, Goodman SA, Robinson N, McCleland ML, Bellinger D, Nichols TC, Arruda VR, Lothrop CD Jr, High KA. Sustained phenotypic correction of haemophilia B dogs with a factor IX null mutation by liver-directed gene therapy. Blood 2002; 99: 2670–6.
9. Nathwani AC, Davidoff AM, Hanawa H, Hu Y, Hoffer FA, Nikanorov A, Slaughter C, Ng CY, Zhou J, Lozier JN, Mandrell TD, Vanin EF, Nienhuis AW. Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno-associated virus encoding the hFIX gene in rhesus macaques. Blood 2002; 100: 1662–9.
10. Snyder RO, Miao C, Meuse L, Tubb J, Donahue BA, Lin HF, Stafford DW, Patel S, Thompson AR, Nichols T, Read MS, Bellinger DA, Brinkhous KM, Kay MA. Correction of haemophilia B in canine and murine models using recombinant adeno-associated viral vectors. Nat Med 1999; 5: 64–70
11. Snyder RO, Miao CH, Patijn GA, Spratt SK, Danos O, Nagy D, Gown AM, Winther B, Meuse L, Cohen LK, Thompson AR, Kay MA. Persistent and therapeutic concentrations of human factor IX in mice after hepatic gene transfer of recombinant AAV vectors. Nat Genet 1997; 16: 270–6.
12. Wang L, Nichols TC, Read MS, Bellinger DA, Verma IM. Sustained expression of therapeutic level of factor IX in haemophilia B dogs by AAV-mediated gene therapy in liver.MolTher 2000; 1: 154–8.
13. Wang L, Takabe K, Bidlingmaier SM, Ill CR, Verma IM. Sustained correction of bleeding disorder in haemophilia B mice by gene therapy.ProcNatlAcadSci USA 1999; 96: 3906–10.
14. Mingozzi F, Liu YL, Dobrzynski E, Kaufhold A, Liu JH, Wang Y, Arruda VR, High KA, Herzog RW. Induction of immune tolerance to coagulation factor IX antigen by in vivo hepatic gene transfer. J Clin Invest 2003; 111: 1347–56.
15. Mount JD, Herzog RW, Tillson DM, Goodman SA, Robinson N, McCleland ML, Bellinger D, Nichols TC, Arruda VR, Lothrop CD Jr, High KA. Sustained phenotypic correction of haemophilia B dogs with a factor IX null mutation by liver-directed gene therapy. Blood 2002; 99: 2670–6.
16. McCarty DM, Fu H, Monahan PE, Toulson CE, Naik P, Samulski RJ. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. Gene Ther 2003; 10: 2112–18.
17. McCarty DM, Monahan PE, Samulski RJ. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene Ther 2001; 8: 1248–54.
18. Nathwani AC, Gray JT, Ng CY, Zhou J, Spence Y, Waddington SN, Tuddenham EG, Kemball-Cook G, McIntosh J, Boon-Spijker M, Mertens K, Davidoff AM. Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. Blood 2006; 107: 2653–61.
19. Wu Z, Sun J, Zhang T, Yin C, Yin F, van Dyke T, Samulski RJ, Monahan PE. Optimization of self-complementary AAV vectors for liver-directed expression results in sustained correction of haemophilia B at low vector dose. MolTher 2008; 16: 280–9.
20. VandenDriessche T, Thorrez L, Acosta-Sanchez A, Petrus I, Wang L, Ma L, De Waele L, Iwasaki Y, Gillijns V, Wilson JM, Collen D, Chuah MK. Efficacy and safety of adeno-associated viral vectors based on serotype 8 and 9 vs. lentiviral vectors for haemophilia B gene therapy. J ThrombHaemost 2007; 5: 16–24.
21. Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, Tai SJ, Herzog RW, Arruda V, Johnson F, Scallan C, Skarsgard E, Flake AW, High KA. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. Nat Genet 2000; 24: 257–61.
22. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, et al. Successful

transduction of liver in haemophilia by AAV-factor IX and limitations imposed by the host immune response. Nat Med 2006; 12: 342–7

23. .Mingozzi F, Maus MV, Hui DJ, Sabatino DE, Murphy SL, Rasko JE, Ragni MV, Manno CS, Sommer J, Jiang H, Pierce GF, Ertl HC, High KA. CD8(+) T-cell responses to adeno-associated virus capsid in humans. Nat Med 2007; 13: 419–22.

24. VandenDriessche T, Mingozzi F, Nair NNN, High K, Chuah MKL. Solving the T-cell problem: generation of immune stealth AAV. Hum Gene Ther 2012; 23: A23.

25. Martino AT, Basner-Tschakarjan E, Markusic DM, Finn JD, Hinderer C, Zhou S, Ostrov DA, Srivastava A, Ertl HC, Terhorst C, High KA, Mingozzi F, Herzog RW. Engineered AAV vector minimizes in vivo targeting of transduced hepatocytes by capsid-specific CD8+ T cells. Blood 2013; 121: 2224–33.

26. Peyvandi, F; Garagiola, I; Young, G (9 July 2016). "The past and future of haemophiliadiagnosis, treatments, and its complications".Lancet. 388 (10040): 187–97.

27. Rangarajan, Savita; Walsh, Liron; Lester, Will; Perry, David; Madan, Bella; Laffan, Michael; Yu, Hua; Vettermann, Christian; Pierce, Glenn F. (2017-12-09). "AAV5–Factor VIII Gene Transfer in Severe Haemophilia A". New England Journal of Medicine

28. van den Berg, H. Marijke (2017-12-09). "A Cure for Haemophilia within Reach".New England Journal of Medicine.

29. Follenzi A, Ailles LE, Bakovic S, Geuna M, Naldini L. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. Nat Genet 2000; 25: 217–22.

30. Pfeifer A, Kessler T, Yang M, Baranov E, Kootstra N, Cheresh DA, Hoffman RM, Verma IM. Transduction of liver cells by lentiviral vectors: analysis in living animals by fluorescence imaging. MolTher 2001; 3: 319–22.

31. VandenDriessche T, Thorrez L, Naldini L, Follenzi A, Moons L, Berneman Z, Collen D, Chuah MK. Lentiviral vectors containing the human immunodeficiency virus type-1 central polypurine tract can efficiently transduce nondividing hepatocytes and antigen-presenting cells in vivo. Blood 2002; 100: 813–22.

32. Park F, Kay MA. Modified HIV-1 based lentiviral vectors have an effect on viral transduction efficiency and gene expression in vitro and in vivo. MolTher 2001; 4: 164–73.

33. Follenzi A, Sabatino G, Lombardo A, Boccaccio C, Naldini L. Efficient gene delivery and targeted expression to hepatocytes in vivo by improved lentiviral vectors. Hum Gene Ther 2002; 13: 243–60.

34. Matrai J, Chuah MK, VandenDriessche T. Preclinical and clinical progress in haemophilia gene therapy. CurrOpinHematol 2010; 17: 387–92.

35. Matrai J, Chuah MK, VandenDriessche T. Recent advances in lentiviral vector development and applications. MolTher 2010; 18: 477–90.

36. Moayeri M, Ramezani A, Morgan RA, Hawley TS, Hawley RG. Sustained phenotypic correction of haemophilia A mice following oncoretroviral-mediated expression of a bioengineered human factor VIII gene in long-term hematopoietic repopulating cells. MolTher 2004; 10: 892–902.

37. Ramezani A, Hawley RG. Correction of murine haemophilia A following nonmyeloablative transplantation of hematopoietic stem cells engineered to encode an enhanced human factor VIII variant using a safety-augmented retroviral vector. Blood 2009; 114: 526–34.

38. Ramezani A, Hawley RG. Correction of murine haemophilia A following nonmyeloablative transplantation of hematopoietic stem cells engineered to encode an enhanced human factor VIII variant using a safety-augmented retroviral vector. Blood 2009; 114: 526–34.

39. Shi Q, Wilcox DA, Fahs SA, Fang J, Johnson BD, Du LM, Desai D, Montgomery RR. Lentivirus-mediated platelet-derived factor VIII gene therapy in murine haemophilia A. J ThrombHaemost 2007; 5: 352–61.

40. Montgomery RR, Shi Q. Platelet and endothelial expression of clotting factors for the treatment of haemophilia. Thromb Res 2012; 129(Suppl. 2): S46–8.

41. Shi Q, Wilcox DA, Fahs SA, Weiler H, Wells CW, Cooley BC, Desai D, Morateck PA, Gorski J, Montgomery RR. Factor VIII ectopically targeted to platelets is therapeutic in haemophilia A with high-titer inhibitory antibodies. J Clin Invest 2006; 116: 1974–82.

42. Gao G, Vandenberghe LH, Alvira MR, Lu Y, Calcedo R, Zhou X, Wilson JM. Clades of adeno-associated viruses are widely disseminated in human tissues. J Virol 2004; 78: 6381–8.

43. Matsui H, Shibata M, Brown B, Labelle A, Hegadorn C, Andrews C, Hebbel RP, Galipeau J, Hough C, Lillicrap D. Ex vivo gene therapy for haemophilia A that enhances safe delivery and sustained in vivo factor VIII expression from lentivirally engineered endothelial progenitors. Stem Cells 2007; 25: 2660–9.

44. Anliker B, Abel T, Kneissl S, Hlavaty J, Caputi A, Brynza J, Schneider IC, Munch RC, Petznek H, Kontermann RE, Koehl U, Johnston IC, Keinanen K, Muller UC, Hohenadl C, Monyer H, Cichutek K, Buchholz CJ. Specific gene transfer to neurons, endothelial cells and hematopoietic progenitors with lentiviral vectors. Nat Methods 2010; 7: 929–35.

45. Brown BD, Cantore A, Annoni A, Sergi LS, Lombardo A, Della Valle P, D'Angelo A, Naldini L. A microRNA-regulated lentiviral vector mediates stable correction of haemophilia B mice. Blood. 2007 Dec 15;110(13):4144-52.