A Review On Genetically Modified Treatment's on HIV/ AIDS

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Abstract- *Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) continue to represent a major global health challenge despite significant advances in antiretroviral therapy (ART). Although ART effectively suppresses viral replication and improves patient survival, it does not provide a definitive cure and requires lifelong adherence. In recent years, genetically modified treatment strategies have emerged as promising alternatives aimed at achieving long-term viral control or functional cure. This review focuses on the current progress and potential of genetically modified therapies for the treatment of HIV/AIDS. The approaches discussed include gene editing technologies such as CRISPR-Cas9, zinc finger nucleases, and TALENs, which target viral genes or host co-receptors like CCR5 to prevent viral entry. Additionally, gene therapy strategies involving genetically modified T cells, stem cells, and viral vectors are explored for their ability to enhance immune responses and confer resistance to HIV infection. The review also highlights the advantages, limitations, safety concerns, and ethical considerations associated with these advanced therapies. Overall, genetically modified treatments represent a revolutionary direction in HIV/AIDS management, offering hope for durable viral suppression and potential cure, although further clinical studies are required to establish their long-term efficacy and safety.*

Keywords: HIV/AIDS, Genetic modification, Gene therapy, CRISPR-Cas9, CCR5 gene editing, Antiretroviral resistance, Stem cell therapy, Zinc finger nucleases (ZFNs), TALENs, Viral latency, HIV cure strategies, Immunotherapy, Genome engineering, Host-directed therapy, Genetically modified treatment's, etc.

I. INTRODUCTION

HIV is a virus that causes AIDS. Normally, our body has immune system that attack viruses And bacteria. Immune system has white blood cells which protect us from infections. White Blood cells contain CD4+ cells which is also known as helper cells or T cells. A person who Is infected will be able to develop. These infections take advantage of body's immune System. These infections cause several health problems and even lead to death of a person. HIV has inability to protect against diseases and count of CD4 cells also decreases in HIV. There is no cure of AIDS but there are certain medicines which are used to slow down the Diseases so you stay healthier for long time. There is no medicine to get rid of diseases [1].

Over the past decade, gene therapy has been able to bring long awaited treatments for immunodeficiency diseases to the clinic [2]. This was accomplished through the development of new gene therapy vectors, in particular, viral based vectors, and genemodified cell therapies [3]. As an example, in indications characterized by severe combined Immunodeficiency (SCID), genetically modified hematopoietic stem cells (HSCs; see Glossary) led to the cure of several children suffering from adenosine-deaminize (ADA) SCID [2]. Genetic modification of autologous HSCs also avoids the high mortality and Morbidity associated with allogeneic bone marrow transplantation [4], particularly in a Setting of paediatric patients with SCID. In order to achieve a prolonged clinical benefit, However, high transduction efficiency into the CD34+-target cell population and durable Expression of the transgene following stable integration of the therapeutic gene expression Cassette, particularly in the hematopoietic progeny, is required. It is very encouraging that This has been achieved in recent clinical gene therapy trials [5]. For several years, stem cell And T cell gene therapies have also been contemplated for infectious diseases, particularly For those caused by HIV. So called 'anti-HIV genes' have been developed, and several of Them have been utilized in combinations transferred by a single gene therapy vector into Either

T cells or HSCs [6]. HSC gene therapy for HIV does promise a functional cure from The disease, if most of these stem cells can be gene modified and successfully engrafted in The infected recipient [7].

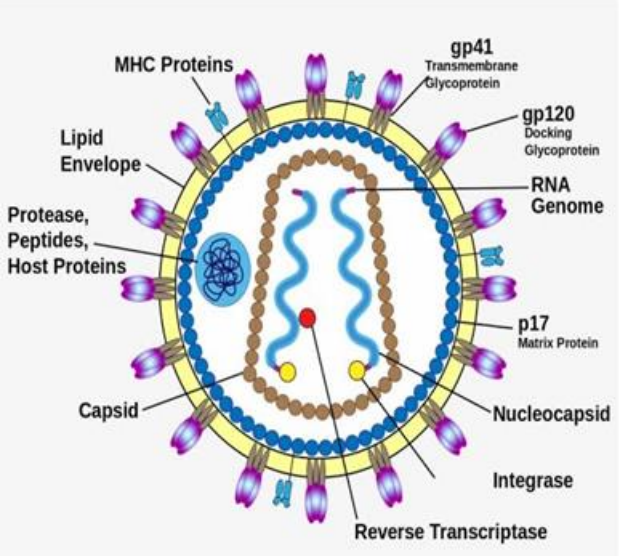

FIG.1: HIV Virus

In period, however, gene therapy for HIV is rather challenging. In order to treat a large population of HIV infected individuals, risks, of novel gene therapeutics very well understood and properly weighed against standard therapy. Additionally, manufacturing and regulatory challenges need to be overcome to make the new therapeutic widely available. In both Europe [8] and the United States, regulatory requirements for gene therapy products, classified as advanced therapy medicinal products (ATMPs) by the European competent authorities in the different member states, are more complex than for the other two product classes within the ATMP category: namely somatic cell therapy medicinal products and tissue engineered Products [9].

LIFE CYCLE OF HIV:

HIV first binds to the cell via gp120 recognizing CD4 on the host cell. This causes a conformational change that exposes the co-receptor binding loop of gp120 and allows it to bind to co-receptor CCR5 or CXCR4. This causes fusion of the viral envelope with the host cell membrane and leads to viral uncoating and reverse transcription. The DNA is imported into the nucleus and integrated into the host cell's genome, where it can then undergo transcription and translation using host cell machinery. New virions are assembled at the host cell membrane, where they are released by budding. For gene therapy approaches, we can either target host dependency factors utilized by the virus to complete this lifecycle or we can target viral genes. SiRNA and ribozymes can target the viral RNA during uncoating and following transcription, leading to degradation of the RNA. Antisense oligonucleotides bind viral RNA after transcription, pre venting translation. Aptamers serve as RNA decoys that can bind the viral proteins, pre venting them from carrying out their function in the viral lifecycle. Single squiggly lines represent RNA, double helix represents DNA, and circles represent viral proteins. [7]

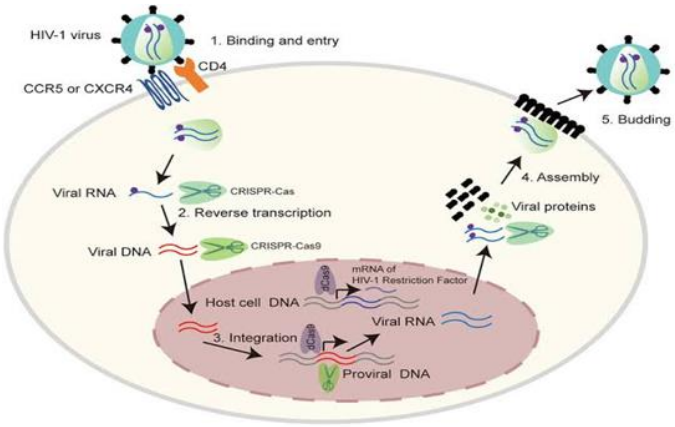

FIG. 2: Life Cycle of HIV

GENETICALLY MODIFIED TREATMENT'S ON HIV/AIDS

These are therapeutic strategies that involve directly altering genetic material. They work by transferring therapeutic gene into somatic cells. These treatment can be done by various approaches.

APPROACHES:

1] DNA Vaccination
2] Intracellular Immunization
3] Gene Editing
4] RNA Based Approaches
5] Immune Based Therapy
6] Stem Cell Based Approaches

1] DNA VACCINATION:

DNA vaccines are contemplated as the third-generation vaccines. These vaccines use an Engineered DNA to encode antigens against bacteria, parasites and viruses capable of inducing Immunologic responses in the host. The expression of these antigens could be regulated by a Strong mammalian promoter embedded on a DNA plasmid. Different strategies such as: I) Codon optimization, ii) removal of bacterial elements, iii) adjuvant formulation and IV) Appropriate delivery method could be used to increase the efficacy of the designed vaccine. DNA vaccines could also be exploited against HIV to induce cellular and humeral immune Responses.

DNA vaccine- plasmid structure; mechanism of action:

In the early 1990s, the first reports of DNA-based vaccines were introduced by Wolff and His co-worker. DNA vaccines are composed of a double-stranded plasmid DNA, which Includes two origins of replications: a prokaryotic origin for replication in bacterial host and A Eukaryotic origin for replication in the vaccination host. Furthermore, they contain an Antibiotic resistance marker to ensure their prokaryotic expression. A special human Cytomegalovirus (CMV) promoter is also included.

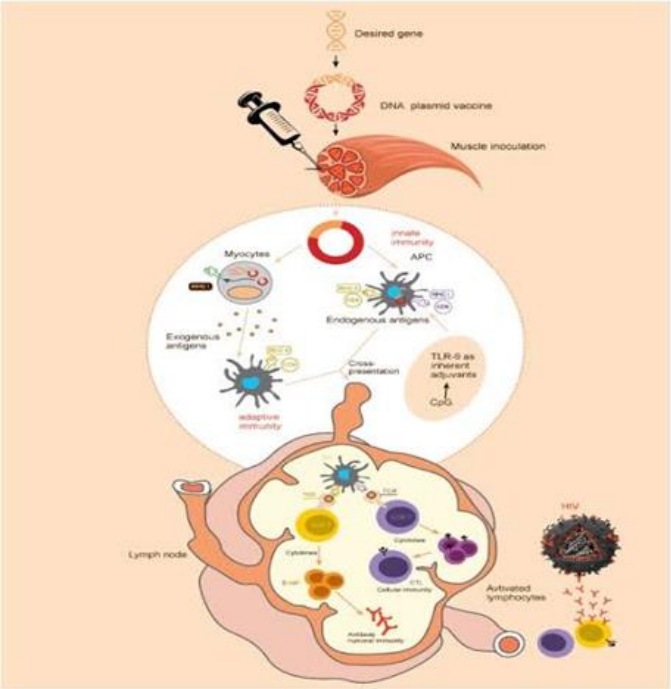

FIG. 3: DNA Vaccination

DNA-as a prime for HIV vaccines; designed on the based on antigens:

HIV is a type of virus which attack to immune cells and lead to destruction of the CD4+ T Cells. It makes the body susceptible to further infections and cancer. HIV-2 virus is different That the HIV-1 in terms of serological or molecular aspects and it has been observed in West Africa, North and South America and Europe. HIV remains to be one of the most serious challenges To the global health. More than 40 types of HIV vaccines have been evaluated among several Thousand volunteers.

Human clinical trials of DNA vaccine:

Infections of HIV are subjected to various therapeutic methods since 1987, especially based On the use of inhibitors of proteases and integrases (1996 and 2007) including Nucleotide Reverse Transcriptase Inhibitors (NtRTIs) and Non-Nucleoside Reverse-Transcriptase Inhibitors (NNRTIs), Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs), Reverse transcriptase inhibitors combined with protease inhibitors (PI or PRI) and integrase Inhibitor (INI). The HIV therapy should be highly.

2] INTRACELLULAR IMMUNIZATION:

Intracellular immunization refers to any forms of gene-transfer-based cellular resistance to viral infection. In contrast with conventional immunization techniques, in which the entire organism is protected against invasion by a pathogen, intracellular immunization consists of the genetic modification of target cells to inhibit or abrogate the replication cycle of a given infectious agent, usually by competing for the binding of proteins that are essential for the replication of this agent. In this strategy, the immunizing moiety is produced inside the cells where it can bind proteins that are usually not accessible by conventional immunization techniques. Thus, the gene encoding the immunizing molecule renders cells resistant to viral gene expression and replication. Intracellular immunization can be accomplished by eitherprotein-based or RNA based approaches. The therapeutic molecules employed so far, in the Context of HIV infection, include transactivation response (TAR) and Rev response element (RRE) decoys, antisense, catalytic RNAs, trans dominant negative mutants and single-chain Antibodies (sFvs).

sFvs:

The humoral immune system is extraordinarily diverse and can form literally millions of Different kinds of antibodies, each capable of binding just one of the millions of different Antigens to which the body may become exposed. Antibodies, displaying high affinity Binding properties, have been exploited for identification, purification and manipulation of Target molecules. An important advance in this field was the discovery that monoclonal antibodies could be Produced by hybridomas, which were made by fusing a single B lymphocyte with an Immortal cell line. Hybridomas can secrete unlimited quantities of a single antibody. Recent Progress in antibody engineering techniques has permitted the isolation of specific antigen-Binding sites of immunoglobulins from hybridomas in vitro.

Single-chain variable fragments are used as genetically modified therapeutic tool. They canSpecifically recognize and block viral proteins.

3] GENE EDITING:

Recently, precision-targeted therapies utilizing gene editing has emerged as an alternative To overcome the limitations of traditional treatments. And the efficiency of gene editing and Its potential to enable a wide treatment has attracted the attention of the scientific Community. Insertion or removal of target protein genes in specific genomes can provide More control over combating viral invasion. Nuclease-mediated gene editing tools, Including zinc finger nucleases (ZFNs), transcription activator. Like nucleases (TALENs), And clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Protein 9 (Cas9) have been widely invested in treatment researches of AIDS [10].

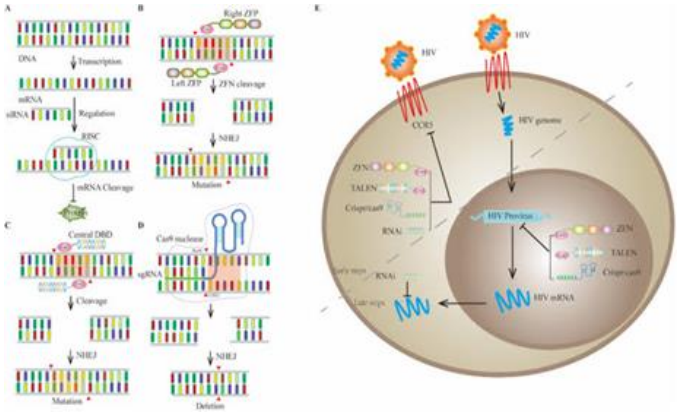

FIG. 4: Gene Editing

A] ZNFs:

Targeted genome editing is made possible by ZFNs (Zinc finger nucleases), whichis a class of DNA-binding proteins that trigger DNA double-strand breaks atspecific user-specified regions. ZFNs mediated a human chemokine (C-C motif)receptor 5 gene (CCR5), whichencoded a coreceptor to entry HIV, modifying in CD4 T cells for protecting the viralinfection. The success of the clinical trial proved that ZFN-CCR5 works [11].

In 2008, the first human clinical trial for using ZFNs to modify protein CCR5 in CD4T cells for curing HIV/AIDS was conducted by Perez et al. The primary co-receptorfor HIV-1 entrance is CCR5, which has a seven-transmembrane chemokine receptor,and also conferring protection to HIV 1 infection is the homozygous 32 deletion inCCR5. After using engineered ZFNs to disrupt the Δ32 in endogenous CCR5, thephenocopy of the Δ32CCR5 null genotype can also prevent the entrance of HIV-1.As a result, CCR5 has been proven to be a primary target for HIV treatment. Perezet al. claimed the experimental design that generating primary human CD4 T cellswhich contain the Δ32CCR5 null genotype for preventing HIV invasion. Throughthe preclinical studies, they found that ZFN-disrupted CCR5 cell enrichment byalmost three times, comparing with cells which weren't modified by ZFNs in theHIV-infected test (27.5% versus 8.5%) [12].

B] TALENs:

TALENs (transcription activator-like nucleases) are similar with the principle ofZFNs, which are also composed of DNA binding and restriction domain.Nevertheless, a study reported that TALE is more potential than ZFNs becauseTALE repeats can be assembled and reconstructed basing on targeting 18 740human protein-coding genes. Scientists intended to use TALENs to disrupt theInteraction between integrase and LEDGF/p75, hence interrupting integration [13].

Theyconducted an experiment that showed the mechanism of ALLINI is independent ofLEDGF/p75 and that focused deletion of the PSIP1 gene has therapeutic promisefor treating HIV-1 illness. Targeted removal of proteins is difficult, but can beachieved withTALENs, which proved its potential in the field of HIV therapy. AsFadel et al. claimed that very few cellular LEDGF is sufficient to interrupt theinfection of HIV.

C] CRISPER/Cas9:

CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-Associated protein 9) is one of the most promising gene editing techniques.CRISPR/Cas9 is a specific and multifunctional gene-editing system, which canHarness to modify and mediate gene editing effectively. The CRISPR-Cas9Accurately cuts DNA, then leaving it to the DNA's own system to recover. The Cas9Helicase can combining with RNA, which is transcribed from host DNA palindromicRepeats, and cleaving incursive DNA linked with RNA that is a transcript from hostDNA short lengths obtained from additional chromosomal elements [14].

CRISPR/Cas9 was first tested in AIDS treatment in 2013, when Ebina et al.successfully used this technology to target HIV LTR for eradicating the expressionof HIV genes in Jurkatcell lines. They measured the capability of CRISPR/Cas9 technology for preventingVital expression through editing the internal DNA of the virus. Transfection of Cas9And gRNA targeting HIV LTR significantly inhibits Tat protein regulates LTR-drivenExpression, which is TAR (trans activation response) binding. Basing on theirExperiment, CRISPR/Cas9 editing LTR-specific components made interference inHIV-1 expression. Two targets were chosen to construct the gRNA-expressingPlasmids, and consequently,

both of which were shown effective disruption of HIV. And the more effective one decreased the average proportion from 45.6% to 20.0%Of the tested positive cells. Essentially, not only did this disruption limit Transcriptionally active proviruses, but it also blocks expression of latent Integrating proviruses [15].

Experimentally, it was demonstrated that cells carrying the D366N mutation gene Showed resistance for HIV attack and survived without intracellular viral replication After infection with HIV strains. However, there is still a lack of clinical trial data.While the current process of CRISPR integration of knocked-in genes is extremely less Efficient than the knocking out, this problem will be solved with advances in Technology [16].

4] RNA BASED APPROACHES:

RNA-based anti-HIV strategies in an ethically acceptable clinical setting includingMarrow ablation. Long-term expression of the RNA transgenes will be necessaryFor success of this procedure, and thus, gene integration will be a requirement inThe progeny cells if the therapeutic effect is to be sustained. In this regard, lentiviralVectors have been promoted as an ideal gene-delivery system since they have beenReported to integrate into non-dividing cells and do not preferentially locate nearGene promoters. We describe here a clinical trial in which autologousHematopoietic progenitor cells (HPCs) are programmed with an expressedInterference RNA (RNAi) in combination with two novel forms of HIV-specific RNA-Based inhibitors (a nucleolar localizing RNA decoy and hammerhead ribozyme) [17].

A] RIBOSOMES:

Ribozymes are small, catalytically active RNA molecules, the most well studied Being the hammerhead and the hairpin ribozymes. The hammerhead ribozyme is One of the smallest ribozymes and is composed of 30 nucleotides with a Conserved core and three stems [18].

Achieving high transduction efficiency and reconstitution with these cells in vivoproved to be challenging despite the promising in vitro data. Vector was detectedin only one patient post-infusion, and no detection of the MLV driven polribozymes was achieved However, in this patient, the U5 ribozyme driven by tRNA(val) promoter was detected at 5- and 7- months post infusion, suggesting that this promoter is better suited for in vivo ribozyme expression. Despite low efficacy of vector, this study marked the firstClinical trial using ribozymes and demonstrated safety of ribozyme transductionInto PBMC.[19] The clinical trials described so far have focused on the use of ribozymes alone. However, the combination of ribozymes with additional RNA-based strategies may Help prevent escape mutants and attack the virus with multiple approaches. Recently, this concept was tested in a phase I clinical trial combining a CCR5 Targeting ribozyme (R5RZ), a TAR decoy, and siRNA targeting a tat/rev exon (ship) [20].

B] RNA APTAMER'S:

Aptamers are single-stranded RNA or DNA molecules that can bind proteins withHigh affinity serving as a decoy. These molecules, normally 15 to 40 bases long,Can be used as decoys to bind viral proteins or as vehicles for targeted delivery ofsiRNAs. These molecules are being explored for their use in vivo as they are non-toxic and non-immunogenic. A recent clinical study discussed above examined theefficacy of a triple construct lentiviral vector expressing a TAR decoy, R5Rz, andshI. [20]Preclinically, Aptamers to gp120 have been used for their dual function in targetingDelivery and inhibiting viral binding. Aptamers to gp120 were fused to siRNATargeting tat/rev and delivered specifically to CHO-gp160 and HIV-infected CEM TCells without triggering an interferon Methods Enzymol. 1999; 306:207-25.

5] IMMUNE BASED THERAPY:

Immune-based gene therapy for HIV is a therapeutic strategy that aims toStrengthen, reprogram, or protect the immune system using genetic modificationsSo that the body can better control or eliminate HIV infection. Unlike antiretroviralTherapy (ART), which suppresses viral replication but does not eradicate the virus, Immune-based gene therapy seeks to provide long-term or permanent resistance toHIV.Provides long-lasting protection (potentially lifelong. Reduces or eliminates theNeed for daily ART. Can create HIV-resistant immune cells. Has potential forFunctional cure (virus control without ART). [21]

A] bNAbs:

Broadly Neutralizing Antibodies (bNAbs) target conserved epitopes of HIV Env andCan neutralize diverse viral strains. Genetic modification strategies have been usedTo optimize their potency and durability.Passive transfer of antibodies against HIV-1 was first tested clinically in the early1990s with pooled polyclonal antibodies. Subsequently, a series of studies testedirst generation monoclonal antibodies in the context of ongoing viremia or duringART interruption. In contrast, animals with the lowest initialviral loads appeared to clear the infection entirely.

After a bNAb wash out period, ART was discontinued and animalswith higher initial levels of SHIVSF162.P3 viremia maintained viral suppression bya CD8+ T cell mediated mechanism. In contrast, animals with the lowest initialviral loads appeared to clear the infection entirely. Whether or not CD8+ T cellscontributed to infection clearance could not be determined [22].

B] CAR-T:

CAR-T therapy, with its remarkable efficacy in treating hematologic malignancies,has sparked interest in its use against HIV. This pioneering approach involvesgenetically modifying T cells to express chimeric antigen receptors (CARs) capableof targeting and eliminating HIV infected cells. The core principle of CAR-T therapylies in its cutting-edge ability to modulate the immune system, priming it to identifyand eradicate HIV infected cells. Thus, this approach has the potential to overcomethe limitations of previous HIV therapies.In HIV research, considerable emphasis is placed on increasing the precision ofCAR targeting to eradicate HIV-infected cells while minimizing collateral damage.The utilization of broadly neutralizing antibodies (bnAbs) to precisely target a widearray of HIV strains is reshnovelstrategies, including the use of multi- CAR and T cell receptor (TCR)-like antibodies,represent promising approaches to combating HIV. The strategic shift from traditional CD4-based targeting to bnAbs represents arevolutionary strategic shift, aims to achieve the precise identification andelimination of HIV-infected cells, especially those harboring the virus in the mostinaccessible sanctuaries. While CD4- based HIV-specific CAR-T cells havedemonstrated long-term safety in clinical trials, theyhave failed to meet expectations for anti-HIV responses, despite multiple optimization efforts. [23].

6] STEM CELL BASED APPROACHES:

Stem cell–based therapy in HIV involves using hematopoietic stem cells (HSCs) orgenetically modified stem cells to rebuild an HIV-resistant immune system. Theidea is to replace or reengineer the patient's immune cells so that HIV cannot infector replicate effectively.Stem cells are taken from a donor who has a CCR5Δ32 mutation (lacking CCR5receptor, needed for HIV entry). Patient's own stem cells are collected, modified toknock out CCR5 or introduce anti-HIV genes, and reinfused. These edited cellsdifferentiate into HIV- resistant T cells and macrophages. Genes for broadlyneutralizing antibodies (bNAbs) or antiviral proteins are inserted into HSCs. Thesecontinuously generate protective immune cell in advanced HIV, stem cell therapyrestores immune function and reduces latent HIV reservoirs. [24].

CCR5:

CCR5 receptor antagonists are a type of tiny chemical that binds to the CCR5receptor and inhibits it. CCR5, a chemokine receptor with a C-C pattern, is involvedin the entry of HIV, the virus that causes AIDS, into cells.As a result, antagonists ofthis receptor are possible entrance inhibitors. In the treatment of HIV infections,there are a variety of therapeutic options. The viral pathway, for example, is one ofthe potential targets for pharmacological therapy in the HIV life cycle. The majorreceptors involved in the HIV entrance process are CCR5 and CXCR4. Thesereceptors are typically expressed on human T cells, dendritic cells, macrophages,and Langerhans cells and belong to the seven Trans membrane G- protein coupledreceptor (GPCR) family. They serve as co receptors, allowing HIV type 1 (HIV1) toconnect to cells prior to viral fusion and entry into host cells. R5 and X4 strains ofHIV isolates can be distinguished. When the virus uses the co-receptor CCR5, it iscalled an R5 strain, and when it uses CXCR4, it is called an X4 strain. BecauseCCR5 receptors are located on the cell surface, big and tiny compounds have theability to disrupt the CCR5- viral interaction and prevent viral entrance into humancellls.

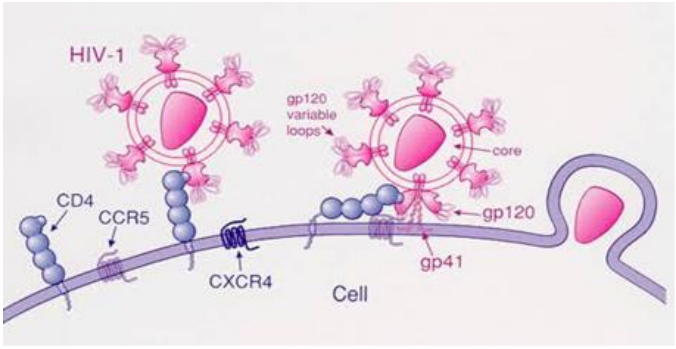

FIG. 5: Mechanism of CCR5

CLINICAL TESTING OF GENETICALLY MODIFIED TREATMENT'S FOR HIV:

Moving to the clinical research phase, major hurdles for academic institutionsinitiating gene therapy clinical trials are the lack of regulatory knowledge and accessto GMP manufacturing facilities. Although GMP manufacturing of the gene therapyvector could be out sourced to commercial facilities, the transduction of theautologous patient cells is ideally performed in a laboratory close to the clinical site,as shipping and transport of the patient cells pre- and post-transduction, whilemaintaining adequate viability and functionality can become problematic. Help withregulatory issues could be obtained from experienced regulatory consultants, amodel that has been implemented successfully in

several academic institutions, butcan add costs that are often not negligible. Partnering with a capable GMPmanufacturer not too far from the clinical site could also be considered[26].

ADVANTAGES OF GENETICALLY MODIFIED TREATMENT ON HIV:

1]Targeted Eradication of HIV Rervoirs: - Gene editing tools (e.g. CRISPR/Cas9, ZNFs, and TALENs) can disrupt proviral DNA integrated into hostgenomes, potentially eliminating latent reservoirs

2]Permanent Protection against HIV Infection: - knockout of CCR5 genemakes immune cells resistant to HIV. Confers HIV resistance in vivo.

3]Reduced Dependency on Lifelong ART: - Genetically modified immune ccells may control HIV without continuous antiretroviral therapy (ART)(Over in costs. Toxicity, and adherence issues.

4]Broad spectrum of approaches: - Gene knockout (e.g. CCR5), Anti-HIV gene insertion Immune enhancement (e.g. CAR-T cell).

5]Restoration Immune Function: - Modified T cell and hematopoietic stemcells (HSCs) can regenerate an HIV-resistant immune system, enhancingCD4+ T cell recovery and immune surveillance.

6]Potential for Functional Cure: - Instead of lifelong viral ssuppressionGenetically Modified Therapies aim for long-term remission or cure, byeither eradicating the virus or creating a resistant immune system.

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