Review

Preventive and Therapeutic Vaccines against HIV: From New Mechanisms to Clinical Trials

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Preventive and Therapeutic Vaccines against HIV: From New Mechanisms to Clinical Trials

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Abstract: As of the end of 2022, despite the disruptions caused by the COVID-19 pandemic, the global HIV epidemic persists, with 39 million individuals living with the virus and 1.3 million new infections reported in that year. Opportunistic infections associated with AIDS claimed the lives of over 630,000 people in 2022, contributing to a cumulative death toll exceeding 40.4 million since the disease's emergence in 1981. Although advances in HIV diagnosis and antiretroviral therapy have transformed the disease into a manageable chronic condition, issues like drug resistance, low compliance, and high treatment costs persist. Until a vaccine becomes available, a comprehensive approach involving prevention and therapy is essential, particularly for high-risk populations. Research shows that prevention strategies are more cost-effective than treatment. The development of antiretroviral drugs has also spurred the creation of antivirals for other infections. The emergence of COVID-19 vaccines has opened new avenues for HIV vaccine development, as lessons from vaccine platform technologies like mRNA, viral vectors, and protein subunits can be applied to HIV. This review explores the most promising prophylactic and therapeutic HIV vaccine trials, emphasizing the need for a multi-pronged approach combining vaccines with non-vaccine strategies. Though significant progress is anticipated, the road to a viable HIV vaccine remains long, especially for high-risk populations, such as medical workers, blood transfusion recipients, and drug users. The combinatorial approach, capitalizing on various aspects of immunity, may hold the key to defeating HIV in the future.

Keywords: HIV; preventive vaccines; therapeutic vaccines; AIDS; vaccine targets

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1. Introduction

According to UNAIDS, at the end of 2022, despite the changes brought about by the COVID-19 epidemic, the number of people living with the human immunodeficiency virus (HIV) in the world was 39 million. In 2022, the number of new HIV infection cases was more than 1.3 million people. More than 630,000 people died from opportunistic infections associated with acquired immunodeficiency syndrome (AIDS) in 2022. At the same time, by the end of 2022, more than 40.4 million AIDS patients have died worldwide since the first cases of infection were recorded in 1981 [1,2].

The problem of HIV infection is still far from being resolved. However, the increasingly available HIV serodiagnosis, combined with highly active antiretroviral therapy, has halted the spread of the epidemic and prolongs the lives of infected patients, making the highly lethal disease a chronic disease. However, the ongoing treatment, nevertheless, does not solve the problems of multiple drug resistance (MDR) formation, low compliance and high cost [3,4]. More virulence and drug-resistant subtypes and circulating recombinant forms (CRF) of HIV are emerging [5,6]. Moreover, the problem of HIV-tuberculosis (TB) co-infection still remains relevant [7].

Until an effective vaccine is available, a comprehensive approach is needed in the fight against HIV infection and AIDS, using all possible means of therapy and prevention (involve all levels), including microbicides [8–11]. Moreover, studies clearly show that HIV prevention in high-risk groups is more cost-effective than therapy [12–14].

The development of antiretroviral drugs for the treatment of HIV has influenced the development of other antiviral drugs targeting different viruses. Some HIV drugs have served as a foundation for the development of drugs against other viral infections, e.g based on HIV nucleoside reverse transcriptase, protease and integrase inhibitors other drugs for the treatment of HCV infection were developed, such as sofosbuvir, telaprevir and bictegravir.

The development of a COVID-19 vaccine opens up opportunities for the development of an HIV vaccine, it can contribute to broader advancements in vaccine development and immunology. The development of COVID-19 vaccines has utilized various vaccine platform technologies, such as mRNA, viral vector, and protein subunit vaccines. These platforms can be adapted and applied to other viral diseases, including HIV. It is noteworthy that in 2023 Nobel laureates in Physiology or Medicine became Dr. Katalin Karikó and Dr. Drew Weissman for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19 [15]. They both hope that mRNA vaccine technology will help create an effective anti-HIV vaccine. Lessons learned from the development of COVID-19 vaccines can help researchers explore and optimize similar platform technologies for HIV vaccine candidates.

The following comprehensive review covers some of the most exciting trials of prophylactic and therapeutic vaccines against HIV with a detailed description of the mechanism, entering a new milestone in their existence.

2. HIV Structure (Briefly)

HIV belongs to the *Retroviridae* family, genus *Lentivirus*, a single-stranded RNA-containing, enveloped, reverse transcribed virus. It causes HIV infection, the terminal stage of which is AIDS [16,17].

HIV isolates are classified into two types: HIV-1 and HIV-2. The main causative agent of AIDS worldwide is HIV-1, which is ubiquitous, while the less virulent HIV-2 is limited to West Africa and South Asia [16–22].

The virion is spherical, 80-100 nm in size, covered on the outside with a shell formed during budding through the plasma membrane of the cell. The envelope contains glycoprotein (gp) spikes consisting of surface gp120* and transmembrane gp41 (cleavage products of gp160 precursor protein). The core of HIV is like a truncated cylinder. Under the shell are p17 matrix protein, p24 capsid protein, nucleoproteins (p7, p6, p9), as well as enzymes (reverse transcriptase - p66 / p51, integrase - p31-32 and protease - p10, p11). The capsid contains two identical plus-strand copies of

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the RNA genome containing the structural genes *gag*, *pol*, *env*, etc. [23]. Structural elements of HIV are potential targets for vaccine candidates (Figure 1).

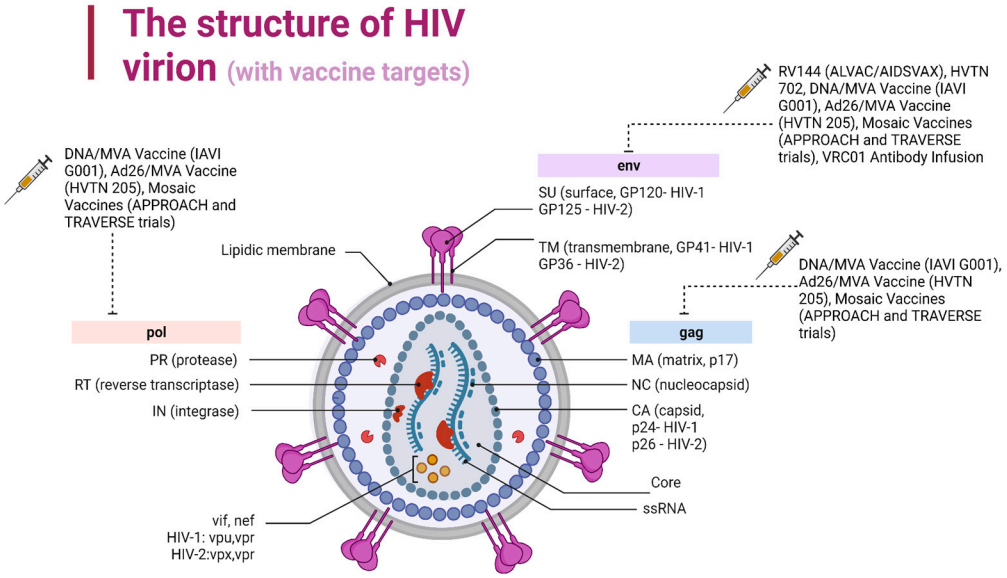

Figure 1. The structure of HIV with vaccine targets. Created with Biorender.com.

2.1. HIV-1 Gene-Encoded Proteins and Their Functions

The gp120 glycoprotein binds to the CD4 receptor and the co-receptor of the plasma membrane of cells. It has constant (C1-C4) and variable (V1-V5) regions that form 9 antigenic epitopes. Hypervariable region V3 interacts with T-lymphocyte; it consists of 4-8 amino acids. HIV-1 is divided into three groups - M, N, O. Most of the isolates belong to the M group, in which 10 HIV subtypes are distinguished (A, B, C, D, F-1, F-2, G, H, J, K). The high variability of HIV is associated with genetic errors that occur during complex multi-stage reproduction of viruses (due to increased mutability of the gene region encoding gp120) (Table 1) [24–27].

Table 1. HIV genes with description of functions.

Gene	Function
Gag (structural)	Encodes p24 (capsid), p7, and p6 core proteins and p17 matrix protein
Pol (structural)	Encodes for reverse transcriptase, integrase, and protease; Reverse transcriptase transforms viral RNA into DNA, integrase incorporates viral DNA into the chromosomal DNA of the host, and protease cleaves huge gag and Pol protein precursors into their components, all of which are required for viral replication.
Env (structural)	Encodes for gp120 and gp41, the glycoproteins of the viral envelope which target the receptors of the cell surface
Tat (regulatory)	Encodes for the Tat protein, which is produced early after infection and increases HIV gene expression
Vif (auxiliary)	Encodes for a small protein called Vif that promotes the infectivity of the viral particles
Vpu (auxiliary)	Encodes for a protein called Vpu that takes part in the arrest of the cell cycle

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Rev (regulatory)	Encodes for a protein called Rev that regulates the nuclear export of the mRNA
Nef (auxiliary)	Encodes for the Nef protein, which modulates cellular signaling and increases the downregulation of the cell surface's CD4 receptors, allowing viral replication.

Gag: group antigen; Env: envelope; Tat: trans-activator of transcription; Vif: virion infectivity factor; Vpu: viral protein U; Rev: regulator of expression of virion proteins; Nef: negative factor; gp: glycoprotein [16].

HIV-2 instead of HIV-1 proteins (gp120, gp41, gp160, p24) contains gp140, gp105, gp36, p26 proteins.

2.2. Mechanism of Infection

The virus binds via the glycoprotein gp120 to the CD4-T-helper receptor and cells of macrophage origin (macrophages, dendritic and microglial cells). The second receptor is the CXCR4 chemokine receptor on T-lymphocytes or the CCR5 chemokine receptor on macrophages. HIV enters the cell by fusion of the envelope with the plasma membrane of the cell through the glycoprotein gp41. After deproteinization of the virion in the cytoplasm of the cell, complementary (proviral) DNA is synthesized on the RNA template using viral reverse transcriptase, which, as part of the preintegration complex, penetrates the nuclear membrane and integrates with the cell DNA using viral integrase. The resulting DNA-provirus included in the chromosomal DNA can be latent or serve as the basis for the synthesis of viral genomic RNA and mRNA, which provide the synthesis and assembly of virions. Virions exit the cell by budding: the core of the virus "dresses" in the altered plasma membrane of the cell. If normally immune cells are activated, they begin to translate and transcribe the proteins necessary for the immune response, then in the presence of HIV in the body, infected immune cells facing any infection transcribe and translate new viruses that are cleaved from the cell membrane and infect more cells [16–21,28,29].

3. Preventive Vaccines

3.1. Vaccines Based on the Induction of Neutralizing Antibodies 1986–2003

Research into developing a vaccine against HIV began in 1986. The first trials were based on the assumption that the activation of neutralizing antibodies would be sufficient to protect against HIV infection. The first HIV vaccines were developed on the basis of recombinant DNA gene modification technology, similar to the hepatitis B vaccine licensed in 1986 [24,30,31]. Between 1988 and 2008, various HIV vaccine designs were developed based on the viral envelope glycoproteins (mainly gp120 and gp160), which are responsible for binding the virus to target cells and serve as the primary targets for neutralizing antibodies. Among the first to begin testing VaxSyn (1988-2003) is the recombinant enveloped glycoprotein (rgpl60) created in the baculovirus-insect cell system. Subsequently, numerous envelope proteins were assessed in an additional 35 studies.

Collectively, these vaccine constructs induced binding and neutralizing antibodies, which were long-lasting, and also stimulated CD4+ T cell responses, but did not produce overt cytotoxic lymphocytes (CD 8+ CTL) responses [32]. The induction of CTL responses, which was already perceived as a desirable characteristic of an HIV vaccine, could be achieved through the use of live vectors, especially poxvirus vectors [33]. Live vectors were very appealing because they could present antigens to the immune system simulating natural infection. In 1986, two different groups reported the expression of the HIV envelope gene in vaccinia vectors [34,35].

One of them was HIVAC-1e (1988), a recombinant vaccinia virus designed to express HIV gp160. In the majority of subjects, T-cell responses were transient and no antibodies to HIV were detected. However, the appearance of a sustained T-cell response in a small proportion of subjects led researchers to the idea that antibody responses would be enhanced by priming with a recombinant vaccinia vector expressing the HIV-1 envelope and then with an envelope booster protein [36]. VaxSyn + HIVAC-1e (1991) – combined approach. This trial was started with HIVAC-1e and then

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boosted with VaxSyn. The prime-boosting approach enhanced both the humoral and cellular immune response and induced neutralizing antibodies [37,38]. At this stage, one of the features of the vector used became apparent: there was a marked decrease in the immunogenicity of the vector in individuals previously vaccinated against smallpox [31,36].

Of course, the further refusal to use the cowpox vector was largely influenced by the fact that the introduction of such a vaccine is contraindicated for people who already have immunosuppression, which means that its use is unsafe in groups at high risk of HIV infection [39,40]. It was decided to use non-replicating poxvirus vectors in the early 1990s, based on two models, namely, a highly attenuated strain of vaccinia virus (NYVAC) or an avian poxvirus, canarypox (ALVAC), that is not able to replicate in mammalian cells [33,41].ALVAC-HIV Vector Vaccine, an HIV vaccine with the ALVAC vector, vCP125, expressing gp160 has been tested alone or as a combination primary booster with the adjuvant gp160 subunit. The vaccine significantly stimulated the response of neutralizing antibodies to protein administration and induced CTL activity [42]. Other ALVAC vectors (vCP205, vCP300, vCP1433, vCP1452, and vCP1521) have been designed not only to express the HIV envelope, but also to express gag and other HIV genes to induce a broader cell-mediated immune response. It was vCP1521 that was then used in the most successful trial of RV144. [43,44]. Vax003-Vax004 – (1994-) vaccines based on bivalent recombinant gp120 and alum provided protection to chimpanzees after HIV infection and were safe and immunogenic in phase 1/2 of human clinical trials [45,46]. As a result, high levels of both neutralizing antibodies (nAbs) binding (in VAX003 and VAX004) and antibody-dependent cellular inhibition of the virus (in VAX004) were observed, but both candidates were not effective in preventing HIV infection due to their narrow specificity [45,46].

3.1.1. Direct Cytotoxicity

Vaccines aimed at stimulating CTLs will cause pre-sensitized T-killers to immediately recognize HIV-infected cells and destroy them by the mechanism of direct cytotoxicity, preventing further replication of the virus. The mechanism of the cytotoxic effect is described below and in Figure 2.

CTLs secrete perforin proteins, which are incorporated into the cell membrane and oligomerize therein to form a pore. Next, CTLs secrete granzyme B, which enters the cell through the previously created pore. The cell cytosol contains procaspase 7, which consists of prodomain, large subunit and small subunit. Granzyme B proteolytically detaches the prodomain procaspase from the large subunit and also detaches the large subunit from the small subunit. Two executioner procaspase 7 molecules are required to form an "active" caspase. As a result, the two large and two small procaspase subunits join together to form a heterotetramer, which is active executioner caspase 7. Executioner caspase 7 starts proteolytically destroying the cell proteins leading to cell death.

It is important to note that proapoptotic proteins such as Bax and Bak are constantly present in the cell. Moreover, Bak is built into the outer mitochondrial membrane and Bax is freely present in the cell cytoplasm. The Bax molecule oligomerizes with Bak on the outer mitochondrial membrane forming a large pore in it through which cytochrome c escapes from the intermembrane space into the cell cytosol and triggers apoptosis. However, in the normal cell cytoplasm there are antiapoptotic proteins such as Bcl2 and BCl-XL, which bind to Bax and Bak preventing their oligomerization. Granzyme B proteolytically destroys Bcl2 and BCl-XL proteins and thus creates conditions for cytochrome c release into the cell cytosol [47].

The cell cytosol contains Apoptotic protease activating factor 1 (APAF1) which has a caspase recruitment domain (CARD) that binds to the active center of APAF1 itself and blocks it. But cytochrome c, once in the cytosol of the cell, pushes CARD from the active center of APAF1 and binds itself to it instead of CARD. Further, about 7 APAF1 molecules with active centers bound by cytochrome c bind to each other using their own CARD domains, forming the apoptosome. Further, CARDs associated with prodomain of seven initiator procaspase 9 interact with the CARDs that are part of the apoptosome. As a result of this interaction, the small subunit detaches from the large subunit of procaspase 9, and the large subunit also detaches from the prodomain. From there, a heterotetramer consisting of two large and two small subunits is assembled with the formation of the

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"active" initiator caspase 9, which itself will start proteolytically activating Caspase 3 and Caspase 6, which are the executioner caspases. Caspase 3 and Caspase 6 produce proteolysis of the cell proteins, which leads to cell death.

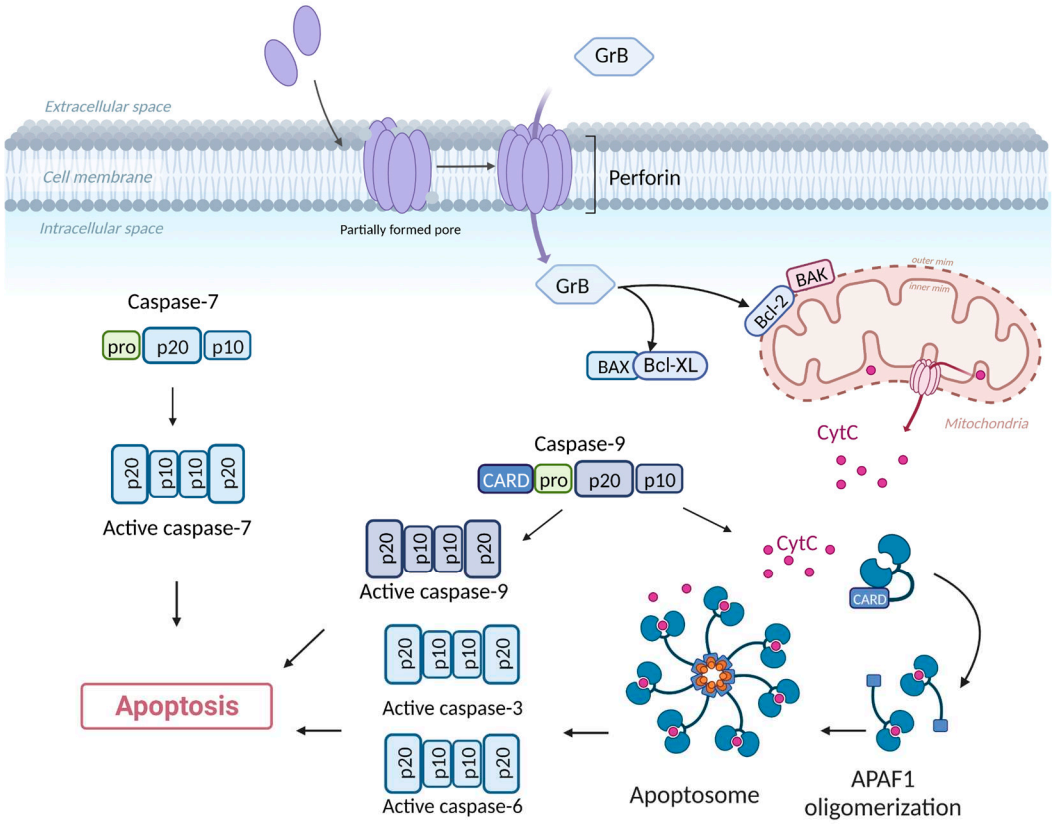

Figure 2. The mechanism of HIV vaccines aimed at stimulating CTLs. GrB - Granzyme B; APAF1 - Apoptotic protease activating factor 1; CARD - caspase recruitment domain; Bcl-2 - B-cell lymphoma 2; BAK - Bcl-2 homologous antagonist killer. Created with Biorender.com.

Perforins form a pore in the plasma membrane. Through this pore Granzyme B enter the cell. Granzyme B activates procaspase 7. Caspase 7 starts proteolytically destroying the cell proteins leading to cell death. Antiapoptotic proteins Bcl2 and BCl-XL bind to Bax and Bak preventing their oligomerization. Granzyme B proteolytically destroys Bcl2 and BCl-XL proteins and thus creates conditions for cytochrome c release into the cell cytosol. Cytochrome c binds to the APAF1 molecule. Further, about 7 APAF1 molecules with active centers bound by cytochrome c bind to each other using their own CARD domains, forming the apoptosome. Apoptosome activates initiator caspase 9, which in turn activates executioner caspases 3 and 6. Caspase 3 and Caspase 6 produce proteolysis of the cell proteins, which leads to cell death [48].

3.1.2. Chemokine-Mediated HIV Suppression

Chemokines based vaccines, especially CCL4 based vaccines will lead to CCR5 desensitization. This would result in HIV having no entry gate for T-helper cells to be infected. Although this type of vaccines will not protect somatic cells from infection, they will prevent the development of AIDS. The mechanism of CCR5 desensitization is described below and in Figure 3.

Being a ligand for CCR5, CCL4 in high amount can overstimulate CCR5, that will lead to the phosphorylation CCR5 by CD28-induced G-protein-coupled receptor kinase 2 (GRK2). b-arrestin binds to the phosphorylated inorganic phosphate molecules. Then, b-arrestin changes its conformation and releases a site for binding to the Adaptor protein complex 2 (AP-2 complex).

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However, this is not enough for the AP-2 complex to join the b-arrestin. The AP-2 complex consists of four proteins called adaptins: two large adaptins (α and β), a medium adaptin (μ), and a small adaptin (σ). The α and β adaptins have so-called 'ears' that form the appendage domain. The remaining part of α and β adaptins, as well as μ and σ adaptins together form the core domain. In general, the AP-2 complex has one core domain and two appendage domains. The core domain binds to synaptotagmin ½, which is embedded in the cell membrane. It is important to know that this interaction is stable only if PIP2 is present in the cell membrane with which the AP-2 complex also binds. After the previously mentioned interactions, the AP-2 complex changes its conformation and is activated, which leads to the interaction of μ-adaptin with b-arrestin.

The cell cytosol contains clathrin protein, which consists of a heavy chain comprising three domains: proximal, distal, and N-terminal, and a light chain bound to the proximal domain of the heavy chain. Clathrin is not found as a monomer in the cell cytosol. The three clathrin molecules bind to each other using the proximal heavy chain domain to form claritine triskelion, which is present in the cell cytosol. The claritine triskelion then binds to the appendage domains of the AP-2 complex, which will lead to polymerization of the triskelion, forming a closed three-dimensional network resembling a soccer ball. As a result of these processes, the plasma membrane is woven inward to form the Clatrin coated vesicle bud [49,50].

The cell requires Ca^{2+} for normal clathrin-dependent endocytosis. Ca^{2+} ions bind to calmodulin, forming the calmodulin/4Ca^{2+} complex. In turn, the calmodulin/4Ca^{2+} complex binds to calcineurin activating it. In the cytoplasm of the cell there is a protein dynamin 1 with which an inorganic phosphate molecule is bound and inhibiting it. Calcineurin dephosphorylates dynamin 1 and thereby activates it. Once activated, dynamin 1 binds to the "neck" of the Clatrin coated vesicle bud and oligomerizes, covering it completely. dynamin 1 has GTPase activity and having enveloped the "neck" of the clathrin coated vesicle bud it begins to hydrolyze GTP to GDP and inorganic phosphate, releasing energy which is used to enhance the torsion of oligomerized dynamin 1 around the "neck", narrowing and eventually cutting it, forming the clathrin coated endocytic vesicle. In the process of cutting the neck, endophilin also plays an important role, which is normally in the homodimerized state and also binds to the "neck" and then polymerizes around it in the form of a ring. This happens because endophilin has "+" charged amino acids, which are attracted to the "-" charged heads of the cell membrane phospholipids.

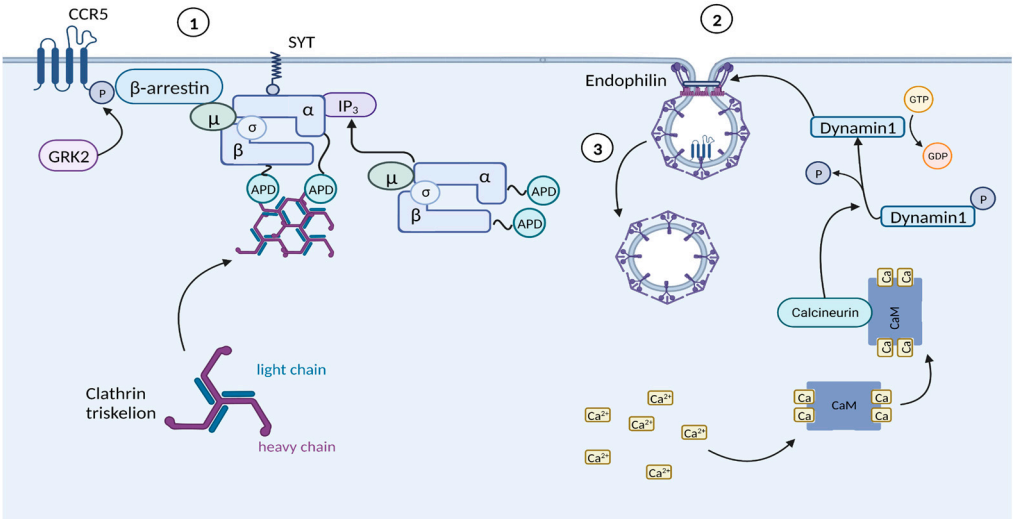

Figure 3. The mechanism of HIV vaccines aimed at chemokine-mediated suppression. CCR5 – C-C chemokine receptor type 5; GRK2 - G-protein-coupled receptor kinase 2; AP-2 - Adaptor protein complex; GTP – Guanosine triphosphate; GDP – Guanosine diphosphate. Created with Biorender.com.

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Phosphorylation of CCR5 by GRK2. B-arrestin binds with the inorganic phosphate molecules of CCR5. Then AP-2 complex binds with B-arrestin. To form a stable complex, AP-2 interacts with SYT and PIP2, embedded in the plasma membrane. Three clatrin molecules bind to each other and form clatrin triskelion. The clatrin triskelion then binds to the AP-2 complex. As a result of these processes, the plasma membrane is woven inward to form the Clatrin coated vesicle bud. Ca^{2+} ions bind to calmodulin, forming the calmodulin/$4Ca^{2+}$ complex. The calmodulin/$4Ca^{2+}$ complex binds to calcineurin and activates it. Calcineurin dephosphorylates dynamin 1 and thereby activates it. Once activated, dynamin 1 binds to the "neck" of the Clatrin coated vesicle bud and oligomerizes, covering it completely. Having enveloped the "neck" of the clathrin coated vesicle bud dynamin 1 begins to hydrolyze GTP to GDP and inorganic phosphate, releasing energy which is used to enhance the torsion of oligomerized dynamin 1 around the "neck", narrowing and eventually cutting it, forming the clathrin coated endocytic vesicle. Endophilin also plays an important role in forming the clathrin coated endocytic vesicle [51,52].

3.2. Stimulation of T-Cell Immune (1995–2007)

Further study of the problem and analysis of the unsatisfactory results of previous years of research prompted scientists to use the T-cell immune response. In the corresponding period of time, the degree of knowledge of the virus also increased. The leading role of CD8+ T-cells in the containment of virus replication at one of the stages of its development in the body has been proven.

Subsequent studies have been conducted with increasing reliance specifically (and often, only) on the T-response [53,54]. Candidate model vaccine vectors used for T-cell vaccines were live recombinant viral vectors, mainly pox and adenovirus vectors (particularly replication-defective adenovirus 5 (Ad5)), as well as DNA vaccines [55].

STEP trial - HVTN 502 – HVTN 503 (Phambili) - recombinant replication-defective adenovirus type 5 (Ad5) vector vaccines: MRKAd5 HIV-1 gag/pol/nef clade B vaccine. Both trials were primarily aimed at reducing post-infection viral load, but were interrupted early because those who were vaccinated had an increased risk of infection [31,56,57].

HVTN 505 - DNA vaccine expressing clade B gag/pol/nef and env from branches A, B and C, which was used to prime rAd5 multiclade vaccines. The goal was to activate both arms of immunity. An increased risk of infection was not observed, but the vaccination schedule was not shown to be effective [58–60].

RV-144 - the study design consisted of a recombinant canarypox vector vaccine, ALVAC-HIV (vCP1521), expressing Env (branch E), group specific antigen (Gag) (branch B) and protease (Pro) (branch B), and AIDSVAX B vaccines /E with an alum adjuvant and a bivalent subunit vaccine based on HIV glycoprotein 120 (gp120). The vaccine showed significant effectiveness in protecting against HIV infection - 31.2% in 42 months after vaccination. Vaccine-induced reactions included binding of IgG antibodies to HIV Env variable loops 1 and 2 (V1V2) and antibody-dependent cellular cytotoxicity (ADCC) in low-IgA vaccine recipients. The study showed that the size and multifunctionality of Env-specific CD4+ T cells play a role in reducing the risk of HIV infection [61,62]. RV305- RV306- Evaluation of the immune response generated in recent booster vaccine recipients compared to RV144 vaccine recipients. The assumption was that late boosters will elicit a sustained immune response [62,63].

HVTN 097 - the schedule included 2 doses of ALVAC-HIV (vCP1521) HIV vaccine followed by 2 booster doses of AIDSVAX B/E. Tetanus immunization was included in the schedule to avoid cross-reaction to the vaccine [64]. The primary booster schedule induced mainly Env-specific CD4+ T cell responses at significantly higher levels compared to RV144 vaccine recipients (RV144 = 36.4%; HVTN 097 = 51.9%). IgG antibodies recognizing the V1V2 region and IgG3 binding antibody responses to gp120 and V1V2 antigens were also significantly higher among HVTN 097 vaccine recipients compared to RV144 recipients. ADCC antibody responses were also higher in HVTN 097 than in RV144 [64].

HVTN 100 is an ALVAC-HIV vector (vCP2438) expressing HIV gp120 Env (C branch of ZM96), gp41 Env, Gag and Pro (all B branches), and the bivalent protein vaccine with gp120 subtype C and

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MF59 adjuvant. The vaccine induced a greater frequency of IgG3 responses to Env gp120, significantly higher CD4+ T cell responses, and gp120 binding antibody responses compared to the RV144 schedule [65]. HVTN 100 exceeded levels that were modeled as necessary for protection.

HVTN 702 - evaluation of the efficacy, safety and tolerability of ALVAC-HIV (vCP2438) plus a bivalent gp120/MF59 subtype C vaccination regimen with primary booster. The vaccination schedule used in HVTN 702 has been modified to improve the efficiency and robustness of immune responses compared to RV144. The study was terminated due to the low efficacy of the vaccine [65,66].

The RV144 trial showed the best result ever for an HIV vaccine development approach, subsequent studies based on RV144 designed to enhance efficacy came to mixed conclusions. However, in all subsequent studies, special attention is paid to the endemicity of the HIV isolate (Table 2) [67].

Table 2. Clinical studies based on RV-144 results.

Study	Vaccine (Immunogen)	Location (site)	Target group	Date	Efficacy
HVTN 305 (NCT05781542)	ALVAC-HIV and AIDSVAX B/E	Thailand (Clade B)	162 women and men	2012-2017	No
HVTN 306	ALVAC-HIV and AIDSVAX B/E	Thailand (Clade B)	360 men and women aged 20–40 years	2012-2017	No
HVTN 097 (NCT02109354)	ALVAC-HIV (vCP1521) and AIDSVAX B/E	South Africa (Clade B/E)	100 black Africans (men and women) aged 18–40 years	2012-2013	No
HVTN 100 (NCT02404311)	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	South Africa (Clade C)	252 men and women	2015-2018	No

3.3. Mosaic HIV Vaccines

Polyvalent HIV-1 Gag, Pol and Env mosaic immunogens expressed by replication-incompetent adenovirus serotype 26 (Ad26) vector and optimized to provide maximum coverage of potential T-cell epitopes. The mosaic HIV vaccine causes the formation of multifunctional antibodies - neutralizing, non-neutralizing and binding. This approach solves the problem of narrow specificity of the candidate vaccine and affects different circulating virus isolates [68]. However, large clinical studies of mosaic HIV vaccines have not shown their effectiveness, although some of these studies are currently underway (Table 3).

Table 3. Largest clinical study of mosaic HIV vaccines.

Study	Vaccine (Immunogen)	Location (site)	Target group	Date	Efficacy
VaxSyn	Recombinant envelope glycoprotein subunit (rgp160) of HIV	Canada (Clade B)	72 adults	1987	No
HIVAC-1e	Recombinant vaccinia virus designed to express HIV gp160	USA (Clade B)	35 male adults	1988	No
VAX003	AIDSVAX B/E	Thailand	2,545 men and	1998-	No

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(VaxGen) (NCT00006327)	(subtype B - MN; subtype AE - A244 rgp120)	(Clade B/E)	women IDUs	2002	
VAX004 (VaxGen) (NCT00002441)	AIDSVAX B/B (subtype B - MN and GNE8 rgp120)	North America (Clade B)	5,417 MSM and 300 women	1999-2003	No
STEP HVTN502	Ad5 expressing subtype B Gag (CAM-1), Pol (IIIB), Nef (JR-FL)	North America the Caribbean South America, and Australia (Clade B)	3,000 MSM and heterosexual men and women	2004-2007	No
Phambili HVTN 503 (NCT00413725)	Ad5 expressing subtype B Gag (CAM-1), Pol (IIIB), Nef (JR-FL)	South Africa (Clade C)	801 adults	2003-2007	No
RV 144 (NCT00223080)	ALVAC-HIV (vCP1521) expressing Gag and Pro (subtype B LAI), CRF01_AE gp120 (92TH023) linked to transmembrane anchoring portion of gp41 (LAI) AIDSVAX B/E Aluminium hydroxide	Thailand (Clade B)	16,402 community-risk men and women	2003-2009	Yes 31%
HVTN 505 (NCT00865566)	6 DNA plasmids - subtype B Gag, Pol, Nef and subtypes A, B and C Env 4 rAd5 vectors - subtype B Gag/Pol and subtypes A, B and C Env	United States (Clade B)	2,504 men or transgender women who have sex with men	2009-2017	No
Uhambo HVTN 702 (NCT02968849)	ALVAC-HIV (vCP2438) expressing Gag and Pro (subtype B LAI), subtype C gp120 (ZM96.C) linked to transmembrane anchoring portion of gp41 (LAI)	South Africa (Clade C)	5,400 men and women	2016-2021	No
IMBOKODO HVTN 705 (NCT03060629	Ad26.Mos4.HIV Subtype C gp140	Sub-Saharan Africa	2600 women	2017-2022	No data

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)		(Clade C)			
MOSAICO HVTN 706 (NCT03964415)	Ad26.Mos4.HIV Subtype C gp140 or bivalent gp140 (subtype C/Mosaic)	Europe North America and South America (Clade C)	3800 MSM and transgender persons	2019-2024	No data
PrEPVacc (NCT04066881)	DNA-HIV-PT123 plasmid and AIDSVAX B/E or DNA-HIV-PT123 plasmid with trimeric CN54gp140; MVA-CMDR (Chang Mai double recombinant) and trimeric CN54gp140 Concurrent PrEP administration of either TAF/FTC or TDF/FTC	Uganda, Tanzania, Mozambique, Republic of South Africa (Clade C)	1668 Adults Men and Women	2020-2023	No data

3.4. mRNA HIV Vaccine

mRNA platforms are designed to deliver a piece of genetic material that instructs the body to make a protein fragment of a target pathogen (such as HIV), which the immune system will hopefully recognize and mount a defense against [69].

mRNA technology may be an important step forward to speed identification of the right target antigens for a protective response, but it alone does not address other challenges associated with HIV vaccine development, such as what antigen will be right. B-cells play an important role in the action of these vaccines, which implement their immune response through the BCR. The mechanism of mRNA HIV vaccine is discussed below and in Figure 4.

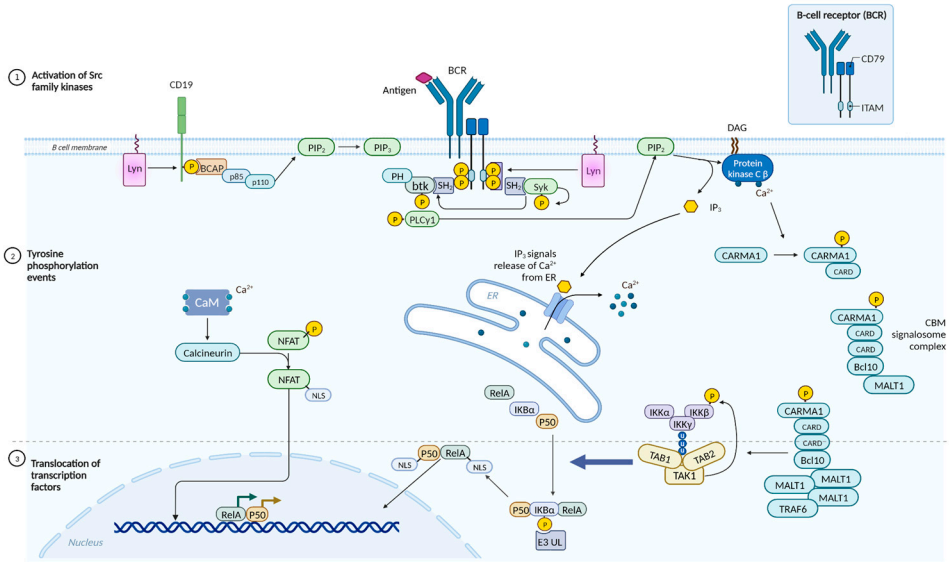

Figure 4. The mechanism of mRNA HIV vaccine. BCR - B cell antigen receptor; SH2 - SRC homology 2; BLNK - B-cell linker protein; BTK - Bruton's tyrosine kinase; PIP3 - phosphatidylinositol (3,4,5)-trisphosphate; PH - pleckstrin homology; IP3 - inositol triphosphate; DAG – diacylglycerol; PLCγ2 -

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phospholipase Cγ2; PIP-2 - phosphotidylinositol diphosphate; PKCβ - Proteinkinase Cβ; NFAT - Nuclear factor of activated T-cells; CARMA11 - caspase recruitment domain family member 11; MALT1 – Mucosa-associated lymphoid tissue lymphoma translocation protein 1; BCL-10 - B cell CLL/lymphoma 10; TRAF6 - tumor necrosis factor receptor-associated factor 6; IKK - IκB kinase; NF-κb - Nuclear Factor kappa b; BCAP - B cell adaptor molecule for PI3K. Created with Biorender.com.

B cell antigen receptor (BCR) is embedded in the membrane of B cells. The BCR is composed of membrane immunoglobulin (mIg) molecules and associated Igα/Igβ (CD79a/CD79b) heterodimers (α/β). The mIg subunits bind antigen, resulting in receptor aggregation, while the α/β subunits transduce signals to the cell interior. BCR aggregation rapidly activates the Src family of Lyn kinases. The cytoplasmic part of CD79a/b molecules contains immunoreceptor tyrosine-based activation motifs (ITAMs). The Lyn protein, which phosphorylates CD79a/b by the OH groups of the two tyrosine residues that make up the ITAMs, is embedded in the membrane of B cells. The Syk protein binds to the phosphorylated tyrosine residues using SRC homology 2 (SH2) domain, after which it phosphorylates itself by the OH groups of tyrosine (autophosphorylation), leading to its own activation. It is important to note that the Lyn protein can also activate Syk by phosphorylation [70].

The adaptor protein BLNK (B-cell linker protein) binds to the phosphorylated tyrosine residues of CD79a/b using the SH2 domain. Next, Syk phosphorylates the BLNK molecule by the tyrosine OH-group, facilitating the binding of phospholipase Cγ2 (PLCγ2) to it. But this is not enough to activate PLCγ2. The fact is that the Bruton's tyrosine kinase (BTK) molecule binds to the phosphorylated tyrosine residues of BLNK protein using its SH2 domain, after which BTK binds to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which is a phospholipid of the inner layer of the cell membrane using pleckstrin homology (PH) domain. The interaction of the PH domain with PIP3 leads to full activation of BTK protein. As a result, BTK phosphorylates and thereby activates PLCγ2. PLCγ2 begins to hydrolyze PIP2 (phosphotidylinositol diphosphate), which is a typical representative of cell membrane phospholipids, to inositol triphosphate (phospholipid head) and diacylglycerol (phospholipid tail). DAG remains in the cell membrane. IP3 binds to IP3 receptors located on the ER membrane and stimulates them. It is important to note that IP3 receptors are Ca^{2+} channels and that after interaction with IP3 they open, promoting the release of large amounts of Ca^{2+} ions from the ER into the cell cytosol along a concentration gradient. Ca^{2+} ions and DAG together activate PKCβ (Proteinkinase Cβ). In turn, Ca^{2+} binds to calmodulin, forming the calmodulin/4Ca^{2+} complex. In the quiescent state, the cell cytoplasm contains the molecule Nuclear factor of activated T-cells (NFAT), which is in a phosphorylated state and is a transcription factor. As long as NFAT is in the phosphorylated state it cannot pass into the nucleoplasm. The calmodulin/4Ca^{2+} complex binds to the protein calcineurine resulting in its activation. Calcineurine, a protein phosphatase, dephosphorylates NFAT and thereby changes its conformation, leading to the availability of nuclear localization signals (NLS) and transport of this transcription factor into the nucleoplasm [71].

The cell cytosol contains a caspase recruitment domain family member 11 (CARD11 or CARMA1), which is in the inactive, "closed" state. PKCβ phosphorylates CARMA1 by the serine/threonine OH-group and thereby promotes a change in the conformation of this molecule and its transition from the "closed" to the "open" state. There is a BCL10-MALT1 heterodimer (B cell CLL/lymphoma 10-MALT1 paracaspase heterodimer) in the cytoplasm. After CARMA1 is in the "open" state, it is bound to BCL10 molecule through caspase recruitment domain (CARD)-CARD domain interactions, which is a part of BCL10-MALT1 heterodimer, forming CBM signalosome complex (The caspase recruitment domain family member 11 (CARD11 or CARMA1)-B cell CLL/lymphoma 10 (BCL10)-MALT1 paracaspase (MALT1)). Once this complex is formed, the MALT1 molecule begins to oligomerize, leading to the recruitment of tumor necrosis factor receptor-associated factor 6 (TRAF6) protein, which is an E3 ubiquitin ligase. The cell cytoplasm contains the molecule IKK (IκB kinase), which consists of three subunits: IKK-α, IKK-β, and IKK-γ (NEMO-NF-kappa-B essential modulator). TRAF6 modifies IKKγ with Lys63-polyUb chains, recruiting the kinase TAK1 through its ubiquitin-binding cofactors TAB2 and TAB3. TAK1 then phosphorylates IKK-β, leading to activation of the IKK molecule. It is important to mention that the B-cell cytosol contains the NF-κb molecule, which consists of three subunits: IkBα (Inhibitor of Nuclear Factor (NF)-Kb α

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isoform), p50, and RelA. In the resting state, IkBα masks the NLS (nuclear localization sequence) of p50 and RelA proteins, preventing transport of these subunits as a heterodimer from the cell cytosol into the nucleoplasm. However, upon activation, IKK starts phosphorylating the IkBα subunit by the OH-groups of the serine/threonine residues. Further, the enzyme E3 ubiquitin ligase binds to the phosphorylated serine/threonine residues, promoting ubiquitination and further proteosomal degradation of IkBα. As a result, NLS is unmasked and the p50/RelA heterodimer is transferred from the cell cytoplasm into the nucleoplasm [72].

The B-cell membrane contains a CD19 protein, the intracellular part of which is phosphorylated by Lyn protein by the OH-group of tyrosine. The phosphorylated tyrosine residues of CD19 are bound to the BCAP (B cell adaptor molecule for PI3K) molecule, which recruits the p85 domain of PI3K protein to itself. This interaction leads to the activation of the p110 domain of PI3K, which with its catalytic activity phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to produce and accumulate phosphatidylinositol-3,4,5-trisphosphate (PIP3). On the one hand, PIP3 is important for binding the BTK molecule to the inner surface of the cell membrane, and on the other hand, it is important for the activation of the Akt/mTOR pathway.

BCR aggregation rapidly activates the Src family of Lyn kinases. The Lyn protein phosphorylates CD79a/b by the OH groups of the two tyrosine residues. The Syk protein binds to the phosphorylated tyrosine residues using SH2 domain, after which it phosphorylates itself by the OH groups of tyrosine (autophosphorylation), leading to its own activation. The adaptor protein BLNK binds to the phosphorylated tyrosine residues of CD79a/b using the SH2 domain. Syk phosphorylates the BLNK molecule, facilitating the binding of PLCγ2 to it. BTK molecule binds to the phosphorylated tyrosine residues of BLNK protein using its SH2 domain, after which BTK binds to PIP3 using PH domain. As a result, BTK phosphorylates and thereby activates PLCγ2. PLCγ2 begins to hydrolyze PIP2 to IP3 and DAG. IP3 contributes to the output of Ca^{2+} form the ER. Ca^{2+} ions and DAG together activate PKCβ. Ca^{2+} binds to calmodulin, forming the calmodulin/4Ca^{2+} complex. The calmodulin/4Ca^{2+} complex binds to the protein calcineurine resulting in its activation. Calcineurine, a protein phosphatase, dephosphorylates NFAT and thereby leads to the transport of this transcription factor into the nucleoplasm. PKCβ phosphorylates CARMA1. CARMA1 binds to BCL-10 molecule, which is a part of BCL-10-MALT1 heterodimer, forming CBM signalosome complex. Once this complex is formed, the MALT1 molecule begins to oligomerize, leading to the recruitment of TRAF6 protein. TRAF6 modifies IKKγ with Lys63-polyUb chains, recruiting the kinase TAK1 through its ubiquitin-binding cofactors TAB2 and TAB3. TAK1 then phosphorylates IKK-β, leading to activation of the IKK molecule. IKK starts phosphorylating the IkBα subunit of NF-κb. Further, the enzyme E3 ubiquitin ligase binds to the phosphate groups of IkBα, promoting ubiquitination and further proteosomal degradation of IkBα. As a result, NLS is unmasked and the p50/RelA heterodimer is transferred from the cell cytoplasm into the nucleoplasm. Intracellular part of CD19 protein is phosphorylated by Lyn. With that phosphate groups BCAP protein binds. BCAP recruits PI3K, which phosphorylates PIP2 to PIP3. On the one hand, PIP3 is important for binding the BTK molecule to the inner surface of the cell membrane, and on the other hand, it is important for the activation of the Akt/mTOR pathway [70,73].

The Table 4 below summarizes ongoing vaccine trials on the mRNA platform. The difference is in the antigens, the viral proteins that the immune system targets.

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Table 4. Ongoing trials of preventive mRNA HIV vaccines.

Trial	Name	Hypothesis	Year	Target group	Site	Vaccine Candidates	Immunogene design	Vaccine Manufacturer
IAVI G002 NCT05001373	A Phase 1 Study to Evaluate the Safety A Phase I Trial to and Immunogenicity of eOD-GT8 and Immunogenic 60mer mRNA Vaccine (mRNA-1644) delivered by an m and Core-g28v2 60mer mRNA Vaccine negative adults (mRNA-1644v2-Core	Sequential vaccination by a germline-targeting prime followed by directional boost immunogens can induce specific classes of B-cell responses and guide their early maturation toward broadly neutralizing antibody (bnAb) development through an mRNA platform	2021-2023	56 adults ages 18 to 50	4 sites in the US (Atlanta; San Antonio; Seattle; Washington, DC)	Two experimental HIV vaccines based on messenger RNA (mRNA) platform: 1. eOD-GT8 60mer mRNA Vaccine (mRNA-1644) 2. Core-g28v2 60mer mRNA Vaccine (mRNA-1644v2-Core)	IAVI Neutralizing Antibody Center (NAC) at Scripps Research	Moderna

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IAVI G003 NCT05414786	A Phase I Trial to Evaluate the Safety and Immunogenicity of eOD-GT8 60mer delivered by an mRNA platform in HIV negative adults	eOD-GT8 60mer delivered by an mRNA platform in HIV negative adults will induce immune responses in African populations as was seen in IAVI G001, which demonstrated this recombinant protein (eOD-GT8 60mer) safely induced immune responses in 97% of recipients, who were healthy U.S. adults	2022-2023	18 healthy, HIV-negative adults	2 sites: Kigali, Rwanda, and Tembisa, South Africa	One experimental HIV vaccine based on messenger RNA (mRNA) platform: 1. eOD-GT8 60mer delivered by an mRNA Vaccine platform (mRNA-1644)	IAVI Neutralizing Antibody Center (NAC) at Scripps Research	Moderna

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HVTN 302 NCT05217641	A Clinical Trial to Evaluate the Safety and Immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV Trimer mRNA Vaccines in Healthy, HIV-uninfected Adult Participants	The BG505 MD39.3 soluble and membrane-bound trimer mRNA vaccines will be safe and well-tolerated among HIV-uninfected individuals and will elicit autologous neutralizing antibodies	2022-2023	108 adults ages 18 to 55 years	11 sites in the US (Birmingham; Boston; Los Angeles; New York City; Philadelphia; Pittsburgh; Rochester; Seattle)	Three experimental HIV vaccines based on messenger RNA (mRNA) platform: 1. BG505 MD39.3 mRNA 2. BG505 MD39.3 gp151 mRNA 3. BG505 MD39.3 gp151 CD4K0 mRNA	Scripps Consortium for HIV/AIDS Vaccine Development (CHAVD) and IAVI Neutralizing Antibody Center (NAC) at Scripps Research	Moderna

4. Therapeutic HIV Vaccines

The idea of developing therapeutic vaccines is to treat already infected patients by enhancing their immune response against the virus [74,75].

4.1. Dendritic Cell-Based HIV Vaccines

One of the problems of our immune system's fight against HIV is the reduced ability of professional antigen-presenting cells to present HIV antigens on their surface. This approach consists in using professional antigen presenting cells (dendritic) to activate T cells, which, in turn, are an important step in specific immune activity in both innate and adaptive pathways. To achieve this goal, antigens are targeted to dendritic cells using different strategies. The most serious trials of dendritic cell-based HIV vaccines are presented in the Table 5. Most studies have been conducted on autologous DCs [76–78].

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Table 5. Ongoing trials of dendritic cell-based HIV vaccines.

Trial	Phase	Registry Identifier	Result	Status	Last Update	Ref.
AGS-004 (personalized therapeutic vaccine utilizing patient-derived dendritic cells and HIV antigens)	IIb	NCT00672191	Induction of CD4 and CD8, reduction of VL, One severe adverse event	Completed	2013	[79]
Autologous HIV-1 ApB DC Vaccine	I/II	NCT00510497	Safe and immunogenic Reduction of HIV blood reservoir	Completed	2016	[80]
Dendritic cell vaccine (DCV-2)	I/II	NCT00402142	Safely induced marginal immune responses, whereas markedly increased Vacc-C5-induced regulatory T cell	Completed	2014	[81]
Dendritic cells loaded with HIV-1 lipopeptides	I	NCT00796770	Safe and showed few CD8 T cell responses	Completed	2017	[82]

The great advantage of these vaccines is the activation of CTLs against HIV-infected cells, which fulfills a therapeutic role. The mechanism of CTLs activation by dendritic cells is described below and in Figure 5.

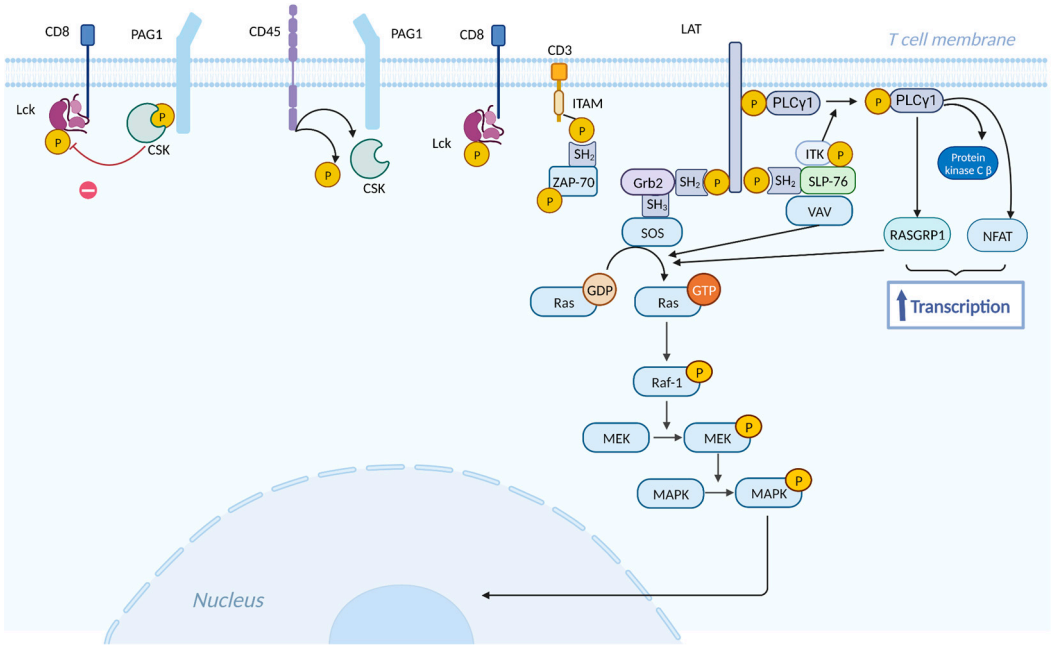

Figure 5. The mechanism of dendritic cell-based HIV vaccine. Lck - lymphocyte-specific protein tyrosine kinase; CSK - C-terminal Src kinase; PAG1 - Phosphoprotein associated with glycosphingolipid-enriched microdomains 1; ITAMs - immunoreceptor tyrosine-based activation motifs; ZAP-70 binds to the phosphorylated tyrosine residues of CD3; LAT - linker for activation of T cells; GADS - GRB2-related adaptor protein of Shc; GRB2 - growth-factor receptor-bound protein 2; PLCγ1 - phospholipase Cγ1; SOS - Son of Sevenless; RAF-1 - proto-oncogene serine/threonine-protein kinase; GDP – Guanosine diphosphate; GTP – Guanosine triphosphate; MEK - MAPK/ERK kinase; MAPK - mitogen-activated protein kinase; ITK - interleukin-2-inducible T-cell kinase; SLP-76 - SH2-

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domain-containing leukocyte protein 76 kDa; RASGRP1 - guanyl nucleotide-releasing protein RAS; DAG - diacylglycerol; NFAT - Nuclear factor of activated T-cells. Created with Biorender.com.

CD8+ T-cells activation plays an important role here. The CD8 glycoprotein is embedded in the Cytotoxic T-cells (CTL) membrane and its cytoplasmic part is reacted by lymphocyte-specific protein tyrosine kinase (Lck). Phosphoprotein associated with glycosphingolipid-enriched microdomains 1 (PAG1) is also embedded in the cell membrane. PAG1 is an adaptor protein whose cytoplasmic part contains inorganic phosphate molecules. C-terminal Src kinase (CSK) interacts with the previously mentioned inorganic phosphate molecules of PAG1. Thus, PAG1 brings CSK in close proximity to the Lck molecule. CSK aids to limit Lck activity via Tyr505 phosphorylation. However, it is important to note that the Protein tyrosine phosphatase, receptor type, C (PTPRC) molecule also known as CD45 is embedded in the cell membrane. After CD8 interacts with the MHC-1 molecule on the APC membrane, CD45 is activated and begins to dephosphorylate the cytoplasmic part of PAG1, resulting in the uncoupling of CSK from the PAG1 molecule. This mechanism results in the distancing of the CSK from the cell membrane and its inability to phosphorylate and inactivate the Lck. As a result, Lck is activated and phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 (cluster of differentiation 3) by the OH group of tyrosine. This creates the opportunity for the SH2 domain of the ZAP-70 protein (Zeta-chain-associated protein kinase 70) to bind to the phosphorylated tyrosine residues. Next, Lck phosphorylates ZAP-70 by the OH-group of tyrosine, which leads to a change in its conformation and activation of the kinase domain [83,84].

The linker for activation of T cells (LAT) protein is built into the membrane of CD8+ T- cells and is a target for ZAP-70 molecule, which phosphorylates LAT on multiple tyrosine residues. The inorganic phosphates on LAT protein serve as docking sites for SH2 domains of GRB2-related adaptor protein (GADS), growth-factor receptor-bound protein 2 (GRB2), and phospholipase Cγ1 (PLCγ1).

The SH3 domain of the GRB2 is bound to the proline-rich region of the Son of Sevenless (SOS) protein, which belongs to guanine nucleotide exchange factors (GEFs). In the quiescent state in the cell cytoplasm, there is RAS protein bound to GDP-inactive state. SOS detaches the GDP molecule from RAS and attaches GTP in its place-active state. Then RAS-GTP goes to the RAF-1 protein (proto-oncogene serine/threonine-protein kinase) and activates it. In turn, RAF-1 phosphorylates and thereby activates the enzyme MEK (MAPK/ERK kinase). MEK phosphorylates the mitogen-activated protein kinase (MAPK) by the OH group of serine/threonine (mitogen-activated protein kinase) and thereby activates it. Ultimately, MAPK phosphorylates and activates specific transcription factors that increase the expression of genes responsible for cytokine synthesis [85].

The SH3-domain of the GADS protein binds to the SH2-domain-containing leukocyte protein 76 kDa (SLP76), then ZAP-70 phosphorylates SLP-76 at critical tyrosine residues. This creates binding sites for the SH2 domain of VAV and ITK (interleukin-2-inducible T-cell kinase). In turn, VAV, being GEF, serves the same function as SOS-MAPK activation.

ZAP-70 phosphorylates ITK at tyrosine residues, which leads to its activation. As we mentioned above, the inorganic phosphate on the LAT protein serves as a docking site for the SH2 domain of PLCγ1. But this is not enough to fully activate PLCγ1. ITK phosphorylates PLCγ1 at tyrosine residues, which leads to its full activation. PLCγ1 activates the inositol triphosphate system, leading to DAG formation and increased Ca^{2+} concentration in the cell. Ca^{2+} by the already known mechanism activates the NFAT pathway. DAG activates and recruits the guanyl nucleotide-releasing protein RAS (RASGRP1) into the plasma membrane. RASGRP1, like SOS, is the GEF responsible for RAS activation in T cells. RAS, in turn, as we mentioned, activates the MAPK kinase pathway. DAG, together with Ca^{2+} ions, activates PKCθ, which acts in the same way as PKCβ in B cells. This will lead to the activation of the NF-κb pathway [83,84].

Cytoplasmic part of CD8 glycoprotein binds with Lck. CSK interacts with PAG1. Thus, PAG1 brings CSK in close proximity to the Lck molecule. CSK aids to limit Lck activity via Tyr505 phosphorylation. After CD8 interacts with the MHC-1 molecule on the APC membrane, CD45 is activated and begins to dephosphorylate the cytoplasmic part of PAG1, resulting in the uncoupling of CSK from the PAG1 molecule. As a result, Lck is activated and phosphorylates the ITAMs of the

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CD3. Next, Lck phosphorylates and activates ZAP-70. ZAP-70 molecule phosphorylates LAT on multiple tyrosine residues. LAT protein serves as docking site for GADS, GRB2 and PLCγ1 molecules. The SH3 domain of the GRB2 is bound to SOS. SOS converts RAS-GDP to RAS-GTP. Then RAS-GTP activates RAF-1 protein. RAF-1 activates MEK and MEK activates MAPK. Ultimately, MAPK phosphorylates and activates specific transcription factors that increase the expression of genes responsible for cytokine synthesis. GADS protein binds to the SLP-76,then ZAP-70 phosphorylates SLP-76. This creates binding sites for VAV and ITK. In turn, VAV, being GEF, serves the same function as SOS-MAPK activation. ZAP-70 phosphorylates ITK. ITK phosphorylates PLCγ1 at tyrosine residues, which leads to its full activation. PLCγ1 activates the inositol triphosphate system. Ca^{2+} activates the NFAT pathway, DAG activates and recruits the RASGRP1 into the plasma membrane. RASGRP1, like SOS, is the GEF responsible for RAS activation in T cells. DAG, together with Ca^{2+} ions, activates PKCθ [83,84].

4.2. Peptide-based HIV vaccines

The approach is to use peptides to elicit a cellular immune response against the most immunogenic or conserved domain of the target protein. The response can be elicited through naturally occurring sub-immunodominant epitopes, and the use of a multi-epitope approach will allow multiple strains and different stages of the virus life cycle to be targeted at once [86–89]. The most serious trials of peptide-based HIV vaccines are presented in the Table 6.

Table 6. Ongoing trials of peptide-based HIV vaccines.

Trial	Phase	Registry Identifier	Result	Status	Last Update	Ref.
Vacc-4x	II	NCT00659789	Induction of CD4 and CD8, reduction of VL, One severe adverse event	Completed	2017	[90]
VAC-3S	I/II	NCT01549119	Safe and immunogenic Reduction of HIV blood reservoir	Completed	2015	[91]
Vacc-C5	I/II	NCT01627678	Safely induced marginal immune responses, whereas markedly increased Vacc-C5-induced regulatory T cell	Completed	2014	[92]
AFO-18	I	NCT01141205	Safe and showed few CD8 T cell responses	Completed	2013	[93]
HIV-v	I	NCT01071031	Safe and can elicit T- and B-cell responses that significantly reduce viral load.	Completed	2012	[94]

4.3. DNA-Based HIV Vaccines

This approach allows both humoral and cellular immune responses to be elicited using small DNA fragments encoding the antigen (plasmids). Expression of the inserted gene of interest can be controlled using a strong mammalian promoter that can be located on a plasmid backbone of bacterial DNA. When target cells are transfected with DNA vaccines, the encoded proteins will be presented in the major histocompatibility complex (MHC) [95–99]. The most serious trials of DNA-based HIV vaccines are presented in the Table 7.

Table 7. Ongoing trials of DNA-based HIV vaccines.

Trial	Phase	Registry Identifier	Result	Status	Last Update	Ref.
Ad26.Mos.HIV + MVA-Mosaic	II	NCT02919306	Not reported	Completed	2018	[100]
MAG pDNA vaccine +/- IL-12	I	NCT01266616	Elicted CD4+ but not CD8+ T-cell responses to multiple HIV-1 antigens.	Completed	2015	[101]

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PENNVAX-B (Gag, Pol, Env) + electroporation	I	NCT01082692	Strong induction of CD8 T cell responses	Completed	2012	[102]

The mechanism of DNA-based HIV vaccines is described in Figure 6. These vaccines are important because they enhance the CD4+T-cell response. The fact is that MHC-II receptors with the antigen epitope are built into the macrophage membrane. Macrophages are approached by naive T-helpers (T0-helpers) whose membrane contains TCR and CD4. CD4 binds to MHC-II receptors, and TCR is necessary to recognize the antigen that is expressed on MHC-II. Once the antigen is recognized, the TCR transmits the signal to the T0-helper (primary signal) via CD3. Furthermore, on the surface of the T0-helper there is CD28, with which CD80/CD86, located on the surface of the macrophage, bind and also activate the T0-helper (secondary signal-activation). In addition, the macrophage secretes IL-1, which binds to IL-1R, which is on the surface of the T0-helper (tertiary activation-coactivation). As a result of all these activations, the T0-helper secretes IL-2, which acts through an autocrine mechanism. In addition to the secretion of IL-2, the T0-helper starts synthesizing and integrating the alpha chain of IL-2R into its membrane (beta and gamma chains are permanently incorporated into the T0-helper membrane), triggering the biochemical cascade discussed below [103].

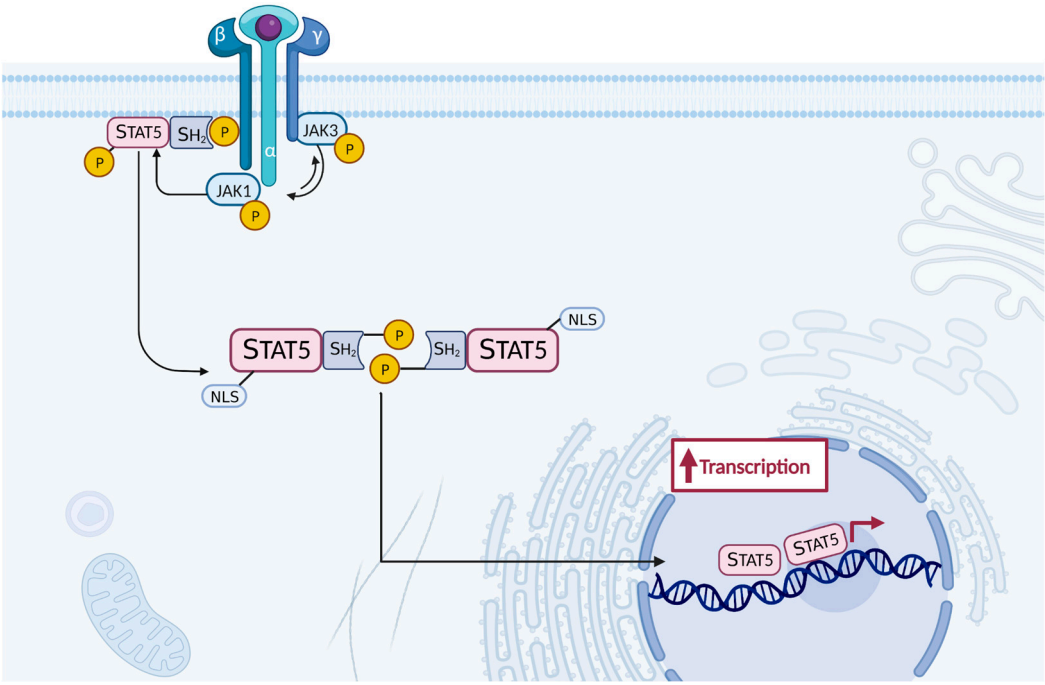

Figure 6. The mechanism of DNA-based HIV vaccine. JAK-1 - Janus kinase 1; JAK-3 - Janus kinase 3; STAT5 - Signal transducer and activator of transcription 5. Created with Biorender.com.

Janus kinase 1 (JAK1) is associated with the cytoplasmic part of the beta chain, and Janus kinase 3 (JAK3) is associated with the cytoplasmic part of the gamma chain. In the resting state, both are inactive. It is important to know that in the heterodimerized state, IL-2Rs have low affinity for IL-2. However, after attachment of alpha chain to beta and gamma chains the affinity of IL-2R to IL-2 increases dramatically. After the interaction of IL-2R with IL-2, JAK1/JAK3 change their conformation, which leads to transphosphorylation of JAK1 and JAK3 by the OH group of tyrosine residues located in a specific site called activation loop. This phosphorylation leads to a repeated change in the conformation of JAK1 and JAK3 so that they gain affinity to the cytoplasmic part of the beta chain and begin to phosphorylate it at the OH-group of tyrosine [103].

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At rest, there is a Signal transducer and activator of transcription 5 (STAT5) protein in the cytosol of the cell that binds to the phosphorylated tyrosine residues of the beta chain using its SH2 domain. Next, JAK1 and JAK3 phosphorylate STAT5 by the OH-group of tyrosine residues and it detaches back from the beta chain. In the cell cytoplasm, STAT5 homodimerizes (phosphorylated tyrosine residues of STAT5 proteins bind to each other's SH2 domains). Once STAT5 is homodimerized it undergoes conformational changes and NLS appears, which plays an important role in the transport of the STAT5 homodimer from the cytosol to the nucleoplasm. STAT5 is a transcription factor that increases the expression of genes responsible for T cell proliferation. This is important because when HIV infection occurs, the pool of CD4+ T-helper cells decreases significantly, which contributes negatively to the immune system's fight against HIV [103].

After the interaction of IL-2R with IL-2, JAK1/JAK3 transphosphorylate each other. This phosphorylation leads to a change in the conformation of JAK1 and JAK3 so that they gain affinity to the cytoplasmic part of the beta chain and begin to phosphorylate it. STAT5 binds to the phosphorylated tyrosine residues of the beta chain. JAK1 and JAK3 phosphorylate STAT5. STAT5 homodimerizes and enter the nucleoplasm.

4.4. Viral Vector-Based HIV Vaccines

The use of viral vectors makes it possible to induce a strong cytotoxic T-lymphocyte response through intracellular antigen expression, leading to the elimination of virus-infected cells.

Adenovirus and Vaccinia virus are the most widely applied vectors due to their potency inducing a robust immune response, specifically including CTL, to the expressed foreign antigens. The most serious trials of viral vector-based HIV vaccines are presented in the Table 8.

Table 8. Ongoing trials of viral vector-based HIV vaccines.

Trial	Phase	Registry Identifier	Result	Status	Last Update	Ref.
DC-HIV04 Comparison of Dendritic Cell-Based Therapeutic Vaccine Strategies for HIV Functional Cure	I	NCT03758625	Not reported	Recruting	2018	[104]
GCHT01	I	NCT01428596	Not reported	Active	2019	[105]
GTU-MultiHIV B-clade + MVA HIV-B (DNA + viral vector vaccines)	II	NCT02972450	Not reported	Not yet recruting	2018	[106]
THV01 (lentiviral vector-based therapeutic vaccine)	I/II	NCT02054286	Not reported	Active	2019	[107]
Ad26.Mos4.HIV + MVA-Mosaic or clade C gp140 + mosaic gp140	I	NCT03307915	Not reported	Recruting	2019	[108]

5. Conclusion

Since none of the previously developed HIV vaccine concepts in isolation achieves a sufficient percentage of efficiency, and the combination of several types of vectors or antigens in some trials increases the possibility of achieving the goal, but does not lead to any stable result, the combinatorial approach should be applied in another direction. The key to defeating HIV may lie in the activation of several links of immunity at once, the combination of vaccine and non-vaccine approaches (pre-exposure prophylaxis, microbicides, multipurpose prevention technologies).

However, there is still a need for a vaccine - the percentage of infected medical workers, blood transfusion patients and drug users is high. Perhaps the COVID-19 pandemic and the subsequent surge in mRNA-based development will lead to significant results.

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