WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 23, 556-569. Review Article ISSN 2277–7105

GENE THERAPY IN HEMOPHILIA

[1]*Sukanya Garje, [1]Kalpana S. Kale and [2]Dr. Megha Salve

[1]Shivajirao Pawar College of Pharmacy, Pachegaon, Maharashtra, India.

[2]Department of Pharmacy, Maharashtra, India.

Article Received on
10 October 2024,

Revised on 31 October 2024,
Accepted on 21 Nov. 2024

DOI: 10.20959/wjpr202423-34764

***Corresponding Author**
Sukanya Garje
Shivajirao Pawar College of Pharmacy, Pachegaon, Maharashtra, India.

ABSTRACT

Hemophilia is a genetic mutation disorder that impacts the coagulation factor VIII or factor IX genes. The preferred palliative treatment is based on the application of safe and effective recombinant coagulation factors. So far, the results have been promising regarding the levels and timing of expression, primarily utilizing adeno-associated vectors. Nonetheless, these treatments are linked to immunogenicity and liver toxicity. Vector serotypes and transgenes (variants) improve coagulation efficiency, thereby increasing the success of these protocols. It's crucial for both doctors and patients to understand the possible advantages and dangers of the new treatments, and a registry of gene therapy patients should be maintained, containing details on the effectiveness and long-term negative effects related to the therapies given. In the field of hemophilia, gene therapy can lead to reduced costs (especially indirect) and promote a more equitable distribution of treatments. For hemophilia A, additional studies are required to determine the best methods for effectively packing the sizable factor VIII gene into the vector. In contrast, for hemophilia B, the focus should be on enhancing both the vector serotype to lower its immunogenicity and hepatotoxicity, as well as the transgene to improve its clotting effectiveness. This approach aims to reduce the total amount of vector used and lower the frequency of adverse events without affecting the protein's expressed efficacy.

KEYWORDS: Hemophilia; gene therapy; immune tolerance induction; viral vectors; CRISP -Cas-9; bispecific antibodies; AAV.

INTRODUCTION

HA occurs in one in 5,000 live births, making it more common than HB, which affects one in

30,000 live births. The severity of HA and HB depends on the functional levels of the relevant circulating factors, where FVIII or FIX levels under 1% are indicative of severe hemophilia, levels from 1 to 5% suggest moderate hemophilia, and levels between 5% and 40% indicate mild hemophilia. Individuals with severe hemophilia often suffer from bleeding into their joints, muscles, or soft tissues after injury or sometimes without any obvious reason. They might also experience life-threatening bleeding events like intracranial hemorrhage. Individuals with mild or moderate factor deficiencies might have spontaneous bleeding, while significant bleeding tends to happen only following trauma or invasive medical procedures. Residual factor activity is typically strongly associated with the clinical features of patients. Nonetheless, people with identical coagulation factor levels can display different bleeding phenotypes. While HA and HB are regarded as clinically indistinguishable, various recent studies have challenged this notion, indicating that patients with HB may be less likely to experience severe bleeding compared to those with HA who share identical plasma levels of residual factor (similar disease phenotypes).[1] Gene therapy is considered the most promising approach for the management of hemophilia A and B. The European Association for Haemophilia and Allied Disorders (EAHAD) reports that there are eleven clinical trials in progress for hemophilia A and B, involving over 300 patients undergoing the intended execution of this therapeutic method is a single vector injection, resulting in stable endogenous synthesis of factor FVIII or IX. The desired therapeutic dose is defined as an FIX level that exceeds the normal value by 1%; in practice, this greatly lowers the chances of bleeding. There are numerous reasons why gene therapy might be a more appealing choice for treating hemophilia. Even a tiny increase in the levels of these clotting factors can significantly affect the overall health of patients. The plasma factor.

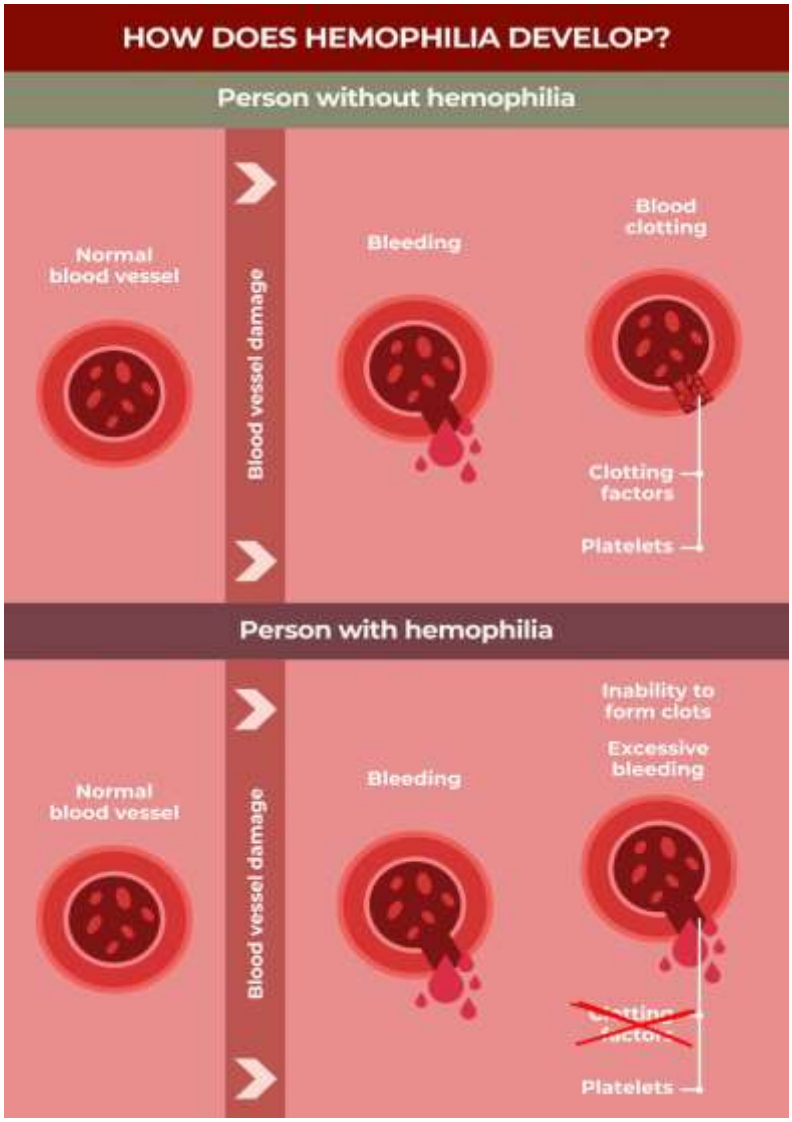

Fig. 1: Hemophilia.[6]

Levels can be evaluated to determine gene expression. Initial research employing an ex vivo gene therapy method via gene transfer in hematopoietic stem cells or autologous fibroblasts led to temporary low levels of FVIII expression.[2]

While gene therapies are set to become accessible soon, there remains a deficit of precise and comprehensive understanding of this new treatment among healthcare professionals and researchers. At present, there are 409 recognized haemophilia centers in Europe. Thorough care for haemophilia involves a multidisciplinary method for treating the condition. Patients have the option to receive extensive care services at a single location: either at a European Haemophilia Treatment Centre (HTC) or a European Comprehensive Care Centre (CCC).[3] The standards that must be met to obtain certification as one of these types of hemophilia center are outlined in the European guidelines for the certification of hemophilia centers

across Europe. Innovative therapies, especially gene therapy, will necessitate modifications in these treatment facilities, along with the implementation of a 'hub and spoke model' featuring systems for long-term safety and efficacy monitoring.[4] Conversely, if the factor level is around or above 100%, it would still be considered safe. Nonetheless, earlier effective gene therapies for various conditions have utilized an ex vivo strategy, like in the case of SCID-ADA3.[5]

Advanced Therapies in Hemophilia

Advanced therapies consist of a range of new and inventive approaches including cell therapy, gene therapy, and regenerative medicine or tissue engineering. They target conditions or disorders that presently do not have a cure or whose treatment needs improvement. As stated by international medical organizations, the items utilized in advanced therapy contexts are human-use medications derived from genes, tissues, or cells, providing novel treatments for various illnesses.[7] In hemophilia, approaches centered on gene and cell therapy have demonstrated significant promise. Clearly effective cell and gene therapy methods are available to tackle both monogenic and polygenic disorders, as well as to enhance the efficacy duration of therapeutic proteins and elevate their expression levels. This is possible due to the accessibility of various types of target cells and transfection vectors, along with the ability to control gene expression and the traits of the transgene, ultimately ensuring its safety. Funding for research into these novel therapies is clearly warranted, given that there are numerous chronic and serious conditions lacking available treatments, as well as others where current options either produce significant side effects or are burdensome and/or impractical, complicating adherence.[8]

Although gene therapy protocols are frequently more effective than cell therapy ones, they come with substantial challenges and a higher incidence of adverse events, primarily linked to the transfer vector. A perfect transfer vector ought to be immunologically neutral; highly specific to tissue and cell types; integrative; able to stably express the transgene and deliver genes to both dividing and non-dividing cells; simple to manufacture in bulk; and able to achieve optimal transgene capacity. Currently, the immunogenicity and hepatotoxicity of the transfer vector, along with the issues related to its integration (insertional mutagenesis), represent the key obstacles that need to be addressed.[9] Nevertheless, it should be noted that retrovirus (RNA virus)-based vectors are essentially missing from clinical applications, despite their considerable transduction efficiency and capacity to sustain transgene expression

long-term. Their disadvantages stem from their significant ability to integrate into the genome and their elevated rate of insertional mutagenesis. Adenoviral (DNA virus) vectors are non-integrative and can efficiently encapsulate the "therapeutic" gene, but they have a significantly high immunogenic potential, which may lead to serious anaphylactic reactions. Adeno-associated virus (AAV) vectors demonstrate the highest promise for clinical applications. They are semi-integrative vectors linked to a reduced risk of insertional mutagenesis. Nonetheless, they exhibit a strong immunogenic reaction to their capsid and can, in certain instances, be hepatotoxic. Finally, lentiviral vectors (LVs), which are derived from single-stranded RNA lentiviruses, integrate into the host genome and have a low risk of causing insertional mutagenesis; furthermore, they do not provoke a notable immune reaction or induce hepatotoxicity. Despite demonstrating the same limited packaging capability as other retroviruses, next-generation LVs, which have been improved and provided with defense against phagocytosis, have been proven to attain optimal FVIII and FIX expression in the liver.[10]

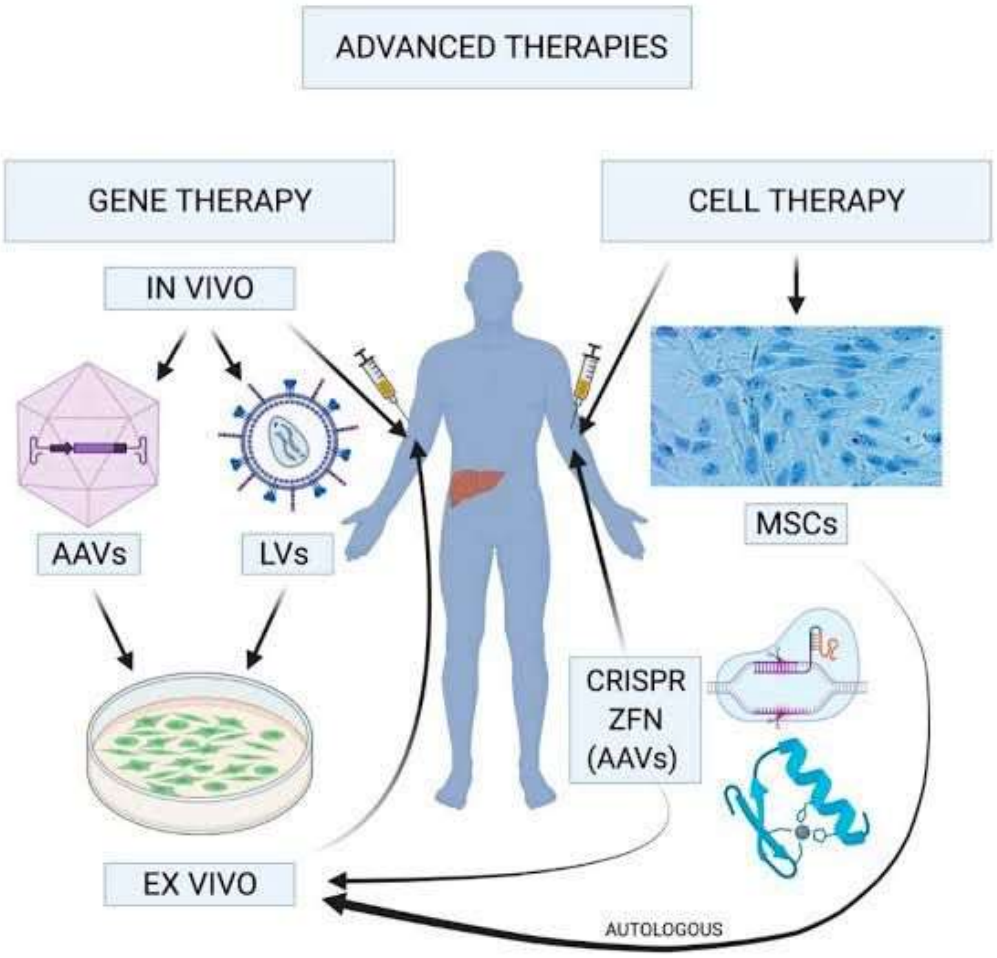

Fig 2: Advanced Therapies in Hemophilia.[11]

The DNA sequences carried by AAV vectors are maintained in an episomal state, which means that sustained expression can only occur when delivered into long-lived postmitotic cells. The DNA of the vector integrates at a minimal rate and is usually eliminated from replicating cells. Recombinant vectors exhibit a preference for various target tissues, including the liver, which leads to their predominant use as gene therapy viral vectors in hemophilia (90%), with in vivo and ex vivo LVs following at 10%. Liver-targeted gene therapy has the potential to change congenital hemophilia from an untreatable condition marked by a severe phenotype into a moderate or possibly mild type of hemophilia. Nonetheless, predicting the percentage of hemophilia patients who might gain from the gene therapy is challenging, as various factors have been identified that could limit its use.[12]

Hemophilia B.

In 2011, Nathwani and colleagues released the initial successful clinical trial of gene therapy for hemophilia B patients. It was a successful proof of concept. In ten patients with severe hemophilia B, gene therapy effectively increased factor IX levels to 1-6%.[13] AAV is a virus with single-stranded DNA. The creation of a double-stranded gene that human hepatocytes can transcribe is essential for expression. The self-complementary structure will speed up this procedure. Luckily, the F9 gene is compact enough for this design, which seemingly increased the length, and it can still be accommodated within an AAV vector. Finally, a key concern regarding the sustainability of expression is the immune elimination of transduced hepatocytes. This typically happens in certain patients approximately 6 to 12 weeks following the infusion of gene therapy. AAV capsids that penetrate hepatocytes are broken down and subsequently displayed on the surfaces of the cells. In certain patients, circulating T cells identify these foreign protein fragments and eliminate the transduced cells similarly to a typical immune reaction to viral infections. In this study, this concern was tackled through the use of steroids. A minor increase in alanine transaminase (ALT) and a decrease in FIX were observed in 4 out of 10 subjects. With brief use of steroids, the immune response was halted and FIX levels were maintained. As the AAV capsid will not replicate any further, the immune response will not pose a problem once all infused vectors have been processed.[14]

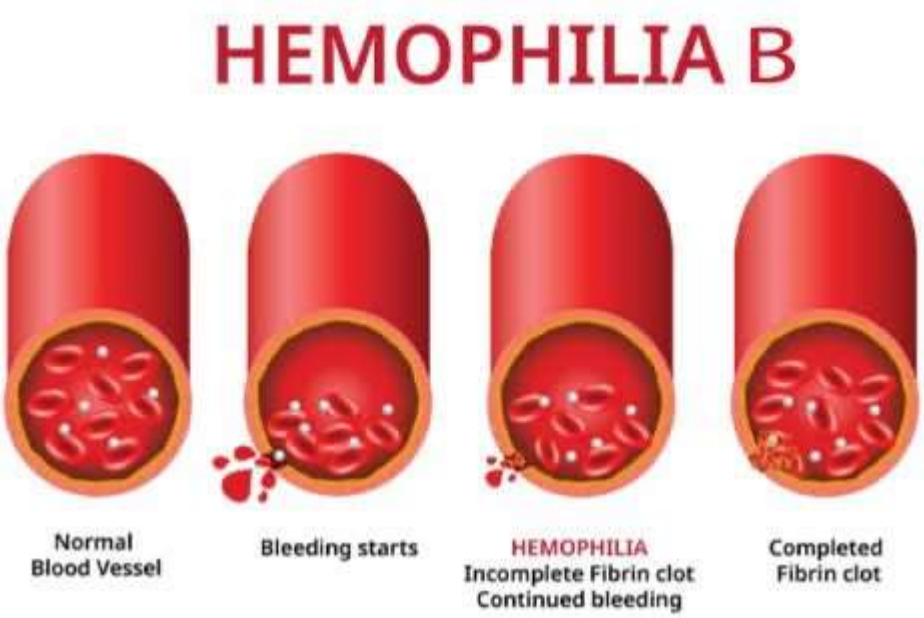

Fig. 3: Hemophilia B.[31]

The researchers assessed the stability and prolonged safety of transgene expression in 10 patients suffering from severe HB, each receiving a single infusion of the AAV8 vector. A vector dose-dependent rise in circulating FIX of 1% to 6% was noted over an average 3-year duration, enabling a decrease in the frequency of prophylactic FIX administration and, in some instances, even its complete suspension. All of the patients, nonetheless, generated antibodies specific to the capsid. The primary negative effect observed was a rise in hepatic enzyme levels (ALT), which was managed with prednisolone. University College London has started a new phase ½ clinical trial targeting HB, using the AAV-F9 vector, FLT180a.[15] However, the maximum vector dose applied in the UniQure Biopharma BV study exceeded the AAV8 vector dose used by Nathwani et al. The authors determined that pseudotyped scAAV5 vectors should be beneficial for treating people with preexisting immunity to AAV5.[16] The patient experienced a temporary asymptomatic transaminitis 4 weeks after the injection, coinciding with a loss of FIX expression. The initial successful gene therapy trial using AAVFIX (NCT00979238) gave recombinant AAV8 with codon-optimized FIX (scAAV2/8-LP1-hFIXco) to six patients with severe haemophilia B, using three varying doses. FIX expression ranged from 2–11% of normal levels for all participants, which was adequate to enhance their bleeding phenotype. In the high-dose group, two participants experienced a temporary, asymptomatic increase in serum aminotransferase levels, which was linked to capsid-mediated cellular immunity in one individual. Transaminase levels were

brought back to normal with a brief period of corticosteroid treatment, and FIX levels remained between 3–11% of the normal range.[17] Subsequently, to achieve increased expression, the AMT-060 FIX transgene was replaced with the Padua transgene, referred to as AAV5-hFIXco-Padua (AMT-061, etranacogene dezaparvovec). A one-time administration of 2 × 10^13 vg/kg AMT-061 resulted in total cessation of bleeding without requiring FIX replacement for as long as 26 weeks (NCT03489291V).[18]

Hemophilia A.

Hemophilia A is five times more common than hemophilia B and requires gene therapy five times more. The main challenge for gene therapy in hemophilia A is that the F8 cDNA is about 7 kb, which significantly exceeds the capacity of an AAV vector. A significant portion of factor VIII, referred to as the B domain, is unnecessary in the active form of factor VIII; however, it is essential for the correct transport of factor VIII within the endoplasmic reticulum and Golgi.[19] An analysis of the 2-year outcomes from the BMN270 gene therapy phase III trial has indicated that the annual joint bleeding rate (AJBR) at various FVIII levels determined by OSA and CSA aligns closely with the predictions made by den Uijl et al. 30 Nevertheless, when FVIII levels drop below 5 IU/dL, as measured by either OSA or CSA (16), the AJBR is somewhat lower than the predictions found in den Uijl's data.30 Additional research is necessary to effectively guide clinical practices concerning surgery or trauma for recipients of gene therapy in the future.[20] Clinical research in hemophilia A has progressed more slowly due to the larger size of the F8 cDNA (7 kb), although the worldwide prevalence is higher. Due to the limited packaging size of recombinant AAV DNA, which is ⩽5 kb, truncation of the F8 cDNA by removing the sequence encoding the non-functional domain (deletion of the B domain, BDD) has enabled its incorporation into AAV vectors.[21]

The initial successful implementation of this method was documented in 2017, employing a single intravenous administration of an AAV5 vector that encodes a BDD F8 (AAV5-hFVIII-SQ) in nine individuals across three dose groups (BMN270, Valoctocogene Roxaparvovec, NCT02576795). In the high-dose group, normalization of FVIII activity was maintained for over a year, along with stabilization of hemostasis and a significant decrease in prophylactic FVIII consumption. AAV-based gene therapies aimed at treating HA are in a more preliminary phase of development compared to those for HB. The primary issue regarding FVIII pertains to the packaging within the vector, as the gene for this protein is significantly larger (7 kb) and surpasses the packaging limit of AAVs, which is approximately 5 kb.

BioMarin Pharmaceutical led the initial clinical trial focused on HA, employing hepatic gene transfer with an AAV serotype 5 (AAV5) vector that expresses B-domain deleted FVIII (BDD-FVIII-DQ, BMN 270).[23] A significant portion of the patients involved in the trial (88.8%) showed a minor rise in their alanine aminotransferase (ALT) levels, which was also associated with a decrease in FVIII activity in one participant. The firm has launched two additional phase 3 trials using vector doses of 4 _ 10^13 and 6 _ 10^13 vg/kg.[24]

Treatments

- Serves as the foundation for hemophilia management. Substitution of fVIII or IX to sufficient plasma levels for hemostatic prevention or management of acute bleeding.
- FRESH FROZEN PLASMA (FFP) - IU FFP consists of approximately 160-250ml of plasma, exhibiting an activity level of around 80%. Rate and total dosage constrained by the possibility of acute or chronic circulatory overload.
- CRYOPRECIPITATE: Obtained by gradually thawing fresh frozen plasma at 2-4°C, followed by collecting the precipitate through centrifugation. Cryo derived from 200ml of FFP holds 80-100 U of FVIII, approximately 250mg of fibrinogen, and beneficial quantities of FXIII and vWF for every 10-15ml of precipitate. Utilize thawed cryo within four hours. Can be kept at -18°C for a year.
- Key indicator - inability to respond to standard replacement therapy.
- Risk factors include the severity of hemophilia, age, race, family history of inhibitors, and significant genetic defects.
- Low titer (<5 BU); typically temporary.
- Elevated titer (>5 BU): ongoing.
- Conduct screenings every 3-12 months or after 10-20 exposure days, and before surgery or when the clinical response to appropriate treatment is less than optimal.[25]
- Gene therapy for hemophilia involves a single infusion intended to enhance a patient's clotting ability by substituting a faulty gene with a working. The objective is to eliminate the need for continuous treatments to improve the quality of life.
- Gene therapy seeks to correct a defective gene or substitute it with a healthy one to attempt to treat a disease or enhance the body's ability to combat illness. It shows potential as a remedy for various conditions, including cancer, cystic fibrosis, heart ailments, diabetes, hemophilia, and AIDS.[26]

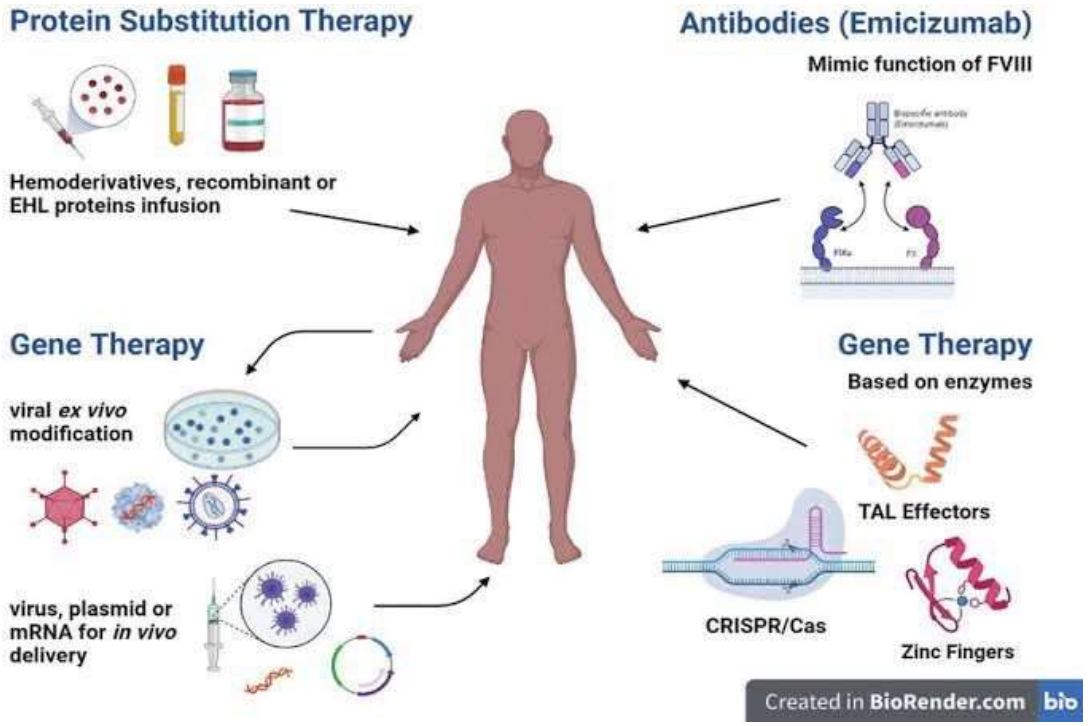

Fig. 4: Treatments of Hemophilia.[32]

Diagnosis

As outlined in section 4.1, there are numerous challenges involved in verifying the diagnosis of PCOS in women exhibiting its characteristic symptoms. While HA testing is the most reliable diagnostic criterion, since it is observed in 60% of women with PCOS, the assessment methods may lead to diagnostic variability. The challenge with hirsutism is that establishing a clear set of traits linked to PCOS is problematic. In a clinical setting, hirsutism is the most common indicator for diagnosing HA. Other factors like acne and alopecia are sometimes considered. Nonetheless, the main disadvantage of using hirsutism as a key indicator of PCOS is its subjective evaluation. Research indicates that women from various ethnic backgrounds exhibit different levels of hirsutism, with symptoms being particularly uncommon among Asian women and not thoroughly comprehended in teenage patients. The second method for diagnosing HA is to assess the levels of circulating androgens.

Diagnosis of PCOS in adolescent girls is still a controversial and widely researched topic. There are many doubts about which features can still be considered physiological for the maturation process, and which go beyond the norm. Diagnostic criteria for adults do not correspond to the diagnosis of this specific group of adolescent patients. There are still ongoing studies on the differences in the diagnosis of adults and adolescents, cut-off points for diagnostic parameters, new parameters that may facilitate diagnosis. To investigate the

differences between PCOS in adult and teenage patients, a study was conducted that compared clinical, hormonal, biochemical and ultrasound parameters. Interestingly, this study did not find a statistically significant difference in PCOS between adults and adolescents. Menstrual cycles of teenage and adult PCOS patients did not differ significantly. In laboratory studies, the mean serum LH/FSH (luteinizing hormone/folliclestimulating hormone), free testosterone, and insulin ratios were significantly higher in both adults and adolescents with PCOS compared to the control group.[29]

A research prepared by Kalra S et al. emphasized the need for early diagnosis of PCOS, referral for appropriate care to improve reproductive, metabolic and general health in adolescents and young adults. For this purpose, a simple questionnaire was created that can be used as a screening tool for PCOS. The questionnaire is divided into three domains: Menstrual/Maternal, Metabolic, 'Misfit masculinity' (dermatological), and each domain consists of 3 possible symptoms that the patient may experience. According to the authors, an affirmative response to any two of the following three domains should prompt PCOS screening and referral to specialist health care. Such a form could be used in primary care to facilitate the identification of patients requiring further diagnosis for PCOS.[30]

Cellular Therapy of Hemophilia

Hemophiliac patients undergoing cell therapy have their stem cells and endothelial progenitor cells altered to produce coagulation factors ex vivo prior to transplantation into their bodies. Fibroblasts, adipocytes, and hepatocytes have also been effectively altered for this aim. This presents various challenges, one of which is maintaining the expression of the transplanted cell blood clotting factor over an extended period. As a result, the primary objectives of the study are to determine the optimal cell type and offer methods for cell transplantation in cell therapy. A clinical trial conducted by Roth et al. was the initial study to document a statistically significant rise in blood clot formation following cell administration. Six people with severe hemophilia A were administered 1–4 × 10^8 fibroblasts that had undergone plasmid transformation to produce FVIII. The FVIII activity increased by approximately 1–5% in the cohort that was administered the highest dosage of cells. However, continuous expressiveness was unattainable.[27] Progress in the study of induced pluripotent stem cells (iPSCs) related to hemophilia therapy has also been achieved. This method is appealing since the group of cells that express coagulation factors is more consistent. In their research, Xu et al. achieved initial outcomes for hemophilia treatment utilizing iPSCs.[28]

CONCLUSION

In individuals with severe hemophilia A, valoctocogene roxaparvovec therapy led to endogenous factor VIII production and notably decreased bleeding and the use of factor VIII concentrate compared to factor VIII prophylaxis. (Supported by BioMarin Pharmaceutical. No gene therapy methods have shown proof of sustained effectiveness in patients. The IM injection of AAV is excessively ineffective. The implantation of fibroblasts is quite challenging and not very efficient. Administering AAV or RV to the liver should be effective.

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