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FUTURE OF GENE THERAPY IN HEMOPHILIA

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ABSTRACT:

Hemophilia is a very uncommon disorder for which the available treatment choices have remained unaltered for a long time. however, in the previous decade there has been fast paced advancement in the treatment choices that are either being developed or have been affirmed for hemophilia, including built coagulations factors and a broad pipeline of new methodologies and modalities. A few of these new modalities, particularly gene therapy, show promising hopes for hemophilia. Gene therapy holds the guarantee of an enduring fix with a solitary drug. Close to-finish amendment of hemophilia A (factor VIII insufficiency) and hemophilia B (factor IX lack) have now been accomplished in patients by hepatic in vivo gene transfer.

Adeno-related viral vectors with various viral capsids that have been built to express significant level, and now and again hyperactive, coagulation factors were utilized. Information bolster that supported endogenous clotting factor production because of gene therapy wipes out the requirement for imbuement of coagulation variables (or elective medications that advance coagulation), and may in this manner at last additionally lessen treatment costs. These progresses, along with better diagnostics, are currently empowering clinicians to improve the standard of care for individuals with hemophilia. The instruments and different techniques utilized in these treatment choices have limitations for their safety and usefulness, which must be balanced with their fruitful utility. This Review centers around the most progressive and inventive methodologies for hemophilia treatment and considers their future use along with brief information about hemophilia and mechanism of coagulation.

KEYWORDS: Hemophilia, Coagulation cascade, Gene therapy, Adeno-associated virus, CRISPR/Cas9 technology

INTRODUCTION:

Blood, the vehicle of the body is in a liquid state for the body to work appropriately yet it is likewise ready to change into a strong state when required to shape coagulation to stop bleeding and hemorrhage.as soon as there is a physical issue prompting skin cut and bleed, the body's profoundly refined system to stop the bleeding comes into action. This system must be balanced to stop the bleeding exactly at the site by forming a clot and keep the rest of the blood streaming as should be expected. This component of the body is called hemostasis. (figure 1) explains the balancing components of the hemostasis. Pathological cluster development at an unrequired site is called Thrombosis.

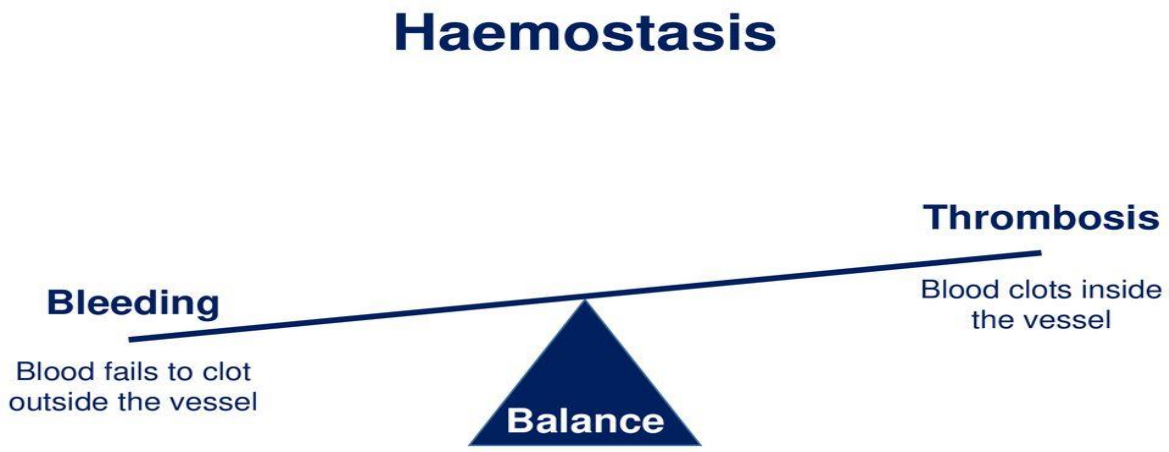

Figure 1 HEMOSTASIS WORKING

COAGULATION CASCADE:

The formation of plug at the laceration site is initiated by a complex and delicate mechanism called as coagulation cascade. The coagulation mechanism involves several soluble inactive proteins, which when activated leads to activation of factors of the cascade in stepwise manner. Factors are donated by roman numerals and activated factors are donated with a suffix `a`. Factors are zymogen and their activated forms are serine protease (Davidson, 1962). Some factors activation involve calcium and phospholipid. Liver is responsible for the production of proteins including coagulation factors, whereas Factor VII is produced by vascular endothelium. Any damage to the liver effects the clotting factors production.

The coagulation in body occurs by two pathways i.e. intrinsic pathway (contact) and extrinsic pathway (or tissue factor). (figure2) The extrinsic pathway is called extrinsic as its activation requires a `extrinsic` component i.e. tissue factor an integral membrane protein released after external trauma. The factors involved in extrinsic pathway are I, II, VII, and X. Factor VII is called stable factor. The extrinsic pathway is clinically measured as the prothrombin time (PT). The intrinsic pathway is activated by factor XII, there is a conformational change in the factor XII when it comes in contact with exposed endothelial collagen. Factors of intrinsic pathway are I (fibrinogen), II (prothrombin), IX (Christmas factor), X (Stuart-Prower factor), XI (plasma thromboplastin), and XII (Hageman factor). The intrinsic pathway is clinically measured as the partial thromboplastin time (PTT). Both pathways converge at a specific point, factor X which is activated to Xa, ultimately leading to fibrin clot formation. Protein C and S keeps a check on coagulation activity and prevent over coagulation.

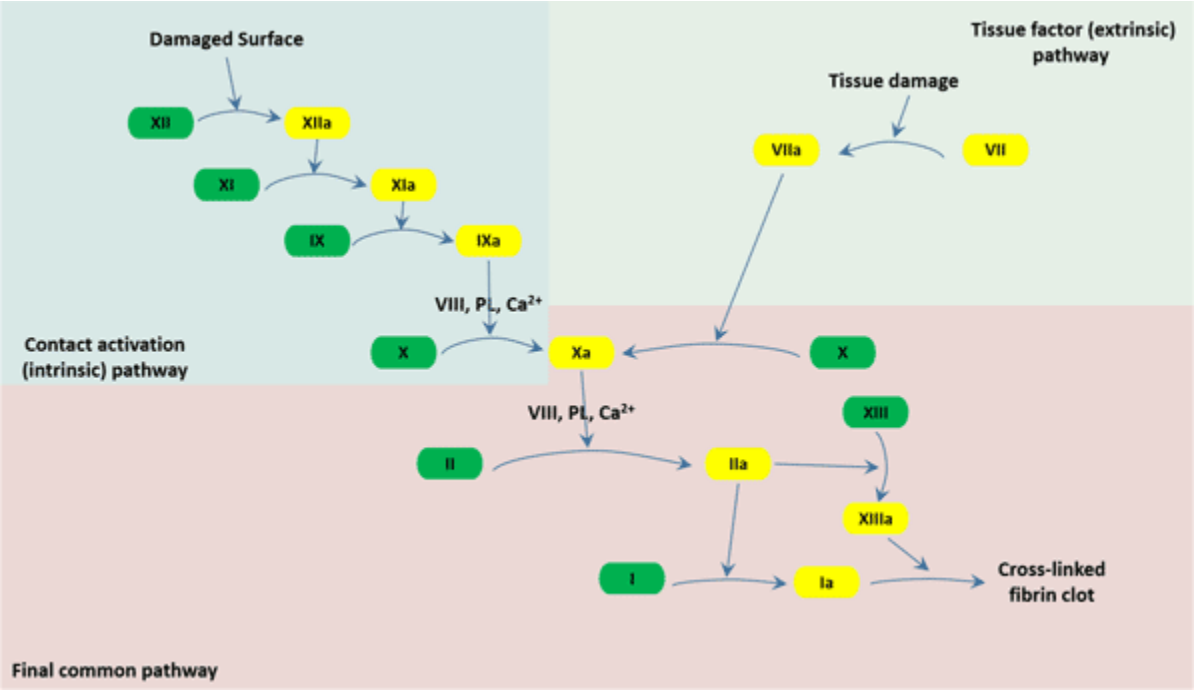

Figure 2 (diagram showing factors taking part in intrinsic pathway and extrinsic pathway, and point of the convergence at a specific point leading to end product` FIBRIN CLOT`)

HEMOPHILIA:

Hemophilia is a hereditary clotting disorder resulting due to altered or missing coagulation protein. This results in disturbance of sophisticated coagulation system and body is unable to form a clot after an injury and there is continuous blood loss from the trauma site. The bleeding is very difficult to manage and can rapidly lead to hypovolemic shock and death if emergency measures are not in time. There are four kind of hemophilia that exist

- *Hemophilia A*
- *Hemophilia B (Christmas disease)*
- *Hemophilia C*
- *Parahemophilia (owren`s disease)*

Hemophilia A results from deficiency in factor VIII and hemophilia B results due to deficiency of factor IX both are X-linked recessive disorders while hemophilia C is caused because of factor XI deficiency and parahemophilia is a rare congenital disorder caused by deficiency of factor V. hemophilia C and parahemophilia are an autosomal recessive mutation. Out of four, hemophilia A and B are more common and parahemophilia being very rare.

With this disorder patient is prone to abnormal bleeding,80% bleeding occurs in musculo-skeletal system and remaining 20% in rest of the body organs. Major complication of hemophilia is bleeding in joints and muscles of the body, pseudotumors and synovitis. Recurrent bleeding episodes results in hemophilic arthropathy which is chronic degenerative changes in the major joints of the body. Muscles injection is not advised in hemophilic patients due to fear of hematoma formations. Synovitis resulting from bleeding episodes in the joint causes inflammation of the synovium and this can result into compression and compartment syndrome. Hemophilic patients should be prescribed COX-2 inhibitors instead of aspirin and NSAID. Preferred anesthesia in hemophilic patients is general because of high risk of bleeding with spinal or epidural anesthesia. In the event that insufficiently treated, continued bleeding will result in decay of the joints and muscles,

extreme loss of capacity because of loss of movement, muscle decay, joint deformation, contractures and torment within the first or second decade of life.

Currently, the treatment available comprises of intravenous injections of plasma-derived or recombinant FVIII or FIX. In spite of the fact that this treatment particularly improves both the future and the personal satisfaction of patients experiencing hemophilia, they are still in danger of life-threatening bleeding episodes and continuous joint harm, particularly since prophylactic treatment is confined by the constrained accessibility and significant expense of purified FVIII and FIX. A major side-effect of prophylactic treatment is that a few patients produce antibodies (inhibitors) against Factor VIII or Factor IX, which render further prophylactic insufficient. Inhibition happen in 10–40% of hemophilia A and in about 5% of hemophilia B patients treated by protein-replacement treatment. Clinically, patients with an inhibitor titer over 5 Bethesda units (1 Bethesda unit is characterized as the measure of counteracting agent that diminishes factor action by half) are never again receptive to factor substitution and require treatment with bypassing agents to look after hemostasis. Customary bypassing agents, for example, actuated prothrombin complex concentrate and recombinant initiated Factor VII, are commonly costly, have short organic half-lives, and are not as powerful as Factor VIII or Factor IX in long haul hemostasis. On the other hand, inhibitor patients can be put on an immune tolerance induction (ITI) convention requiring continuous infusion of high physiological effect coagulation factor until inhibitors are diminished or wiped out and patients can continue factor substitution therapy. (Mariani, Siragusa, & Kroner, 2003) (Michele, 2011) Although powerful in roughly 66% of patients with hemophilia A with inhibitors, ITI frequently must be stopped in patients with hemophilia B due to the advancement of hypersensitivity and nephrotic syndrome (Dimichele, 2007).ITI treatment is costly and places a huge weight on the patient, and the long span of treatment builds the hazard for bleeds (Kempton & Meeks, 2014). Considering the high lifetime costs, frequencies of mixtures, and potential wellbeing trouble, there is a requirement for elective financially savvy treatments with decreased hazard and improved viability for hemophilia.

TREATMENT OPTIONS FOR HEMOPHILIA:

There are number of treatment options available for hemophilia each having its own limitation. (i) concerntarte of clotting factor injections (ii) Extended-half-life drugs (these increase the half life of clotting factors by protecting them from degradation) (iii) Non–coagulation factor–based treatment for hemophilia (works by supressing anticoagulants) (iv) Gene therapy

IS GENE THERAPY THE ULTIMATE TREATMENT:

The gene therapy gives a practical duplicate of the ailment causing a gene that is either missing or produced as a nonfunctional protein; in this manner, it tends to be profoundly compelling in treating a monogenic ailment, for example, hemophilia. The underlying obstacle of delivering the helpful gene into target cells and tissues was achieved through the viral vectors got from mammalian viruses that have naturally advanced delivery system of their hereditary freight into cells and tissues. These vectors contain insignificant wild-type viral groupings, and their pathogenic, replicative, and basic viral qualities are supplanted with the remedial gene cassette. Throughout the years, hepatic in vivo gene therapy utilizing adeno-related viral (AAV) vectors have indicated the best accomplishment in preclinical and clinical investigations, with a few clinical investigations for both hemophilia A and B selecting patients for stage 3 testing.

Hemophilia is appropriate for treatment by gene therapy on the grounds that the phenotype is receptive to a wide scope of factor levels, and control is not obligatory. Further, as clotting proteins are released into the blood circulation, it is conceivable to address the bleeding diathesis with gene delivery to a small number of hepatocytes. Factor VIII and Factor IX can be incorporated into nonnative cells and tissues. For instance, despite the fact that Factor VIII is normally released by specific endothelial, for example, LSEC and extrahepatic endothelial cells, expression in hepatocytes create a practical protein that has reestablished hemostasis in animal models and human patients. Lastly, for patients in developing nations with comorbidities and mortalities because of insufficient factor supply, the gene therapy could prove to be a huge advantage by giving a continuous supply of coagulating factors from a single treatment.

VECTORS USED FOR GENE THERAPY IN HEMOPHILIA:

The vectors used for gene therapy classify into two groups, one being viral and the other being no viral.there are three kind of viral vectors has been utilized for the clinical trials of hemophilia gene therapy namely: Adenovirus, retrovirus (lentivirus), Adeno-associated virus.

Adenovirus:

Adneovirus is 36kb dsDNA in size, non enveloped and non intergated vector. The main advantages of this vector are large genome, high titer production with ease and infecting multiple cell types. The biggest and only diadvantage with this vector used id high immunological response.

Retrovirus:

The size of retrovirus is 8 Kb ssRNA which is enveloped and Integrating. Advantages include larger genome, high infection efficiency and stable gene transfer. Disadvantage associated with this vector is insertional mutagenisis.

AAV:

AAV size is 4.7 Kb ssDNA (which is the main limitations of this vector), non-enveloped and non-integrating. The properties of AAV and adenovirus are very similar except the difference in size. Advantages of AAV are low immunogenicity, Infects many cell types and provide long-term gene transfer.

ADENO-ASSOCIATED VIRUS (AAV) TRANSDUCTION:

The gene therapy technology which replace defective gene with a functional gene requires means to transfer the genetic material to the site of intent. These means (vehicles) are called Vectors. The most studied vector in gene therapy is AAV. Adeno-associated vector as the name indicates it is dependent on coinfection with other viruses mainly adenovirus for its replication, AAV is engineered from parvovirus. As the figure represent the gene of interest has to be inserted between the promoter and terminator. So, the package overall contains ITR at both ends, promoter, gene of interest and terminator (figure 3).

The total size of this package should be less than 5kb. This is the reason that there has been more research with AAV for hemophilia B (gene of interest size 2.8kb) than hemophilia A (gene of interest size 4.8kb).

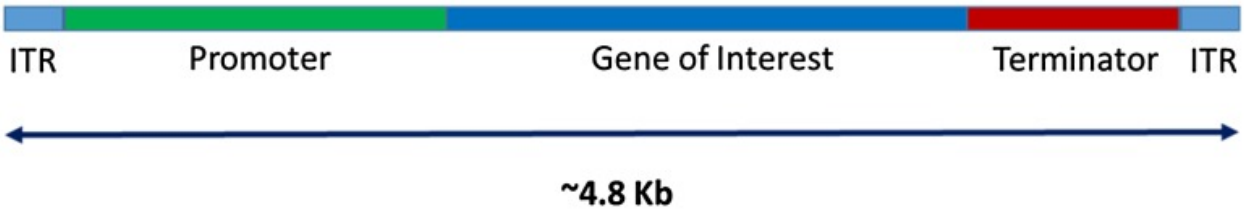

Figure 3 schematic presentation of gene package inserts inside AAV vector (ITR, inverted terminal repeats)

Limitations associated with AAV vector:

It is an excellent vector, but it comes with number of limitations.

I. AAV has the limitations of maximum capacity of ~5kb including the Inverted Terminal Repeat (ITR)
II. Thus far, the AAV-based hemophilia preliminaries have focused on either the muscle or the liver. Pre-formed neutralizing antibodies to AAV, even at unassuming titers, can forestall fruitful transduction after vector delivery into the circulatory system. As a result, 40% of grown-up hemophilia patients might be ineligible to partake in liver based AAV preliminaries.
III. a humoral invulnerable reaction against the transgene item, the AAV capsid or both might be mounted.
IV. Once inside the cell core, most of AAV genomes are balanced out transcendently in an episomal structure, which makes them vulnerable to dilution if the cell replicates. Episomes will integrate at an exceptionally low frequency and along these lines the potential danger of insertional mutagenesis exists. The capsid proteins introduced on the cell surface may likewise signal the transduced cells for destruction.

CRISPR/Cas9 AND HEMOPHILIA:

CRISPR/Cas9 technology has changed the way scientist looked at certain diseases, treatment options and available avenue for research. The innovation not just end up being unrivaled (cheap, fast, effective) than all other available nucleases but also opened new avenue in the field of biomedical science. CRISPR is not only making achievements possible in biomedical world but also brought revolution in agricultural, animals and therapeuticial world. the technology works in simple and easy steps involving cellular repairing mechanism. CRISPR/Cas9 was discovered in 1987 in E. coli, a natural defence mechanism to prokaryotes from invading viruses and plasmids.

Genome editing is alteration of genetic makeup of living organism by deleting, replacing or inserting a DNA sequence to bring out a better, improved or disease-free genome. CRISPR/Cas9 stands for clustered Regularly interspaced short palindromic repeats (these are found in bacteria`s DNA)/CRISPR associated system9.CRISP/Cas9 was discovered in eubacteria and archaea (prokaryotic organisms) which provides acquired immunity to the organisms from invading plasmids and viruses. scientists made use of this naturally occurring defense system of prokaryotic organisms to bring revolution in therapeutic, agricultural and medical grounds. CRISPR/Cas9 genome editing is use of CRISPR/Cas9 to remove a region in genome which is later repaired by cellular mechanism. This system compries of a Cas9 nuclease and a guide RNA (gRNA) which together are used to create a double strand DNA break at a specific target site.

Genome editing technology (also known as gene editing) is based on the use of programmable nucleases, that gives scientists the ability to change an organism’s DNA, which is made up of genes and produce specific changes in regions of interest in the genome. The double-strand breaks (DSBs) that are produced as a result of nucleases are later repaired. The repair mechanisms can follow any of the two pathways, the non-homologous end-joining (NHEJ) and the homology-directed repair (HDR). The NHEJ can lead to an error while HDR is free of any error. There can be insertions, deletions or substitutions done in the target area which can eliminate or correct the defects in genes.

The possibility of correction of genome defect opens new horizon for correction of inherited diseases especially those of monogenic origin e.g hemophilia,sickle cell disease duchene muscular dystrophy etc

The CRISPR/Cas9 simplicity, accuracy, effectiveness and time saving qualities has highlighted its importance in the gene therapy for hemophilia and seems a promising revolution in inherited diseases correction. The system includes a complex of a guide RNA (gRNA) and a Cas9 endonuclease. The gRNA directs the Cas9 nuclease(scissor) to create a double strand break at target site of genome (figure 4).

The mechanism is very simple, starting with gRNA which is the molecule that can read the correct sequence of DNA, this gRNA guides the Cas9 to the specific site of the DNA where a cut is desired. The Cas9 locks and unzips the DNA. The locking allows the gRNA to make connection with the target DNA region and then Cas9 acting like a knife cuts both strands of targeted DNA. The cell, sensing the problem, initiates a repair at the break site. Normally the repair take place by gluing back the loose ends which can lead to errors but sometimes can prove to be useful. This repair gives researcher the opportunity to access certain gene function by comparing mutated and non-mutated gene and to make desired alternation for better outcomes.

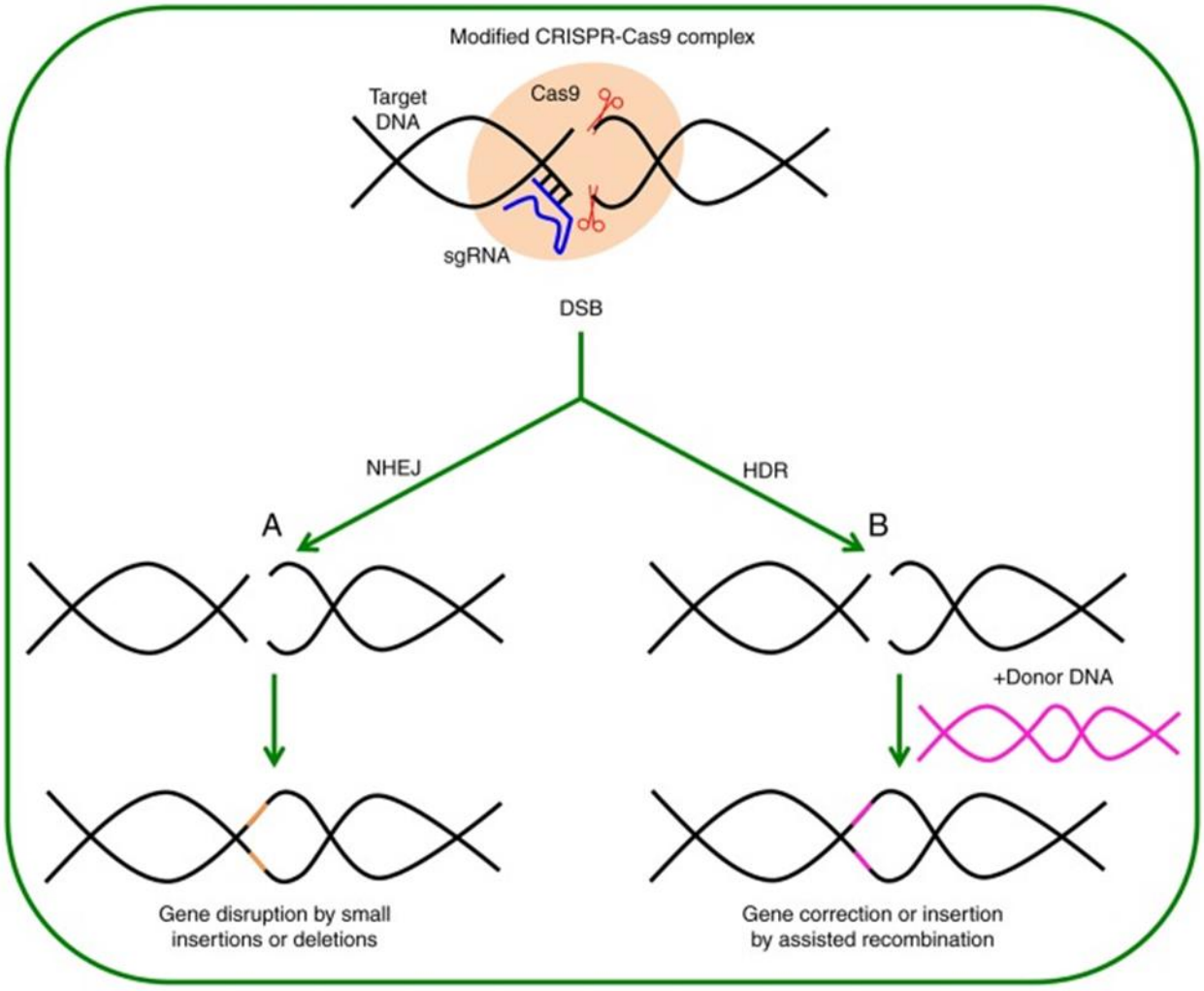

Figure 4: Genome editing through CRISPR/Cas9. gRNA, guide RNA; DSB, double-strand break; NHEJ, non-homologous end joining; HDR, homology-directed repair.

The technology comes with its limitation, the main limitation being the off target, which can lead to genetic instability, hindering advancement and application in clinical procedures.

as the CRISPR technology uses the repair mechanism of the cell to introduce the desired editing that are high chances of targeted alleles carrying additional modification like duplication, deletion, partial insertion resulting in genomic instability.

CONCLUSION:

Following a 30-year time of in vitro experimentation and preclinical evaluation, the field of gene therapy is starting to show strong proof of clinical advantage in a scope of hereditary diseases. The ongoing achievement of gene therapy for hemophilia B features the capability of this remedial methodology for the management of coagulation pathologies. As additional proof of the guarantee of gene therapy activities, the association in hemophilia gene therapy preliminaries by the biopharmaceutical business has expanded drastically in the previous 2 years. All things considered, before gene therapy can be reached out to broad clinical utility, a few basic obstacles should be removed. To begin with, is the improvement of gene therapy vectors that can be created in enormous amount with high and reproducible quality. Evidence that the AAV vectors utilized in an ongoing FIX clinical preliminary contained just ≈10% of transgene containing particles outlines the requirement for improved and progressively proficient vector creation conventions. With current clinical preliminaries being restricted to contemplate populaces of 5 to 10 patients, there is far to go before the broad use of gene transfer can be envisaged.

Next is the issue of deterrents to effective gene transfer. With levels of pre formed antibodies to current AAV based vectors extending from 30% to 60%, many otherwise qualified patients are disqualified from this type of treatment. Regardless of whether AAV capsid or the utilization of novel AAV serotypes will bypass this deterrent is not yet clear. Correspondingly, the utilization of other vector types, for example, lentiviral develops, would significantly lessen this issue. Beside the issues presented by immune reactions to the vector, insusceptible responses to the novel transgenic protein may likewise entangle a few uses of gene transfer, especially when the transgenic protein presents peptide successions that are novel to the beneficiary.

Although quick antagonistic impacts of gene transfer utilizing AAV and lentiviral vectors have been very minute, the longterm result of gene therapy will require formal checking, especially for genotoxicity results. Studies were done in small, animal models with more noteworthy life span have not indicated any proof of an upgraded occurrence of constant pathologies (in particular, no proof of malignant growth development). Nevertheless, these perceptions should be fortified by formal long haul, multi-year reconnaissance in human gene therapy beneficiaries. At long last, the adequacy of gene therapy rather than at present available or next-generation factor substitution treatments should be assessed in randomized clinical preliminaries.The guarantee of genetic treatments for improved management of the coagulation disorders is currently starting to be figured out.

Despite the fact that gene substitution systems are the most efficient of these methodologies, the utilization of inhibitory oligonucleotides and little inhibitory RNA molecules to modify the hemostatic equalization has exhibited how other nucleic acid-based systems have demonstrated extensive potential in late clinical preliminaries. With leading access to genome altering advances, this energy toward translational advantages is likely to proceed.

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