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Gene therapy in Hemophilia: Current Developments and Future Perspectives

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ABSTRACT
Hemophilia is a rare X-linked bleeding disorder caused by a deficiency of coagulation factors Factor VIII and Factor IX. Hemophilia can also be acquired due to autoimmune antibodies developed against factor VIII and factor IX. Management of acute bleeding mainly by coagulation factor replacement therapy is the core stone treatment of hemophilia. Development of antibodies(inhibitors) against FVIII or FIX concentrates is a major complication. Hemophilia replacement therapy requires extensive time period and constant monitoring of patients on factor levels, development of inhibitors,etc. Many researches and clinical trials are ongoing in order to develop a cost effective single dose therapeutic product which can offer a permanent recovery. The creation of gene therapy is a recent invention. In order to introduce a copy of the gene that codes for the clotting factor that is missing in people with hemophilia, modified viruses such adeno-associated virus and lentivirus (which do not cause disease) are used as a vector. Following treatments,patients produce their own coagulation factors normally after receiving viral treatment. A single intravenous administration may lead to long-term expression of FVIII/FIX, maintenance of steady-state plasma concentrations, and reduction (or perhaps eradication) of bleeding episodes over the course of the recipient's lifetime. Clinical trials have evaluated several gene treatments, with promising results. However, there are major obstacles to overcome, includingimmunogenicity towards viral vectors, hepatotoxicity, cost and affordability of new genetic products. This literature review includes reviewing a summary of some of the research in the advances of gene therapy, limitations, future perspectives, and other advances in hemophilia therapy including Platelet-Targeted FVIII Gene Therapy, RNAi therapeutic targeting antithrombin, placental cell-based gene therapy,Non-Gene Therapy bi-specific antibodies, etc.

Methodology: *The information is collected from secondary sources published in various scholarly journals selected from google scholar. The research articles systematically reviews advances, limitations, future perspectives, and other advances in hemophilia therapy.*
Results/Findings: *Based on a systematic review, advances in gene therapy, limitations, future perspectives, and other advances in hemophilia therapy are identified. Many of clinical trials have evaluated several gene treatments, with promising results. Considering the rapid developments in hemophilia gene therapy clinical trials, there is a critical need for education to prepare the hemophilia care team for the potential integration of gene therapy into the therapeutic options available to those with the condition.More studies in the field will help to overcome current challenges in hemophilia therapy, expectingcost-effective, productive, less immunogenic gene therapy products to be approved and available in markets soon.*
Keywords: *Hemophilia, Gene therapy, Hemostasis,Adeno-associated virus vector, Lentiviral vector*

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I. INTRODUCTION

Mutations in the genes producing coagulation factor VIII (FVIII) and factor IX result in the rare X-linked bleeding disorders hemophilia A(FVIII) and hemophilia B. (FIX) [1][2]. Hemophilia can also be acquired, a rare autoimmune disorder caused by the development ofantibodies against factor VIII or factor IX [3]. Enhancing haemostasis to a level that prevents and controls acute bleeding is the cornerstone of the treatment of hemophilia. Historically, preventive replacement therapy regimens, including regular intravenous

injections of FVIII or FIX concentrates, could only partially achieve this [4]. Hemophilia A is more common compared to Hemophilia B .Both men and women without a history of bleeding can develop acquired haemophilia A, a rare bleeding disorder brought on by neutralising autoantibodies against coagulation factor VIII (FVIII);similar to hemophilia B where antibodies against factor IXdevelops. In patients with AHA, controlling acute bleeding and preventing injuries that could result in bleeding are major considerations [5].However,thedevelopment of antibodies against FVIII or FIX concentrates, also known as inhibitors, is a major complication of factor replacement therapy[6].Many studies and clinical trialson gene therapy and bi-specificFVIII-mimetic therapeutic antibodies,etc, are being conducted to address these problems. Extended half-life FVIII & FIX concentrates, that facilitate more flexible and individualized preventative treatment than standard FVIII , FIX products, non-replacement therapy with an FVIII-mimicking monoclonal antibody or molecules that can rebalance coagulation, potentially curative gene therapy, and other novel and potentially transformative treatment modalities had been added to the therapeutic options[3].With advances in hemophilia gene therapy, including AAV-directed gene therapy and lentiviral gene therapywith significant potential, the possibility of using gene editing to fix the underlying mutation is on the horizon.Through the induction of the production of the deficient coagulation factors (FVIII and FIX, respectively) in human cells, gene therapy has the potential to offer individuals with HA and HB the best possible bleeding protection. This novel method can be carried out by either introducing functional factor genes into cells or by modifying the genome already there. The transfer of FVIII/FIX genes into hepatocytes by adeno-associated viruses is the more popular of the two approaches for factor gene delivery that are currently being researched (AAVs). Another choice is intravenous delivery of autologous stem cells that have had their genomes altered by lentiviral vectors [7].Hemophilia will be less of a burden for many of the eligible patients because to the first-generation gene therapies, which have started the regulatory review process. People who are seropositive or cross-reactive to multiple AAV serotypes, children, people with comorbid illnesses, and residents of countries where even the most basic plasma-derived clotting factors are not reliably available are just a few of the many people who are not qualified. Despite the fact that first-generation gene therapies will considerably lessen the burden of hemophilia, there are still a lot of questions concerning the technology's reliability, effectiveness, and safety as it is administered to hemophiliacs all over the world [8].A single therapeutic injection of gene therapy for hemophilia offers the possibility of a permanent recovery. Despite obstacles to general adoption, there is a great deal of expectation about fulfilling this promise with the start of phase 3 gene therapy research for both hemophilia A and hemophilia B using modified trans-genes, including FVIII-BDD and FIX-Padu. Hopefully, future developments will eventually make gene therapy available to allhemophiliacs around the world [9].

Here, we examine the developments and limitations that led to recent successful clinical studies, related works and indicate the directions that will enable gene therapy and other advances in hemophilia therapy to be used more widely.

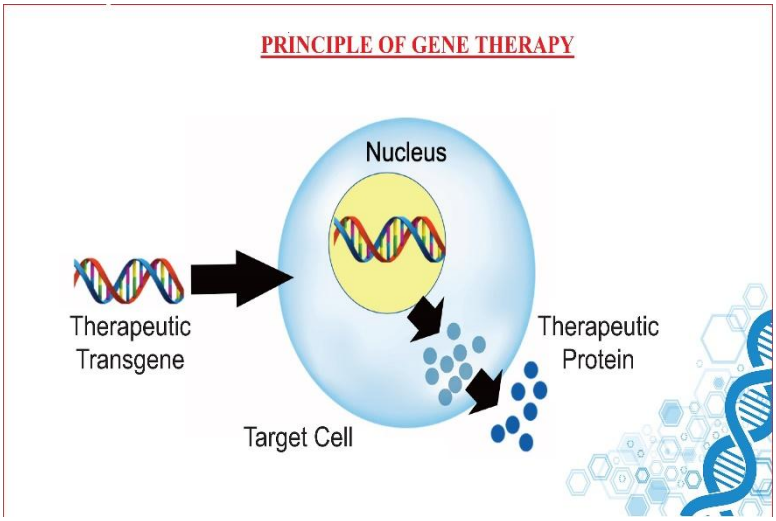

Fig. 1: Principle of gene therapy

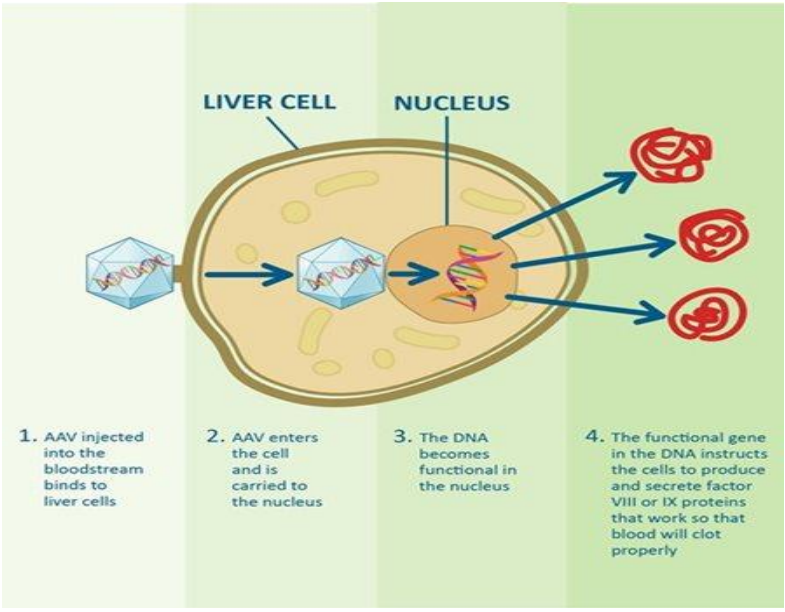

Fig. 2: Principle of AAV associated gene therapy

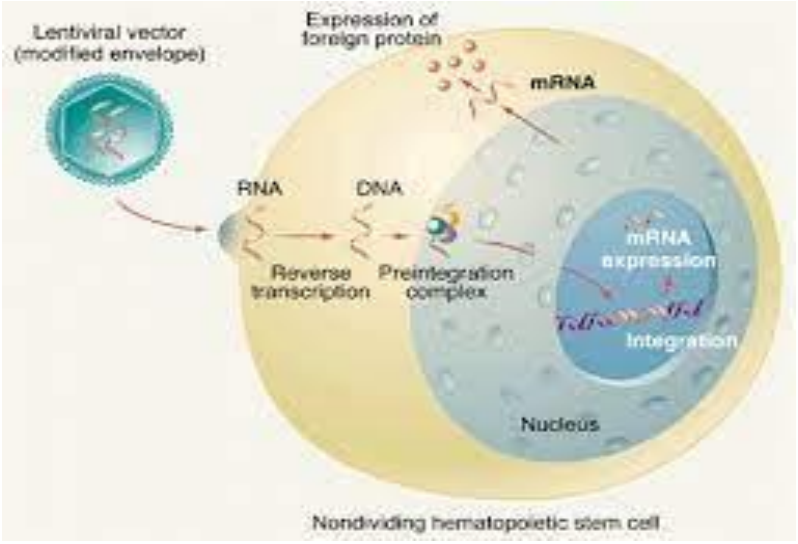

Fig. 3: Principle of Lentiviral associated gene therapy

II. OBJECTIVES: CURRENT STATUS OF GENE THERAPY IN HEMOPHILIA; AND UPDATES ON NEW INITIATIVES:

The following section reviews

1. Advances in hemophilia gene therapy, including AAV-directed gene therapy and lentiviral gene therapy
2. Immunogenicity and genotoxicity of gene therapy
3. Limitations of gene therapy
4. Other advances in hemophilia therapy

III. METHODOLOGY :

The literature evaluation was constructed using the most recent data gathered from a variety of secondary sources, including published literature from many scholarly journals. Using Google Scholar, a computerized search was used to choose relevant articles. Recent secondary sources were exclusively explored for literature evaluation in order to gain a better understanding of the present status and limitations of gene therapy.

IV. WHAT IS CURRENTLY KNOWN ABOUT GENE THERAPY FOR HEMOPHILIA? RELATED WORKS.

Table 1: Published works on gene therapy and other advances in hemophilia therapy.

SL NO	RESEARCH AREA	OUTCOME	REFERENCES
1	AAV-directed gene therapy	Over the past 20 years, adeno-associated viral vector-mediated gene therapy for hemophilia A and hemophilia B has been thoroughly studied in preclinical models. As hemophilia gene therapy becomes more widely used, it becomes increasingly obvious that many components of the procedure need important laboratory support to ensure safe and beneficial outcomes from his novel therapeutic approach. These contributions from the lab cover a wide range of activities, including assessments of the gene therapy vehicle, evaluations of the patient's immune response to the vector, and ultimately the execution of assays to ascertain the hemostatic benefit of the gene therapy and possibly its long-term safety on the host genome. The safe and effective delivery of gene therapy will necessitate an informed and coordinated contribution from laboratory professionals, as with many elements of previous hemophiliatreatment.	Paul Batty et al.[2021][10].
2	AAV-directed gene therapy	The review article by Benjamin J et al. [2020] reviewed that the evidence collected from various sources, they explained that, after liver-directed AAV(adeno associated viral) gene therapy, continuous uninterrupted production of FVIII and other transgenes can influence the immune system to induce immunological tolerance. Hence, this fact should be considered in order to apply this strategy in clinical trials of factor VIII gene therapy	Benjamin J et al. [2020][11]
3	AAV-directed gene therapy	George Q et al [2019] summarized an update on clinical trials on gene therapy on hemophilia. Gene therapy presents the possibility of a long-lasting treatment with a single drug injection, in contrast to other different therapies for the X-linked bleeding condition haemophilia that are now in clinical research. Hepatic in vivo gene transfer has already nearly fully corrected haemophilia A (factor VIII deficiency) and haemophilia B (factor IX deficiency) in patients. Developed are adeno-associated viral vectors with various viral capsids that have been modified to express highly active coagulation factors at high levels. Patient data show that prolonged endogenous generation of clotting factors as a result of gene therapy reduces the need for coagulation factor infusions (or alternative medications that enhance coagulation), which may ultimately also lower treatment costs.However, some individuals getting high vector dosages have experienced mild liver toxicity. Immune suppression was used as a result of the toxicities' correlation with a T-cell response targeted at the viral capsid in some but not all cases. Additionally, due to innate immunity to viral capsids, not all patients can be treated. Nevertheless, research on hemophilia in animal models indicates that the method can potentially be used to induce immunological tolerance and prevent or get rid of antibodies that are inhibitory to coagulation factors. These are serious therapeutic complications that might develop during conventional protein replacement therapy.	George Q et al. [2019][12]
4	AAV-directed gene therapy	Gene therapy gives hemophilia patients the opportunity to have a single treatment and maintain consistent factor levels for extensive periods, as opposed to being dependent on recurrent administration at frequent intervals and a steady supply of medication. Adeno-associated virus (AAV) vector-mediated gene transfer phase III trial stage for hemophilia A and B has begun . The use of factor VIII and factor IX genes in gene editing techniques or lentiviral gene transfer are presently being tested in clinical settings. The initial gene therapy treatments for FVIII and FIX are expected to be approved and available in markets soon.	Reiss et al.[2021][13]
5	AAV-directed gene therapy	K. John Pasi et al. [2020] conducted a multiyear follow-up of Adeno-Associated Virus (AAV)-mediated gene therapy and discovered that AAV5-hFVIII-SQ vector gene therapy in hemophilia A patients resulted in consistent, clinically significant benefit as measured by a significant decrease in annualized rates of	K.John Pasi et al [2020][13]

		hemorrhagic events and a complete cessation of prophylactic factor VIII usage in all participants who received 4 or 6 x 10^{13} vg per kg of the gene therapy	
6	AAV directed gene therapy	The first adeno-associated virus (AAV) gene therapy vector centered on the AAVhu37 serotype is BAY 2599023 (AAVhu37FVIII), and it is currently in the clinical stage. A single-stranded DNA genome expressing a B-domain-deleted FVIII is present in the non-replicating AAV vector BAY 2599023, which is controlled by a liver-specific promoter/enhancer pair that is ideal for transgenic expression. The AAVhu37 capsid, a member of the hepatotropic clade E family, was chosen because preclinical studies revealed that it effectively transferred the FVIII gene to the liver and that it exhibited excellent biodistribution and long-lasting FVIII expression. However, a patient's eligibility may be limited by pre-existing humoral immunity to AAV capsids.	Steven pipe et al. [2020][14]
7	AAV-directed gene therapy	Due to the wide variety of AAV serotypes, therapeutic vector doses, and production methods, it is difficult to compare therapeutic products. Despite encouraging outcomes, gene therapy is still not at the point where it can be utilised to treat everyone with haemophilia because of the limitations imposed by these exclusion criteria. Immunosuppression, plasmapheresis, the addition of empty capsids to serve as decoys, innovative bioengineered capsids, localised vector infusion, and other attempts to destroy pre-existing NAbs have not totally eradicated the NAbs and have only marginally decreased titers. Inhibitors to the infused protein can form in up to 30% ofpatients with severe HA and 5% of patients with severe HB, which can reduce or prevent the achievement of hemostasis with replacement protein.Efforts to overcome these obstacles along with continuous education should broaden the availability of gene therapy for the vast majority of patients with hemophilia.	Bhavya Doshi et al. [2018][15]
8	AAV directed gene therapy ***(**ValoctocogeneRoxaparvovec an investigational AAV5 gene therapy marketed as ROCTAVIAN)	A clinical trial conducted by Brian R. Long et al. (2020) Patients with pre-existing humoral immunity to AAV5 or a history of FVIII inhibitors were excluded. Following dosage delivery, blood plasma and peripheral blood mononuclear cell (PBMC) samples were taken at regular intervals to evaluate the humoral and cellular immune responses to the AAV5 vector and the transgene-expressed HFVIII-SQ. The production of antibodies directed to the AAV5 capsid that were cross-reactive with other common AAV serotypes was the main immunological response brought on by ValoctocogeneRoxaparvovec (BMN 270 treatment.)	Brian R. Long et al. [2020][16]
9	AAV-directed gene therapy	The first study to demonstrate a persistent dose-dependent increase in FIX levels in patients with severe haemophilia B after a single injection of adeno-associated viral (AAV) vectors was the St. Jude/UCL phase 1/2 experiment, which was published in 2011. Over a 7-year follow-up period, transgenic FIX expression in the high-dose group has remained steady at >=5% of normal, leading to a marked decrease in spontaneous bleeding and FIX protein use without harm. Following a single injection of AAV vectors, formerly seriously impaired patients saw clotting factor activity that was approaching normal or near-normal levels and "zero bleed rates," which represents unequaled advancements in gene therapy for hemophilia A and B. As a result, AAV gene treatments are anticipated to change the way hemophilia A and B are treated. The review by Amit et al. [2019] examined recent developments and challenges that need to be resolved before this innovative therapy for inherited bleeding diseases may be made more widely available.	Amit et al. [2019][17]
10	AAV-directed gene therapy	Valder R et al. [2020] addressedfacts and issues with gene therapy for hemophilia in the twenty-first century and stated that research on gene therapy vectors based on adeno-associated viruses (AAV) has given the foundation for early-stage clinical trials, first for haemophilia B (HB), and now for haemophilia A.	Valder R et al. [2020][18]
11	AAV-directed gene therapy	The model created by Keziah Cook et al. [2020] predicts that intervention with single-dose administration valoctocogene and roxaparvovec would really be cost-saving for people with	Keziah Cook et al. [2020][19]

		hemophilia A at a price level comparable to other currently offered gene therapy products because it has the potential to lower FVIII utilisation, direct medical costs, lifetime bleeds, and accumulated joint damage. However, due the scarcity of data, the model depends on evidence-based hypotheses for clinical inputs. Modeling population heterogeneity, regarding lifetime bleed rates, joint injury progression, and long-term gene therapy durability, helped to reduce this uncertainty.	
12	AAV-directed gene therapy	In the trial by M C Ozelo et al. [2022] in patients with severe hemophilia A they detailed that Valoctocogeneroxaparvovec treatment provided endogenous factor VIII production and dramatically decreased bleeding and factor VIII concentrate consumption compared to factor VIII prophylaxis	M C Ozelo et al. [2022][20]
13	AAV-directed gene therapy	According to Sylvia Fong et al [2022] article , patients with severe haemophilia A who had a single intravenous infusion of valoctocogeneroxaparvovec (AAV5-hFVIII-SQ) demonstrated that clinical advantages that lasted for five years.	Sylvia Fong et al [2022][21]
14	AAV-directed gene therapy	A minimally sized, highly effective AAV-fVIII vector with three distinctive components was developed by Harrison et al. [2018]. These components are a liver-directed 146-nt transcription regulatory module, a target-cell-specific codon optimization algorithm, and a high-expression bio-engineeredfVIII variant. The AAV-fVIII vector genome can be as small as 4,832 nt , and the tissue-directed codon optimization strategy promotes increased fVIII transgene product expression in target cell types, such as hepatocytes, compared to conventional genome-level codon optimization strategies. They used an ancient and orthologous fVIII sequence element that was previously demonstrated to support enhanced biosynthesis through post-translational mechanisms as a tertiary strategy.These technologies work together to create an AAV-fVIII vector that provides persistent, therapeutic levels of fVIII in haemophilia A mice at a low dose.	Harrison et al. [2018][22].
15	AAV-directed gene therapy	Adeno-associated virus type 5 (AAV5) vector and a liver-specific promoter are combined in AMT-060 to express a codon-optimized wild-type human FIX gene. The trial with a single infusion of AMT-060 produced persistent and clinically significant improvements in FIX activity, a significant decrease in spontaneous bleeding, and the use of FIX concentrate without the presence of detectable cellular immune reactions to capsids	Miesbach et al. [2018][23]
16	Lenti viral directed gene therapy	The major obstacle in developing factor VIII therapy is the development of the immunogenicity of the product. Jie Gong et al,[2021] introduced a synthesized shortened factor VIII gene (F8-BDD) and cloned it into lentiviral vector(LV). Two novel F8-BDD genes were generated; one restored all the B domain's N-glycosylation sites (F8-299) while the other restored N-glycosylation sites (F8-N8) in mice .F8-299 modification shows reduced immunogenicity of factor VIII protein despite of overall reduced protein expression, expecting that this study will aid in creating gene treatments against hemophilia A	Jie Gong et al,[2021][24]
17	Lenti viral directed gene therapy	AAV vectors have drawbacks, such as their primarily episomal nature in target cells' nuclei and the pervasive preexisting immunity against the parental virus in people. Utilizing transcriptional and microRNA-mediated control, lentiviral vectors (LV) intended for liver-directed gene therapy are created to precisely target transgene expression to liver cells. A possible benefit for creating long-term expression, particularly in paediatric patients where the liver experiences significant growth, is that LV incorporates into the target cell chromatin and is preserved while the cells duplicate their genome.	Cantore et al. [2021][25]
18	Lenti viral directed gene therapy	Long-term production of FVIII in endothelial cells by lentiviral vector mediated gene therapy holds the promise of a single treatment since available treatments based on repeated FVIII infusions do not provide a permanent solution. LV-corrected blood outgrowth endothelial cells were identified, grown, and transduced	Chris Doering et al. [2021][26]

		with an LV containing FVIII regulated by an endothelium-specific promoter using GMP-like techniques from HA patients and healthy donors. Either FVIII-corrected HA BOECs were administered into a Cell Pouch implanted subcutaneously in NSG-HA mice, or were directly implanted into the peritoneal cavity. The first preclinical investigation demonstrating the safety and viability of implantation of GMP-like produced LV-corrected BOECs within an implantable device for the long-term therapy of HA found that FVIII secretion was adequate in both cases to improve the mouse bleeding phenotypes for ongoing therapy for HA	
19	Hematopoietic stem cell gene therapy for hemophilia A using bio-engineered factor VIII	Hematopoietic stem and progenitor cell lentiviral gene therapy is a potential approach toward the permanent treatment of hemophilia. Immunogenicity is a major issue of factor VIII gene therapy. The study by Athena L et al. [2021] described protocol in a mouse hemophilia A gene therapy model, a non-genotoxic conditioning protocol using an immunotoxin targeting CD117 (c-kit) to deplete endogenous hematopoietic stem cells and a combination of monoclonal antibodies to temporarily inhibit the immune system against the transgene product is used which helps in reducing immunogenicity.	Athena L et al. [2021][27]
20	Placental Cell-Based Gene Therapy	Administration of factor VIII (FVIII) through gene and/or cellular platforms emerged as a promising hemophilia treatment. The efficacy of human placental cells (PLCs) as FVIII delivery vehicles was examined by Nadia et al. [2020] and identified that the optimal FVIII transgene to produce/secrete therapeutic FVIII levels from these cells. They used three PLC cell banks to show that PLCs naturally released low levels of FVIII, indicating that they could be used as a transgenic FVIII manufacturing platform	Nadia et al. [2020][28]
21	Human Alphoid^{tetO} Artificial Chromosome	The research by Sergey V et al. [2020] explains the introduction of the human artificial chromosome Alphoid^{tetO}-HACvector as a useful tool for introducing genes into eukaryotic cells. Human induced pluripotent cells were developed from FVIIIY/−mutant mice fibroblasts, Therapeutic alphoid^{tetO}-HAC-FVIII transferred into theFVIII^{Y}/–iPSCs displayed the constitutive FVIII expression.	Sergey V et al. [2020][29]
22	Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc)	Congenital X-linked bleeding disorder haemophilia A (HA) is caused by deficency of coagulation factor VIII (FVIII). Currently, recombinant (r-) or plasma derivative (pd-) FVIII concentrates are used to treat HA by replacing the FVIII deficiency. The gold standard treatment for people with severe haemophilia is repeated infusions of FVIII given on a regular basis, or prophylaxis.	Giruad et al. [2021][30]
23	Platelet-Targeted FVIII Gene Therapy	The treatment of haemophilia A mice with platelet-targeted FVIII gene therapy is successful even when inhibitors are used, according to findings from preclinical studies involving animal models. Through peripheral tolerance mechanism platelet-targeted genetic therapy can encourage immunological tolerance to specific antigens. Numerous critical factors, such as platelets' ability to prevent the immune system from recognising the neoprotein, the presence of the von willebrand factor in the platelets, and appropriate preconditioning before gene transfer, may be responsible for the success of platelet gene therapy in haemophilia A with inhibitors. Yuanhua et al. [2020] concluded that platelet-targeted gene therapy offers a novel strategy for haemophilia A gene therapy even in the presence of inhibitors since it can deliver therapeutic protein and trigger antigen-specific immune tolerance.	Yuanhua et al. [2020][31]
24	RNAi therapeutic targeting antithrombin	Antithrombin (AT) in the liver is the target of the RNA interference drug fitusiran, which inhibits AT translation by binding to and degrading messenger RNA-AT. This silences AT gene expression and prevents AT production. When fitusiran is administered subcutaneously on a weekly or monthly basis, Nicoletta Machin et al. [2018] found that AT knockdown causes dose-dependent AT reduction. Fitusiran dose escalation has been shown in clinical trials to enhance thrombin production and clinical hemostasis as indicated by a decrease in annualised bleed rate. Contrary to currently approved medications, this improvement was seen in patients with hemophilias A as well as B, with or without inhibitors.	Nicoletta Machin et al. [2018][32]

25	Emicizumab—a humanised, bispecific monoclonal antibody that mimics activated FVIII	Gene therapy represents a paradigm change and could establish itself as the new gold standard for the care of haemophilia A patients. Following this, the creation of emicizumab—a humanised, bispecific monoclonal antibody that mimics activated FVIII—was advanced in the field of medicine.The recurrent and ongoing prescription of exogenous FVIII and emicizumab has a detrimental effect on patient quality of life.	Castaman et al. [2022][33]
26	Tocilizumab: a Novel Immunomodulatory Regimen for Hemophilia Gene Therapy	According to the findings of the study by Klaudia et al. [2021], short-term prophylactic inhibition of IL-6 signalling was safely used in non-human primate models without causing any harm and may be used to reduce immunological responses that are frequently seen after AAV vector infusion. These findings are in favour of tocilizumab's ongoing research as a tailored immunomodulatory regimen in hepatic gene therapy for hemophilia.	by Klaudia et al. [2021][34]
27	Bispecific Antibodies and Developments in Hemophilia Non-Gene Therapy Options	Patients with haemophilia still face challenges related to FVIII inhibitor development and frequent intravenous infusions. A bi-specific antibody imitating activated FVIII developed in Japan to address these unmet demands. . In spite of the presence of inhibitors, phase 3 studies showed a substantial decrease in bleeding rates and a high proportion of patients with no treated bleeds. Though isolated thrombotic microangiopathic and thrombo embolic problems were identified in the studies, emicizumab generally found to be well tolerated.	Shima M et al.[2020][35]

Table 2: Getting closer to the first licenced product for haemophilia.[36]

Hemophilia A Clinical Gene Therapy Trials 2020			
Gene Therapy	Product Name	Clinical Trial Stage	Sponsor
1. BMN-270	AAV5	Phase 3	Biomarin
2. SB-525	rAAV2/6	Phase 3	Pfizer (Sangamo)
3. SPK-8011	AAV-Spark200	Phase 3	Roche (Spark)
4. BAY-19429	AAVhu37FVIII	Phase 1/2	Bayer
5. Spark-8016	AAV-Spark200	Phase 1/2	Spark
6. Spark-8016 (inhib)	AAV-Spark200	Phase 1/2	Spark
7. Go-8	AAV2/8 FVIII-V3	Phase 1	UCL-St. Jude
8. ET3	HSC lentivirus	Phase 1	Expression Therapeutics
9. YUVA-GT-F801	HSC/MSC - lentivirus	Phase 1	SGIMI
10. Pleightlet (MUT6)	Autologous CD34 - lentivirus	Phase 1	Med College Wisconsin
Hemophilia B Clinical Gene Therapy Trials 2020			
Gene Therapy	Product Name	Clinical Trial Stage	Sponsor
1. AMT-061	FIX Padua AAV5	Phase 3	CSL Behring/UniQure
2. SPK-9001	FIX Padua - AAV-Spark100	Phase 3	Pfizer (Spark)
3. FLT180a	FIX Padua AAVS3	Phase 1/2	Freeline
4. AMT060	WT FIX AAV5	Phase 1/2	UniQure
5. SB-FIX	AAV6 ZFN targeted	Phase 1/2	Sangamo
6. YUVA-GT-F901	FIX-Lentivector	Phase 1	Shenzhen Geno-Immune Medical Institute (SGIMI)

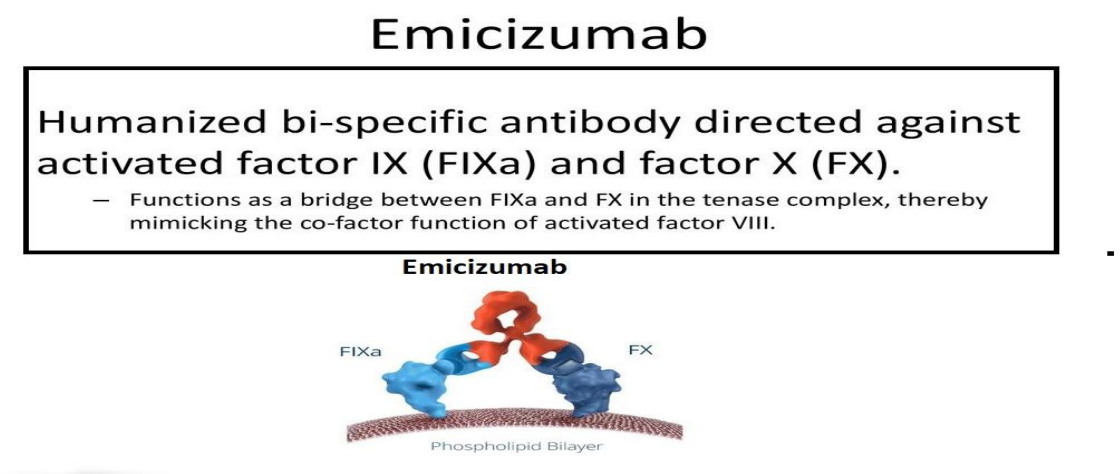

Fig. 4: Emicizumab: —a humanised, bi-specific monoclonal antibody that mimics activated FVIII

V. HEMOPHILIA GENE THERAPY MAJOR CHALLENGES :

Major challenges involved in implementing gene therapy include:

1. Immune response directed against viral vectors.

2. Costs and affordability of gene therapy.

3. Lack of randomized controlled trials.

4. The limitations of the traditional quality of life measures in hemophilia.

5. The immaturity of the evidence regarding the durability of benefits.

Immune response directed against adeno-associated viral (AAV) vector gene therapy is the major challenge in gene therapy. In order to overcome the challenges, Paul E. Monahan et al. [2021] described various solutions including.

1. Administration of immunomodulating agents
2. Using low vector-engineered doses,
3. Long-term follow-up of hemophilia patients undergoing AAV therapy [37].

Hepatotoxicity and immunogenicity are linked to gene therapy. The viability of these techniques will increase as a result of improving the vector serotypes and the transgene (variants). Gene therapy may lead to cost reductions (especially indirect ones) and a more equal distribution of therapies in the setting of hemophilia. for hemophilia A, more research is required to determine the best way to incorporate the large factor VIII gene into the vector; for hemophilia B, the focus should be on optimizing the vector serotype to reduce its immunogenicity and hepatotoxicity and the transgene to increase its clotting efficacy in order to reduce the amount of vector administered and lower the incidence of adverse events without compromising the effectiveness of the protein expression [38]

Louis et al. [2021] identified and discussed the main methodological issues that need to be addressed in order to evaluate the effectiveness of gene therapy for hemophilia. These issues included the lack of randomized controlled trials, the limitations of the traditional quality of life measures in hemophilia, the immaturity of the evidence regarding the durability of benefits, the lack of definition and valuation of a cure for chronic diseases, and the choice of perspective [39].

The objective of the study done by Renske et al. [2021] was to conduct an early cost-effectiveness analysis of valoctocogeneroxaparvovec (valrox; Roctavian) compared to factor VIII prophylaxis or emicizumabin severe hemophilia A patients without FVIII-antibodies. The study stated that patients and doctors welcome the introduction of new therapeutic options, but they come at a high price. Although it sounds appealing to provide incremental health benefits at lower costs, there are other factors that demand further consideration [40].

RESEARCH GAP :

The creation of gene therapy is a recent invention. Most of the clinical trials in gene therapy are in the initial phases only. The long-term impact of gene therapy on hemophilia patients remains unclear. Even though gene

therapy provides fresh insight into the management of hemophilia, providing hemophilia gene therapy products at a low cost is challenging.

VI. CONCLUSION :

Developments and challenges in gene therapy from recent literatures were reviewed in this literature review. Based on our study, we have summarized that.

Gene therapy can improve the lives of hemophilia patients not only by reducing bleeding incidents, but also by increasing their participation in active and sporting activities. Many clinical trials have evaluated several gene treatments, with promising results. Considering the rapid developments in hemophilia gene therapy clinical trials, there is a critical need for education to prepare the hemophilia care team for the potential integration of gene therapy to the therapeutic options available to those with the condition.Gene therapy is one of several cutting-edge methods being tested in clinical studies to cure hemophilia, along with non-replacement and in vivo and ex vivo gene therapy. To enable equitable personalization of care for individuals with hemophilia, the availability of these pricy innovative therapies would necessitate evolution in clinical and financial healthcare services. Even though immunogenicity against viral vectors in gene therapy isa major challenge, using immunomodulatory medications or chemicals can help to reduce immunological responses and train the immune system to become tolerant to FVIII,this challengewill be addressed by more clinical trials. Our study examined recent developments and challenges that need to be resolved before this innovative therapy for inherited bleeding diseases may be made more widely available. Expecting more research and clinical trials answers a lot of questions concerning the technology's reliability, effectiveness, and safety as it is administered to hemophiliacs all over the world. Along with viral vector gene therapy, other innovations in hemophilia therapy including Platelet-Targeted FVIII Gene Therapy, RNAi therapeutic targeting antithrombin,placental cell-based gene therapy Non-Gene Therapy bi-specific antibodies, etc provide fresh insight into the treatment of hemophilia. More studies in the field will help to overcome current challenges in hemophilia therapy expecting a cost-effective,productive,less immunogenic gene therapy products to be approved and available in markets soon.

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