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A REVIEW ON NEW DEVELOPMENT IN HAEMOPHILIA GENE THERAPY

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ABSTRACT :

Hemophilia is an X-linked inherited bleeding disorder, resulting from defects in the F8 (hemophilia A) or F9 (hemophilia B) genes. Persons with hemophilia have bleeding episodes into the soft tissues and joints, which are treated with self-infusion of factor VIII or IX concentrates. Hemophilia provides an attractive target for gene therapy studies, due to the monogenic nature of these disorders and easily measurable endpoints (factor levels and bleed rates). All successful, pre-clinical and clinical studies to date have utilized recombinant adeno associated viral (AAV) vectors for factor VIII or IX hepatocyte transduction. Recent clinical data have presented normalization of factor levels in some patients with improvements in bleed rate and quality of life. The main toxicity seen within these studies has been early transient elevation in liver enzymes, with variable effect on transgene expression. Although long-term data are awaited, durable expression has been seen within the hemophilia dog model with no late-toxicity or ontogenesis. There are a number of phase III studies currently recruiting; however, there may be some limitations in translating these data to clinical practice, due to inclusion/exclusion criteria. AAV-based gene therapy is one of a number of novel approaches for treatment of hemophilia with other gene therapy (in vivo and ex vivo) and non-replacement therapies progressing through clinical trials. Availability of these high-cost novel therapeutics will require evolution of both clinical and financial healthcare services to allow equitable personalization of care for persons with hemophilia.

INTRODUCTION :

Since the clotting factor genes were among the first to be cloned in the early 1980s, the haemophilia quickly became candidates for somatic cell gene therapy since they are recessive characteristics. To produce sustained expression of therapeutically appropriate amounts of factor VIII (FVIII) and factor IX (FIX) in animal models of hemophilia, several

approaches have been employed during the last three decades. Indeed, a variety of gene transfer techniques have been successful in providing a long-term "cure" for hemophilia A and B in mice and, to a lesser extent, in big mammals. But until recently, comparable achievements in human illness have not been reported. Several important facets of the science of hemophilia gene therapy will be covered in this State-of-the-Art overview. Initially, with the recent Given the effectiveness of gene therapy in a very small number of individuals with hemophilia, what obstacles stand in the way of this therapy's broader use? Second, what unique problems does FVIII gene transfer present? Lastly, what chances are offered by a different strategy for gene transfer that uses lent viral vectors

Prevalence of Haemophilia in the EU

The World Federation of Haemophilia (WFH) 2018 survey was used to determine the prevalence of people with haemophilia A and B in Europe. Literature data for countries that were not included were also used (Table 1). For all severities of hemophilia A, a recent meta-analysis [1] estimated 2.46 instances per 10,000 men, and for all severities of hemophilia B, an estimate of 0.5 cases per 10,000 males. Although these estimations are larger than those previously mentioned, definitions still classify hemophilia as a rare disease. The World Federation of Haemophilia (WFH) 2018 survey was used to determine the prevalence of people with haemophilia A and B in Europe. Literature data for countries that were not included were also included (Table 1).

An estimate of 2.46 instances per 10,000 men for all severities of hemophilia A and 0.5 cases per 10,000 males for all severities of hemophilia B was put up by a recent meta-analysis7. Despite being higher than previously reported estimates, the EU's criteria of rare illnesses (less than 5 cases per 10,000 people; Regulation [EC] No. 141/2000 of the European Parliament and of the Council) nevertheless classify hemophilia as such.

TABLE 1 Haemophilia A and B prevalence in European countries

Country	Population	People with haemophilia A	Prevalence (per 10 000)	People with haemophilia B	Prevalence (per 10 000)
Albania	2 876 101	161	0.56	33	0.11
Austria	8 747 358	658	0.75	117	0.13
Belgium	11 348 159	970	0.85	242	0.21
Bulgaria	6 981 642	560	0.80	68	0.10
Cyprus	1 172 458	43	0.37	-	-
Czech Republic	10 561 633	937	0.89	136	0.13
Denmark	5 731 118	410	0.72	102	0.18
Estonia	1 316 481	97	0.74	10	0.08
Finland	5 495 096	150	0.27	33	0.06
France (metropolitan)	66 896 109	5864	0.88	1498	0.22
Germany	82 667 685	3686	0.45	628	0.08
Greece	10 746 740	873	0.81	184	0.17
Hungary	9 817 958	893	0.91	230	0.23
Ireland	4 773 095	617	1.38	243	0.51
Italy	61 680 122	3992	0.65	886	0.14
Latvia	1 960 424	129	0.66	21	0.11
Lithuania	2 872 298	147	0.51	24	0.08
Netherlands	16 877 351	1026	0.61	125	0.07
Norway	5 232 929	325	0.62	90	0.17
Poland	37 948 016	2413	0.64	428	0.11
Portugal	10 324 611	539	0.52	112	0.11
Romania	19 705 301	1615	0.82	210	0.11
Slovakia	5 428 704	521	1.00	79	0.15
Slovenia	2 064 845	207	1.00	30	0.15
Spain	47 042 984	1679	0.36	-	-
Sweden	9 798 871	860	0.88	195	0.20
United Kingdom	65 637 239	6559	1.00	1518	0.23

Gene therapy for hemophilia :

Gene therapy is the process of altering and transferring genetic material to make up for improperly altered genes. By causing the transplanted gene to express at therapeutic levels over an extended period of time, gene therapy seeks to treat or even prevent hereditary illnesses . As a genetically well-understood hereditary illness, hemophilia is a prime candidate for gene therapy. Furthermore, precise control is not required because the coagulation factors' plasma levels have little effect on the severity of the bleeding phenotype. The limited therapeutic half-life of the currently known therapies is their biggest drawback, necessitating repeated intravenous infusions and considerable attempts to create more potent gene therapy techniques [2] Expert Comments on Biological Therapy 1101 The majority of gene therapy techniques used today always use two types of vectors. While lentivirus vectors are used for outside-the-cell gene transfer into hematopoietic and other stem cells, adeno-associated viral (AAV) vectors are commonly used for in body gene transfer into post mitotic cells. [34] Clinical investigations using lent vectors for viruses have not yet started because of the difficulty in producing the volume of vector needed for in vivo injection [4](wt) the

wild form AAV, a bitsy, single-stranded DNA infection belonging to the Parvovirus family, is nonpathogenic and replication-imperfect, which means it cannot cause disease. Current clinical research on gene therapy for hemophilia uses recombinant Viral (rAAV virus) vectors to directly carry clotting factor genes into liver cells. The transgenic product is subsequently produced and released into the circulation by these hepatocytes once they have changed into protein bio factories. Once the target cell has been transduced, the rAAV therapeutic sequences of genes are mostly found as concatemeric episomes that have little integration into the host's genomic DNA.

Gene therapy for haemophilia A

The ultimate objective of gene therapy for hemophilia is to give those who have the disorder a safe and long-lasting remedy, reducing the likelihood of bleeding incidents and the expense of hemophilia treatment. Even though hemophilia gene therapy has advanced significantly in recent years, there are still a number of problems that need to be resolved. The initial attempts at clinical trials for hemophilia gene therapy were carried out between the 1990s and the beginning of the 2000s. A number of tactics were investigated, including three fruitless hemophilia A trials: Autologous fibroblasts transfected ex vivo without the use of viruses .[5] intravascular delivery of an adenovirus vector and an intravenous retroviral vector. The most encouraging outcomes, however, came from two studies for hemophilia B that used an adeno-associated viral (AAV) vector and were first given to the muscle and then to the liver. These studies established the groundwork for later clinical trials on hemophilia that concentrated on liver-directed AAV carrier gene therapy

Gene therapy for haemophilia B

The safety and efficacy of liver-targeted adeno-associated virus (AAV) vector gene therapy has been studied in recent clinical studies for delivering a functional copy of the F9 gene to hepatocytes in order to synthesize the therapeutic protein. The gene that produces FIX is significantly smaller than the F8 gene, which affects their secretion and plasma concentration levels, and it is primarily synthesized in hepatocytes and not in liver sinusoidal endothelial cells (LSECs), such as FVIII. The selection process for these trials is similar to that for haemophilia A, but oddly, some trials have included moderate haemophilia patients, and a few have accepted patients who already have neutralizing antibodies against AAV, which are normally excluded. Early clinical trials in haemophilia B gene therapy used either human wild-type or codon-optimized FIX . In 2011, the first successful clinical experiment showed that intravenous treatment of scAAV2/8-LP1-hFIXco gene therapy increased FIX levels in a dose-dependent manner. [3]

Hemophilia Gene Therapy Awareness :

The purpose of the CHC-defined patient journey and Q&A resource presented here is to assist HCPs in understanding the concerns and questions that PWH are likely to have at various periods of life as well as when talking about gene therapy choices. New treatment alternatives, mounting evidence, and changing personal preferences, objectives, goals, and risk tolerance will all be encountered along the way for each patient. This tool will assist in filling in the knowledge gaps of PWH and HCPs by identifying and offering essential information to support these gene therapy talks. Only 6% of PWH believe they have an advanced comprehension of gene therapy, according to a recent global study distributed to patient groups [6] As gene therapy emerges, it will be critical to meet the knowledge gaps of HCPs and PWH. Edu, even though the majority (68%) say they have a basic understanding . For HCPs, a similar trend was seen: just 12% reported an advanced knowledge while 44% recorded a basic or advanced knowledge [7] With 40% of doctors directly in hemophilia treatment claiming limited ability to discuss gene therapy with their patients showing physicians, nurses, and other health care teams who care for prematurely pregnant women will better enable them to meet the needs of their patients through helpful and informative discussions. This is consistent with results of another international survey of doctors and scientists. [6] While most PWH respondents have good impressions of gene therapy in relation to such issues as yearly bleeding rates, factor levels, long-term effects, utilization of factor replacement, and treatment expenses [8] Many feel that new products—such as gene therapy and other factor/non-factor replacement treatments—would not help them. HCPs will thus have to inform PWH about all potential treatment choices as they include them in shared decision-making.

Gene therapy as a treatment modality for hemophilia

The discovery of a genetic therapy for hemophilia has advanced significantly over the last decade, but these developments have also presented new challenges. Therefore, it is not entirely surprising that the biotechnology and pharmaceutical industries have ceased to participate in gene therapy clinical trials for this disease, at least for the present. The very small number of hemophiliac patients and the high cost of conducting such trials have surely contributed to this tendency. Nonetheless, there is strong evidence to suggest that the biomedical and general hemophilia communities must successfully adopt gene therapy treatments for hemophilia. The Hemophiliacs have participated in talks on the viability of gene therapy approaches to treat hemophilia for more than ten years. It may be argued that the success of this treatment strategy is even more scientifically required. How likely is it that gene therapy will be effective in treating more complicated genetic disorders if it is unsuccessful in this exceptional example of a straightforward monogenic disorder Lastly, there are unmistakable ethical requirements that motivate the achievement of this objective. Since 80% of hemophilia suffer reside in poor nations and do not currently have access to an effective treatment for this curable condition, success in this case must be determined by the creation of an affordable treatment option. Prophylactic or immediately therapy substitution using either plasma-derived or recombinant coagulation factor concentrate are the current therapeutic options for treating hemophiliacs in the industrialized world. Because clotting factor prophylaxis is more successful at avoiding the development of chronic hemophilic arthropathy, it is currently advised for patients who are severely afflicted. Despite their higher cost and the remarkable safety profiles and hemostatic performance of the existing plasma-derived product, recombinant blood clotting factor concentrates have recently become more popular. This is caused, at least in part, by the perception that plasma-derived factor concentrates carry a danger of spreading unique blood-borne infections. According to the current standard of care for hemophilia, a patient with severe hemophilia can now expect a nearly normal lifespan and the level of life, instead of a limited lifespan and crippling chronic arthropathy. Therefore, it would seem that the objective of using gene therapy to enhance hemophilia treatment has amazing hopes. However, there are still a number of restrictions on factor replacement treatment. In the end, only 20% of hemophilia patients worldwide have access to this level of therapy due to the high cost and restricted availability of factor concentrates Furthermore, factor concentrates have a short half-life, thus necessitating regulari.v.injections with their accompanying inconvenience. In a primary prophylaxis context, in very young children, this has resulted in the increasing use of central venous catheters with their associated complications of infection and line-related thrombosis [8]

Trial results on gene therapy for hemophilia A :

Another obstacle was the creation of a trial medication for hemophilia A gene therapy. The primary location of FVIII production in the liver is the sinusoidal endothelial cells, whereas hepatocellular are the physiological site of FIX synthesis. The B-domain of FVIII was omitted since the vector only functions with gene constructs of a specific size. In 2017, the findings of the first successful gene therapy study for hemophilia were released (BMN-270). Over the course of a year, six out of seven patients in the high-dose group demonstrated persistent normalization of factor VIII activity (mean, 93% ± 48), which resulted in hemostasis stability and a relatively significant decrease in yearly factor VIII usage from 5286 IU/kg to 65 IU/kg. An rise in alanine amino transfer to 1.5 times the upper limit of the usual range or below was the main adverse event. Over a period of up to six years, several articles showed that VIII expression was consistently dropping. There were no serious side effects, no patients ever returned to FVIII prophylaxis permanently, and their quality of lidecision-making

Trials results on gene therapy for hemophilia B :

In 2011, six individuals with hemophilia B showed the first positive outcomes of a trial on the intravenous delivery of an AVV-based gene therapy for hemophilia. All subjects showed dose-dependent expression of the factor IX transgene ranging from 2 to 11 IU/dL. Even after eight years, the dosage cohorts showed persistently elevated FIX activity in the range of 2–5% trial (AMT-060). In 10 hemophilia B patients, a mean increase in FIX activity of 4.4 IU/dL was achieved in the lower and of 6.9 IU/dL in the higher dose cohort Stable FIX levels were also recorded in both cohorts at 5 years [9] The discovery of the Padua variation of the FIX gene, which was first discovered in familial thrombophilia and has five to ten times greater activity, marked an additional step in the treatment of genes for hemophilia

B. Although the cofactor reliance, activation, and inactivation molecular regulation in this case is comparable to that of the FIX wild type, the increased rate of X factor activation causes hyperactivity and noticeably elevated factor level [10]

Current Gene Therapy Clinical Trials in Hemophilia :

The fact that the patient might have a therapeutic response without the circulating factor concentrations reaching normal levels is a crucial aspect of hemophilia therapy. In fact, lowering the risk of morbidity and death only requires a modest rise in plasma levels (to over 1% of the reference value). Since restoring circulating factor levels to 100% is not required, this lowers expectations for the goal factor correction. Some ongoing clinical studies on HA or HB that are listed in the clinical trials.gov repository served as the basis for the analysis in this article. and the Clinical Trials Register of the European Union. On the topic of hemophilia, there are now more than 40 ongoing gene therapy clinical studies, with HA and HB being split around 50/50. The recombinant viral vectors utilized in the various HA and HB clinical trials are listed in Table 1, and the most important clinical trials currently underway are included in Table 2. The Oxford University Hospitals NHS Foundation Trust is funding one of these clinical trials, which will examine the possible effects of gene therapy on the lives of hemophiliacs and their loved ones. [4] A multiple-cohort study will be carried out among various hemophiliac groups whose lives may have been affected by gene therapy. The purpose of the project is to enable patients and their households to use narrative narratives to share their own life stories. Following the implementation of the new treatments, the stories will provide a genuine exchange of experiences and provide insight into how these individuals and their families have managed hemophilia .

Novel therapeutics in hemophilia care:

Targeting natural anticoagulant pathways, FVIII mimic (bispecific antibodies), longer half-life FVIII/FIX concentrates, and gene therapy/editing are some of the novel therapies that are quickly developing to enhance care for people with hemophilia. By fusing FVIII/FIX recombinant concentrates to polyethylene glycol (PEG) (FVIII & FIX), IgG1-Fc (FVIII & FIX), or albumin (FIX), half-life extension has been achieved. Due to FVIII's need for von Wille brand factor (VWF) chaperoning, these changes have resulted in a considerable decrease in dosage for regularity in hemophilia B (B) patients (half-life: 82–102 h), with less pronounced improvements in hemophilia A patients (half-life: 14–19 h). As a result, recombinant FVIII-Fc linked to the VWF D'D3 binding domain and two XTEN linkers (BIVV001) has been developed. The EXTENA study's (NCT03205163) preliminary findings for this drug have reported half-life extensions to 38 (25 IU/kg) and 44 (65 IU/kg) hours, which might drastically alter the way concentrate prophylaxis is given to hemophilia A patients [11] Currently, all factor concentrate-based methods necessitate intravenous infusion, are ineffective for individuals on FVIII/FIX inhibitors, and cause alloantibody development in certain patients (about 30% in hemophilia A and 3% in hemophilia B). A new discovery that has generated a lot of enthusiasm in the hemophilia A community is Emicizumab, a bispecific monoclonal antibody that is injected subcutaneously and binds to FIX/IXa and Factor X/Xa, functioning as a partial functional mimic of FVIIIa. This agent's phase 3 clinical trials (HAVEN 1–4) have shown a significant decrease in bleeding rates in adults as well as kids. with and without inhibitors. However, further safety and effectiveness data must be provided through post-marketing studies/registries and "real-world" scenarios. Additionally, preclinical (protein S and activated protein C) and clinical (anti-thrombin and tissue factor pathway inhibitor) research are developing novel medicines that target natural anticoagulant proteins. [12] Even though there are many agents being studied, all of these methods will only, at most, improve the bleeding phenotype; in the case of breakthrough bleeding, severe trauma, or surgery, patients will still need traditional factor concentrate. [13]

Advanced Therapies in Hemophilia

Cell therapy, gene therapy, regenerative medicine, and tissue engineering are examples of novel and imaginative methods that make up advanced therapeutics. They target illnesses or situations for which there is presently no cure or whose treatment demands optimization. International medical organizations define advanced treatments as pharmaceuticals for use by humans that are derived from genes, tissues, or cells and that provide novel

approaches to the treatment of certain illnesses. [14] (Figure 1) Gene and cell therapy-based regimens have demonstrated significant promise in hemophilia. There are unquestionably successful cell and gene therapy strategies to treat both monogenic and polygenic disorders, as well as to extend the therapeutic proteins' useful duration and enhance their expression levels. The availability of a large variety of target cell types and transfection vectors, as well as the capacity to control the transgene's properties and gene expression, ultimately ensuring its safety, allow for this to be accomplished. .There are several severe and chronic conditions for which there is currently no treatment, and others for which the current treatment either has severe side effects or is time-consuming and/or difficult, making adherence challenging. For these reasons, investments in researching these new therapies are obviously justified. [15]

The intravenous injection of live autologous, allogenic, or xenogeneic non-germline the cells that have undergone minimal ex vivo processing or manipulation through selection, expansion, propagation, pharmacological treatment, or some other alteration of their biological properties is known as somatic cell therapy. Restoring a lost or nonexistent function in a live body is the aim of somatic therapy.

Typically, stem cells are employed in cell therapy because they possess a number of unique properties, including the capacity to self-renew, differentiate into distinct cell lines or embryonic layers, and remain undifferentiated. [16]

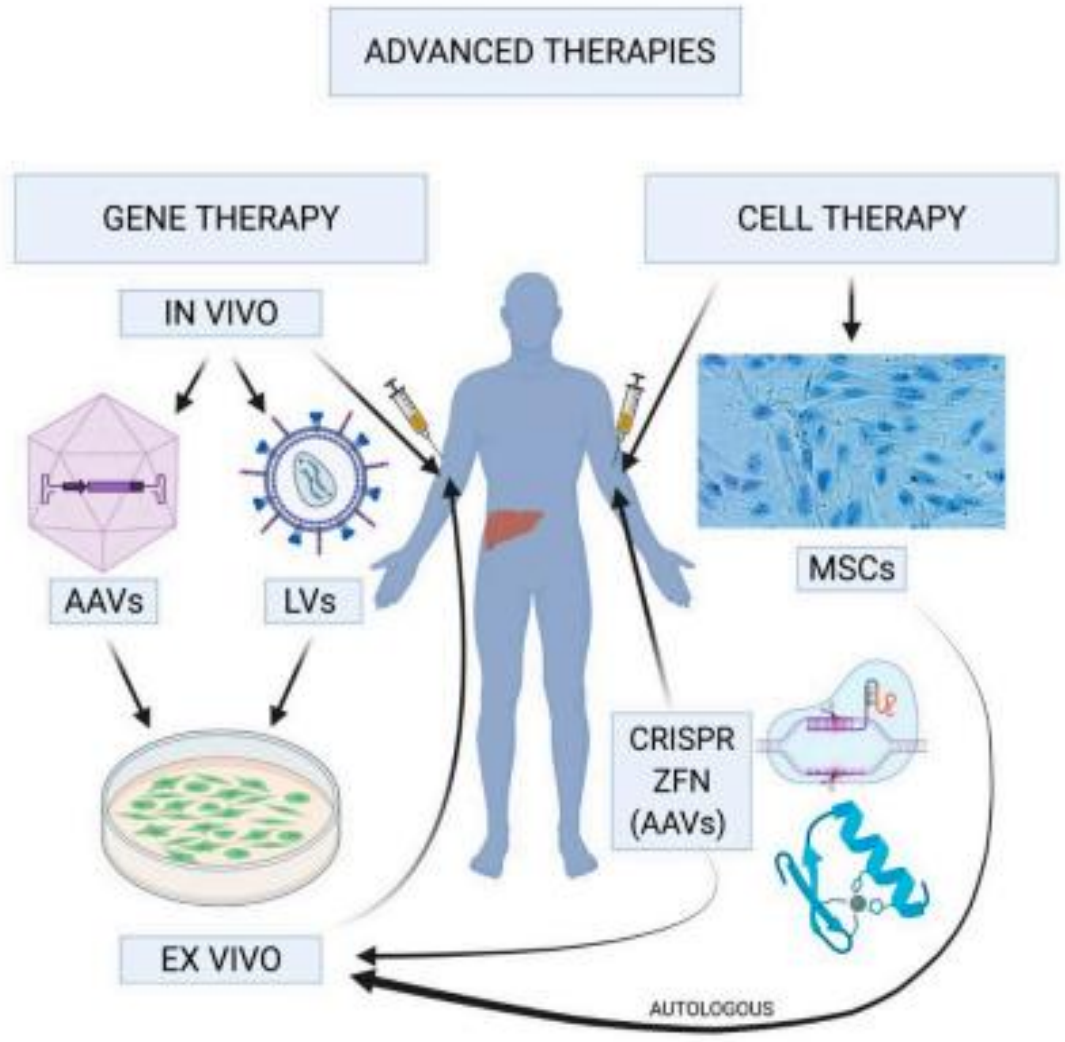

Figure 1. [17]

sophisticated therapeutic techniques. In vivo gene therapy involves systemically administering the "therapeutic gene"-carrying adeno-associated viral (AAV) or lent viral (LV) vectors, which are usually organ-specific. Self-produced or allogenic cells are transfected in vitro and then rein fused into the patient after growing in the culture medium in ex vivo gene therapy. [18] Stem cells or previously differentiated cells, whether transfected or not, are used in cell therapy to address a physiological function deficit in the patient. Correcting the faulty genes that cause the illness is an additional option. Numerous gene-editing methods, including CRISPR/Cas9 and ZFNs (zinc finger nucleases), are available to do so. Guide RNAs and other elements required for the correction are inserted using adeno-associated viral vectors. Mesenchymal stem cells, or MSCs. [17]

Table 2. Main clinical trials underway in the field of hemophilia A (HA) and hemophilia B (HB).

Title	NCT Number	Intervention	Sponsor
Gene therapy study in patients with severe HA with antibodies against AAV5 [a]	NCT03520712	Adeno-associated virus serotype 5 vector containing a B-deleted variant of FVIII [b] (valoctocogene roxaparvovec)	BioMarin Pharmaceutical
Gene therapy study in patients with severe HA	NCT02576795	Valoctocogene roxaparvovec-BMN 270	BioMarin Pharmaceutical
Study evaluating the efficacy and safety of valoctocogene roxaparvovec in patients with HA	NCT03370913	Valoctocogene roxaparvovec	BioMarin Pharmaceutical
Study evaluating the efficacy and safety of volactocogene roxaparvovec combined with prophylactic administration of corticosteroids in HA	NCT04323098	Valoctocogene roxaparvovec	BioMarin Pharmaceutical
Single-arm study evaluating the efficacy and safety of a dose of 4×10^{13} vg/kg of valoctocogene roxaparvovec in patients with HA	NCT03391974	Valoctocogene roxaparvovec	BioMarin Pharmaceutical
Gene therapy for HA	NCT03001830	New adeno-associated virus serotype 8 capsid vector pseudotype encoding FVIII-V3 (AAV2/8-HLP-FVIII-V3)	University College, London/Medical Research Council

Limitations and risks associated with gene therapy in hemophilia

The F8 transgene enclosed in a viral capsid is administered intravenously as part of gene therapy for hemophilia Because of the structure of the liver's capillaries, intravenous injection causes the transgene to be preferentially targeted to the hepatocyte. [19]Following recognition of the AAV capsid by the host cell's glycosylated receptors on the cell's surface, the virus is taken up via clathrin-mediated endocytosis and moved through the cytoskeleton. [20] To prevent lysosomal destruction and to facilitate its transport to the nucleus and subsequent uncoating via conformational modifications of pH-sensitive endosomolytic viral proteins, the AAV must exit the endosome at the ideal moment. [21] The rAAV genome's viral inverted terminal repeats cause either intramolecular or intermolecular recombination, also known as concatemerization, to create circularized genomes that endure in the nucleus as episomes. [22] The absence of replication of episomal AAV genomes during cell division is a significant drawback of the Av-based therapeutic gene therapy strategy. When employing this strategy, it's crucial to take into the possible loss of protein expression as well as the effects of liver expansion and transduced hepatocyte dilution in younger individuals. [2]

recent development in gene transfer for haemophilia :

The use of adenovirus vectors to achieve sustained expression both of Factor VIII and Factor IX is presently being investigated. Although some hepatotoxicity is still visible and may make it more difficult to translate the results Into clinical trials, improved liver-specific promoters and additional production method redesign led to long-term expression of Factor VIII in canine models of hemophilia A In particular, the safe use of adenoviral vectors for stable gene transfer in humans is challenging due to inter-subject variance and a modest therapeutic index. Adenoviral vectors are increasingly being employed as vaccine delivery vehicles due to their intrinsic immunogenicity it is more likely that new advancements in the realm of retroviral vectors will be used to treat hematological illnesses. The creation of lent viral vectors, which offer several benefits over first-generation retroviral vectors, has been one of the most significant developments. They are more suited for transduction of hepatocytes and hematopoietic stem cells, for instance, since they can transduce non-dividing cells. Second, lentivirus vectors exhibit a random integration pattern into the open-reading frames of genes, whereas retroviral vectors preferentially integrate their genomes close to transcriptional start sites [23]

Future Directions :

Ideally, the resource would be updated and published as needed to provide the latest relevant information and address any feedback from the community. This work would benefit from even wider stakeholder engagement including surveying a larger, more diverse group of PWH. It would incorporate findings of clinical trials and other research (such as from registries) that would be of potential relevance to decision-making (eg, probability of expressing FVIII, levels of FVIII expression, duration of expression, probability of side effects) and shared and reviewed with patients as it becomes available . [24] Additionally, although this resource offers a tool for education and decision support to facilitate conversations between healthcare professionals and patients, obstacles to delivering the necessary clinical and biological learning about gene therapy to healthcare professionals (such as time and travel expenses) should also be recognized and addressed. [4]

CONCLUSION :

An important turning point in the history of genetic medicine has been reached with the development of hemophilia gene therapy from a promising idea to a workable therapeutic strategy. Haemophilia, a genetic condition marked by poor blood coagulation, has historically presented significant difficulties for patients and medical professionals. In the past, treatment was based on routine infusions of the clotting factor focuses, which were successful in stopping bleeding episodes but

frequently failed to provide a long-term fix. A paradigm shift has occurred with the advent of gene therapy, which attempts to treat the underlying genetic reasons of the illness in order to not only manage it but maybe cure it. Adeno-associated virus (AAV) vector development is essential to the progress of hemophilia gene therapy. Targeting the liver, which is where coagulation factors are produced, these vectors work as delivery vehicles, delivering therapeutic genes into the patient's cells. Because AAV vectors may provide consistent, long-term expression of the target gene without triggering strong immune reactions, they are very appealing. With some patients reaching near normal levels of clotting factor in their circulation for prolonged periods of time, recent clinical trials employing these vectors have shown encouraging outcomes. These results highlight the potential for gene therapy to be a game-changing treatment for those with hemophilia. Nevertheless, a number of obstacles still exist in spite of these encouraging developments. The possibility of immunological reactions to the AAV vectors or the recently added clotting factor is one of the main worries. Some individuals' pre-existing immunity to AAV may reduce the therapy's efficacy. The use of immunosuppressive drugs and the creation of next-generation AAV vectors that are more adept at avoiding immune detection are two strategies that researchers are now investigating to lessen these immune reactions. Furthermore, because gene therapy is tailored, treatment must be carefully planned because patient variability might have a big influence on the results of the treatment.

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Reference:

1. Iorio A, Stonebraker JS, Chambost H, et al. Establishing the prevalence and prevalence at birth of hemophilia in males a meta-analytic approach using national registries. *Ann Intern Med*. 2019;171(8):542-546. doi:10.7326/M19-1208

2. Trinchero A, Sholzberg M, Matino D. The Evolution of Hemophilia Care: Clinical and Laboratory Advances, Opportunities, and Challenges. *Hamostaseologie*. 2020;40(3):311-321. doi:10.1055/a-1175-6530

3. Spencer HT, Riley BE, Doering CB. State of the art: gene therapy of haemophilia. *Haemophilia*. 2016;22:66-71. doi:10.1111/hae.13011

4. Pierce GF, Pasi KJ, Coffin D, et al. Towards a global multidisciplinary consensus framework on haemophilia gene therapy: Report of the 2nd World Federation of Haemophilia Gene Therapy Round Table. *Haemophilia*. 2020;26(3):443-449. doi:10.1111/hae.13971

5. Oth AAR, Awa IET, Oanne J, et al. The New England Journal of Medicine NONVIRAL TRANSFER OF THE GENE ENCODING COAGULATION FACTOR VIII IN PATIENTS WITH SEVERE HEMOPHILIA A. *N Engl J Med*. 2001;344(23):1735-1742. www.nejm.org

6. Peyvandi F, Lillicrap D, Mahlangu J, et al. Hemophilia gene therapy knowledge and perceptions: Results of an international survey. *Res Pract Thromb Haemost*. 2020;4(4):644-651. doi:10.1002/rth2.12326

7. Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for

Hemophilia A. *N Engl J Med*. 2020;382(1):29-40. doi:10.1056/nejmoa1908490

8. Iorio A, Skinner MW, Clearfield E, et al. Core outcome set for gene therapy in haemophilia: Results of the coreHEM multistakeholder project. *Haemophilia*. 2018;24(4):e167-e172. doi:10.1111/hae.13504

9. Leebeek FWG, Meijer K, Coppens M, et al. AMT-060 Gene Therapy in Adults with Severe or Moderate-Severe Hemophilia B Confirm Stable FIX Expression and Durable Reductions in Bleeding and Factor IX Consumption for up to 5 Years. *Blood*. 2020;136(Supplement 1):26-26. doi:10.1182/blood-2020-139225

10. Samelson-Jones BJ, Finn JD, George LA, Camire RM, Arruda VR. Hyperactivity of factor IX Padua (R338L) depends on factor VIIIa cofactor activity. *JCI Insight*. 2019;4(14):1-14. doi:10.1172/jci.insight.128683

11. Konkle BA, Shapiro A, Quon D, et al. BIVV001: The First Investigational Factor VIII Therapy to Break Through the VWF Ceiling in Hemophilia A, with Potential for Extended Protection for One Week or Longer. *Blood*. 2018;132(Supplement 1):636-636. doi:10.1182/blood-2018-99-113575

12. Weyand AC, Pipe SW. New therapies for hemophilia. *Blood*. 2019;133(5):389-398. doi:10.1182/blood-2018-08-872291

13. Dorle MD, Bhivasane A, Sanap GS. REVIEW : CUTANEOUS MUCORMYCOSIS. 2023;12(7):57-69. doi:10.20959/wjpr20237-27831

14. Shukla V, Seoane-Vazquez E, Fawaz S, Brown L, Rodriguez-Monguio R. The Landscape of Cellular and Gene Therapy Products: Authorization, Discontinuations, and Cost. *Hum Gene Ther Clin Dev*. 2019;30(3):102-113. doi:10.1089/humc.2018.201

15. Sanap G, Dama G, Hande A, et al. Preparation of transdermal monolithic systems of indapamide by solvent casting method and the use of vegetable oils as permeation enhancer. *Int J Green Pharm*. 2008;2(2):129. doi:10.4103/0973-8258.41189

16. Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. *Respiration*. 2013;85(1):3-10. doi:10.1159/000345615

17. Khan WS, Hardingham TE. Mesenchymal stem cells, sources of cells and differentiation potential. *J Stem Cells*. 2012;7(2):75-85.

18. Sanap GS, Mohanta GP. Development of miconazole nitrate controlled release formulations based on sln and NLC for topical delivery. *Int J Pharm Pharm Sci*. 2014;6(4):393-399.

19. Duan D. Systemic delivery of adeno-associated viral vectors. *Curr Opin Virol*. 2016;21:16-25. doi:10.1016/j.coviro.2016.07.006

20. Pawar S, Manikpuriya S, Sanap G. The phytochemical and pharmacological screening of Withania coagulans. 2023;16(October):109-117.

21. Lowell JA, Mah KM, Bixby JL, Lemmon VP. AAV8 transduction capacity is reduced by prior exposure to endosome-like pH conditions. *Neural Regen Res*. 2021;16(5):851-855. doi:10.4103/1673-5374.299272

22. 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. doi:10.1038/s41573-019-0012-9

23. Sanap GS, Mohanta GP. Solid lipid nanoparticles (SLN) -based hydrogel formulation for topical delivery of miconazole nitrate. *Int J Pharma Bio Sci*. 2013;4(2):21-33.

24. Kaczmarek R, Pierce GF, Noone D, O'Mahony B, Page D, Skinner MW. Eliminating Panglossian thinking in development of AAV therapeutics. *Mol Ther*. 2021;29(12):3325-3327. doi:10.1016/j.ymthe.2021.10.025