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Article

Universal mRNA-Based HIV Vaccine for Acute and Latent Infections

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Abstract: Human Immunodeficiency Virus (HIV) infection and its latent recurrent infection make vaccine development a significant challenge for traditional vaccines; however, the mRNA delivery of multiepitope antigens capable of creating antibodies against the surface proteins of HIV-1 and HIV-2 along with their respective Nef proteins, responsible for the latent infection can bring a most effective, as well as affordable solution to the unresolved epidemics of HIV. In this paper, we present the complete design of a multiepitope vaccine to prevent acute infections and inactivate Nef proteins that block the MHC-1 and MHC-2 pathways to hide the virus for later reinfection.

Keywords: HIV-1 1; HIV-2 2; surface proteins 3; Nef proteins 4; mRNA delivery 5; Universal vaccine

1. Introduction

HIV is a lentivirus that primarily infects hosts and cells through bodily fluids or pregnancy communication. HIV infects cells essential to the immune system, such as CD4+ T cells, macrophages, and dendritic cells, ultimately causing cell death. When the rate and magnitude of cell death cause essential cell levels to fall below critical levels, it becomes increasingly more difficult for the host to mount an effective immune response, leading to acquired immunodeficiency syndrome (AIDS) [1]. Without treatment, the average survival time after infection with HIV is estimated to be 9 to 11 years. In terms of HIV vaccine development, the unique challenge has been the virus's extreme genetic diversity and propensity for rapid mutation. However, the versatility of nucleoside-modified mRNA vaccines opens the door to creating multivalent vaccines that can target multiple strains of HIV simultaneously. Furthermore, as these vaccines can be produced with remarkable speed, they offer a promising solution to react quickly to the evolution of the virus within the population [2].

HIV-1 lineages are divided into three main groups: M (for Major), O (for Outlier), and N (for New, or Non-M, Non-O). Most strains found worldwide belong to the group M. Group O seems to be endemic to and largely confined to Cameroon and neighboring countries in West Central Africa, where these viruses represent a small minority of HIV-1 strains. A limited number of isolates from Cameroonian persons represent group N. Group M is subdivided into 9 clades or subtypes (A to D, F to H, J, and K) [3].

Modern combination antiretroviral therapy (cART) can bring HIV-1 in blood plasma to a level undetectable by standard tests, prevent the onset of acquired immune deficiency syndrome (AIDS), and allow a near-normal life expectancy for HIV-infected individuals. Unfortunately, cART is not curative, as within a few weeks of treatment cessation, HIV viremia in most patients rebounds to pre-cART levels. The primary source of this rebound, and the principal barrier to a cure, is the highly stable reservoir of latent yet replication-competent HIV-1 proviruses integrated into the genomic DNA of resting memory CD4+ T cells [4].

In contrast, the quest for an effective HIV vaccine has been challenging due to the virus's ability to evade the immune system. Initial vaccine designs attempted to stimulate neutralizing antibodies against the virus's envelope proteins. The RV144 trial [5] underscored the necessity of inducing

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antibodies and cellular immune responses. This led to the exploration of broadly neutralizing antibodies (bnAbs), which some individuals naturally produce against HIV.

The development of an effective vaccine for HIV has been significantly more challenging compared to other pathogens, such as SARS-CoV-2, which causes COVID-19. Unlike COVID-19, for which highly effective vaccines were developed swiftly, HIV's vast genetic diversity and its ability to integrate into the human genome present unique obstacles. These complexities necessitate a vaccine that can elicit antibodies capable of neutralizing all viral particles. One approach to achieving this is mRNA vaccines, particularly wherein multiple epitopes of surface proteins can be combined to elicit a broad immune response.

The HVTN 302 study evaluates the safety and immune response of three experimental HIV mRNA vaccines, which focus on the spike protein of HIV. This protein is crucial for the virus's ability to enter human cells. By targeting this spike protein, the vaccines aim to prevent HIV from infecting human cells [6]. By focusing on the surface proteins of HIV-1 and HIV-2, these vaccines target the mechanisms by which the virus enters human cells.

Although HIV-2 infection is less geographically dispersed than HIV-1, and the HIV-2 epidemic is primarily focused on West Africa, HIV-2 is not uncommon in the outbreak in Europe and India because of travel between West Africa and Europe or India. HIV-1 and HIV-2 genomes share about 60% homology in conserved genes such as gag and pol and 35–45% homology in the env genes. The core proteins of HIV-1 and HIV-2 display frequent cross-reactivity, whereas the envelope proteins are more type-specific [7].

The HIV-1 envelope glycoprotein gp160 (P03377 · ENV_HV1BR) and HIV-2 (gp125 Q74432 · Q74432_9HIV2) oligomerizes in the host endoplasmic reticulum into predominantly trimers. In the second step, gp160 transits in the host Golgi, where glycosylation is completed. The precursor is then proteolytically cleaved in the trans-Golgi and thereby activated by cellular furin or furin-like proteases to produce gp120 and gp41.

The surface protein gp120 attaches the virus to the host lymphoid cell by binding to the primary receptor CD4. This interaction induces a structural rearrangement, creating a high affinity binding site for a chemokine coreceptor like CXCR4 and/or CCR5 that acts as a ligand for CD209/DC-SIGN and CLEC4M/DC-SIGNR, which are respectively found on dendritic cells (DCs), and on endothelial cells of liver sinusoids and lymph node sinuses [8]. These interactions allow these cells to capture viral particles at mucosal surfaces and then transmit them to permissive cells. HIV subverts the migration properties of dendritic cells to gain access to CD4+ T-cells in lymph nodes.

Virus transmission to permissive T-cells occurs either in trans (without DCs infection, through viral capture and transmission) or cis (following DCs productive infection, through the usual CD4-gp120 interaction), thereby inducing a robust infection. In trans infection, bound virions remain infectious over days, and it is proposed that they are not degraded but protected in non-lysosomal acidic organelles within the DCs close to the cell membrane, thus contributing to the viral infectious potential during DCs' migration from the periphery to the lymphoid tissues [9].

On arrival at lymphoid tissues, intact virions recycle back to DCs' cell surface, transmitting the virus to CD4+ T-cells. Transmembrane protein gp41 acts as a class I viral fusion protein. Under the current model, the protein has at least 3 conformational states: pre-fusion native state, pre-hairpin intermediate state, and post-fusion hairpin state. During the fusion of viral and target intracellular membranes, the coiled coil regions (heptad repeats) assume a trimer-of-hairpins structure, positioning the fusion peptide near the C-terminal region of the ectodomain. The formation of this structure appears to drive apposition and subsequent fusion of viral and target cell membranes. Complete fusion occurs in host cell endosomes and is dynamin-dependent; however, some lipid transfer might occur in the plasma membrane. The virus undergoes clathrin-dependent internalization long before endosomal fusion, thus minimizing the surface exposure of conserved viral epitopes during fusion and reducing the efficacy of inhibitors targeting these epitopes. Membrane fusion leads to the delivery of the nucleocapsid into the cytoplasm [10].

Nef Protein

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The case of HIV becomes very different from other infections due to the involvement of Nef protein. This regulatory protein plays a crucial role in the virus's ability to form latent reservoirs, making the disease harder to cure.

The Nef protein, encoded by the Nef gene in HIV-1, HIV-2, and simian immunodeficiency viruses (SIV), plays a critical role in the pathogenicity and progression of HIV to AIDS, contradicting its initial classification as a "Negative Factor." This multifunctional protein is instrumental in evading the host's immune defenses by downregulating CD4 receptors and MHC class I molecules on the surface of infected cells, thereby hindering the immune system's ability to detect and eliminate the virus. Furthermore, Nef enhances the infectivity and replication of HIV by manipulating various cellular mechanisms, including altering cell signaling pathways to create an environment that favors viral replication [11].

The Nef interacts with the cellular sorting machinery, particularly the dileucine-based sorting pathway, to down-regulate CD4 and enhance viral infectivity [12]. Nef also modulates the immune synapse by promoting viral replication and persistence [13]. It binds to Argonaute-2, functioning as a viral suppressor of RNA interference [14]. Furthermore, Nef downregulates the natural killer cell-activating ligand PVR, preventing NK cell-mediated lysis of infected cells [15]. It intersects the macrophage CD40L signaling pathway to promote resting-cell infection [16]. These findings underscore the importance of Nef in HIV pathogenesis and suggest its potential as a target for therapeutic intervention.

The HIV-Nef protein promotes evasion from CTL recognition by downmodulating MHC-I HLA-A and -B. Nef disrupts MHC-I cell surface expression by binding to the cytoplasmic tail and stabilizing the interaction between MHC-I and clathrin adaptor protein 1 (AP-1). The formation of this complex results in the targeting of MHC-I to the lysosome for degradation [17].

Nef protein bypasses host T-cell signaling by inducing a transcriptional program nearly identical to anti-CD3 cell activation. Interaction with the TCR-zeta chain up-regulates the Fas ligand (FasL). It increases surface FasL molecules and decreases surface MHC-I molecules on infected CD4+ cells, sending attacking cytotoxic CD8+ T-lymphocytes into apoptosis [18]. Extracellular Nef protein targets CD4+ T-lymphocytes for apoptosis by interacting with CXCR4 surface receptors [19].

In infected CD4+ T-lymphocytes, the Nef proteins down-regulate the surface MHC-I, mature MHC-II, CD4, CD28, CCR5, and CXCR4 molecules. Mediates internalization and degradation of host CD4 through the interaction with the cytoplasmic tail of CD4, the recruitment of AP-2 (clathrin adapter protein complex 2), internalization through clathrin-coated pits, and subsequent transport to endosomes and lysosomes for degradation [20]. Nef protein diverts host MHC-I molecules to the trans-Golgi network-associated endosomal compartments by an endocytic pathway to finally target them for degradation. MHC-I down-regulation may involve AP-1 (clathrin adapter protein complex 1) or possibly Src family kinase-ZAP70/Syk-PI3K cascade recruited by PACS2. Consequently, infected cells are masked for immune recognition by cytotoxic T-lymphocytes [21]. Decreasing the number of immune receptors also prevents reinfection by more HIV particles (superinfection). It down-regulates host SERINC3 and SERINC5, excluding these proteins from the viral particles. Virion infectivity is drastically higher when SERINC3 or SERINC5 are excluded from the viral envelope because these host antiviral proteins impair the membrane fusion event necessary for subsequent virion penetration [22].

Given the well-defined role of Nef protein, it is essential that any vaccine would address removing it to avoid latent recurring infections.

Capsid Proteins

Capsid proteins play crucial roles in the HIV lifecycle, making them potential targets for vaccine development alongside surface proteins. The capsid p24 (Q9WMV8 · Q9WMV8_9HIV1) protein is an HIV core protein comprising the virus's structural core. It's often used as a marker in viral load tests to measure the amount of HIV in a person's blood.

While surface proteins, particularly the envelope protein, are highly variable and mutate frequently, making vaccine development challenging, capsid proteins are more conserved,

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potentially offering a more stable target for the immune system. A vaccine targeting capsid and surface proteins could elicit a broader immune response, increasing the chances of preventing viral replication even when the virus mutates. However, capsid proteins are internal, making them less accessible to antibodies than surface proteins, which presents significant challenges in vaccine design. Moreover, since the immune system predominantly targets external structures, inducing an effective response against internal proteins like capsids may require innovative approaches. The concern about off-target effects is valid when considering the inclusion of capsid protein antibodies in an HIV vaccine. Off-target effects refer to the unintended actions of antibodies (or any therapeutic agents) on proteins or cells other than their intended targets, which can lead to adverse effects or toxicity.

In the case of antibodies against the HIV capsid protein, off-target effects could occur if the antibodies bind to human proteins that are similar in structure to the HIV capsid protein or if they interfere with cellular processes that are crucial for normal cell function. This can happen due to the phenomenon known as molecular mimicry, where a part of the HIV capsid protein resembles a sequence or structural element of a host protein.

These unintended interactions can lead to various complications, including immune responses against the body's own tissues (autoimmunity), inflammation, or other cellular dysfunctions. Therefore, when designing and testing capsid protein antibodies for inclusion in HIV vaccines, researchers must thoroughly evaluate their specificity, binding affinity, and potential for off-target effects.

Epitope Selection

The surface proteins HIV-1 gp160 P03377 and HIV-2 gp125 Q74432 offer the most optimal and broad coverage of B-cell and T-cell epitopes. B cell epitopes from globular proteins range from 5 to 30 amino acids and are usually conformational. The length and flexibility of the epitope ensure high-affinity binding to B cell receptors or circulating antibodies [23]. Linear epitopes are recognized by T cells. These epitopes are often internal hydrophobic amino acid sequences processed by macrophages and presented to T cells in the context of human leukocyte antigen (HLA) class I and II molecules. Processed epitopes containing 7 to 17 amino acids are presented to T lymphocytes by antigen-presenting cells.

The choice of MHC alleles for HIV infection epitope prediction considers several factors specific to the virus's immunology and the population affected by the disease. HIV mutates rapidly; therefore, identifying epitopes conserved across multiple strains can be crucial for effective vaccine design or therapeutic interventions [24]. For HIV, CTL (Cytotoxic T Lymphocyte) responses are crucial, and these responses are mediated by peptides presented on MHC class I molecules. MHC class I alleles are associated with better control of HIV infection: HLA-B*57 and HLA-B*27. The alleles have been associated with slower progression to AIDS, suggesting that epitopes presented by these alleles could effectively elicit protective T-cell responses. HLA-B*35: This allele, in contrast, is often associated with rapid disease progression, and understanding the epitopes presented by this allele might help in understanding mechanisms of immune escape [25].

For HIV infection, understanding which MHC Class II alleles to focus on for epitope prediction is essential for designing effective therapies and vaccines. These alleles present antigens to CD4+ T-helper cells, which are crucial for orchestrating the immune response, including producing antibodies and activating cytotoxic T cells. The selection of class II alleles would similarly focus on common alleles and those associated with HIV disease progression or control. Some MHC Class II alleles that have been noted in the context of HIV research include DRB1*01: This allele has been associated with slower disease progression in some studies; DRB1*03: This allele group has shown to be important in the context of HIV, though the associations can be different depending on the specific allele; DRB1*04: Some alleles in this group are also important in HIV infection and can be associated with differences in disease progression; DRB1*07: This allele has shown some protective effects in certain populations; DRB1*11: Associations with slower progression of disease have been seen with this allele in some studies; DRB1*13: This allele is also notable in some studies for its role in HIV infection; DRB1*15: This allele may have a role in influencing the immune response to HIV. DQB1

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and DPB1 alleles are also part of the MHC Class II region and can present antigens to CD4+ T cells. Some specific alleles within these loci may also affect the immune response to HIV [26].

Table 1 reports the selected B-cell and T-Cell epitopes of HIV-1 and HIV-2 surface protein and their related Nef proteins.

Table 1. Selected epitopes of HIV-1 and HIV-2 proteins and related Nef proteins. [US Patent pendin].

HIV-1 gp160 P03377
B-Cell Epitope EKYQHLWRW; PNPQEVVLVNVTENFNMWKNDM; IRGKVQKEYAFFYKLDIIPIDND; ITQACPKVSFE; NNNTRKSIRIQ; KQSSGGDPEI; TWFNSTWSTEGSNNTEGSD; KQFINMWQEVGKAMYAPP; LGVAPTKAKRRVVQREK; EAQQHLLQLTVWGIK; QEKNEQELLELDKWASL
MHC Class-1 T-cell epitope KSLEQIWNNMTW; KAYDTEVHNVW; IPIDNDTTSY; STQLFNSTW; TAVPWNASW; RVKEKYQHLW; TTAVPWNASW; ARILAVERY
MHC Class-2 T-cell epitope IKQLQARIL
HIV-1 NEF P05856
B-Cell epitope KWSKRVTGWPT; PQVPLRPMTY; LKEKGGLDGLIYSQKR; GYFPDWQNYTPGPGIRY; PEKIEEANKGEN; QHGMDDPER; HLAFQHYARELHPEY ,
MHC Class 1 T-cell epitope IRYPLTFGW; GIRYPLTFGW; KRQDILDLW; HTQGYFPDW
MHC-Class 2 T-cell epitope LVPVEPEKI; MRRAEPAEL
HIV-2 gp125 Q74432
B Cell epitope ERDKKKLYNETWYS; ENSSDSKTANKSKDN; ESCDEHYW; TNYSGFEPKCSKVVAST; VKHPRYKGTNDTRN; AAQGKGSD; LNWIENKTGRGQKQH; ATLNRTNITFSAEVAELYRLELGDY; FAPTPEKRYSSDHGRP
MHC Class 1 T-cell epitope HIRQIVNTW; KRGVFVLGF; HSQPINRRPRQAW; GSDPEVEYMW; RAENRTYIYW; RMMETQTSTW; KGSDPEVEYMW; FRYCAPPGY
MHC Class 2 T-cell epitope YIYWHGRDN; WFGFNGTRA
HIV-2 NEF- P15829
B-cell epitope QGGLEGMYYSERRH; IVSGWQNYTHGPGIRYP; PAATREEEE; ISSWDDIHG
MHC Class-1 T-cell epitope IRYPKYFGW; RRHRILDTY; NRFPEEFGY; ARFPEEFGY; SSWDDIHIW; GRYPKYFGW; FRFPEEFGY
MHC Class-2 T-cell epitope FENEEGIVS; YGRLSGERR

Messenger RNA (mRNA)

The nucleoside-modified mRNA technology heralds a new era in vaccine development, standing on the shoulders of prior art but pushing the boundaries into novel territories of immunization strategies [27]. The mRNA is structurally composed of a 5'UTR, a signal peptide for

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efficient translation, the open reading frame that encodes the antigen, a 3'UTR for stability, and a polyA tail. This structure ensures efficient translation and antigen presentation for an immune response. Using pseudouridine in the mRNA is a modification to avoid innate immune sensing and enhance translation efficiency [27]. An open reading frame (ORF) is a continuous stretch of DNA or RNA beginning with a start codon (e.g., methionine (ATG or AUG)) and ending with a stop codon (e.g., TAA, TAG or TGA, or UAA, UAG or UGA). An ORF typically encodes a protein. It will be understood that the sequences disclosed herein may further comprise additional elements, e.g., 5' and 3' UTRs.

The nucleoside modifications within the mRNA are crucial because they help to evade the host's innate immune responses, which can often degrade mRNA before it achieves its purpose [28]. These modifications can enhance the translational capacity and stability of the mRNA, leading to higher and more prolonged protein expression of the vaccine antigen within the body. As a result, these vaccines can induce robust and sustained immune responses, which are critical for preventive and therapeutic vaccine strategies. Our vaccine against HIV comprises surface proteins of HIV-1 and HIV-2 and their Nef proteins based on selected epitopes (Table 1).

The epitopes (Table 1) are linked by a linker group comprising Alanine, Asparagine, Glutamic Acid, Glycine, Leucine, Lysine, Phenylalanine, Proline, Serine, and Threonine, or a combination thereof, singularly or in multiple additions, and preferably Glycine-Serine- Glycine-Serine-Glycine-Serine- Glycine-Serine [29]. The selection of linkers is readily evaluated by studies that show the type and extent of antibodies created [30]. We have selected GGGSGGGS as our preferred linker based on our experience creating mRNA sequences with multiple epitopes [31].

Table 2 lists the complete design of the mRNA vaccine that can be readily brought to patients, as governed by the regulatory aspects described below.

Table 2. Sequences of mRNA structure [US Patent Pending].

Element	Description
Cap (2)	A modified 5'-cap1 structure (m7G+m3'-5'-ppp-5'-Am): GA
5'-UTR (52)	The 5'-untranslated region derived from human alpha-globin RNA with an optimized Kozak sequence: GAATAAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAACCCGCCACC
Signal peptide (48)	S glycoprotein signal peptide (extended leader sequence) guides translocation of the nascent polypeptide chain into the endoplasmic reticulum: ATGTTCGTGTTCCTGGTGCTGCTGCCTCTGGTGTCCAGCCAGTGTGTG
Coding region (38	Codon-optimized sequence (ORF): GAAAAAΨAΨCAGCAΨCΨGΨGGCGCΨGGGGCGGCGGCAGCGGCGGCGGCAGCCCGAACCCGCAGGAA GΨGGΨGCΨGGΨGAACGΨGACCGAAAACΨΨΨAACAΨGΨGGAAAAACGAΨAΨGGGCGGCGGCAGCGG CGGCGGCAGCAΨΨCGCGGCAAAGΨGCAGAAAGAAΨAΨGCGΨΨΨΨΨΨΨΨAΨAAACΨGGAΨAΨΨAΨΨ CCGAΨΨGAΨAACGAΨGGCGGCGGCAGCGGCGGCGGCAGCAΨΨACCCAGGCGΨGCCCGAAAGΨGAGC ΨΨΨGAAGGCGGCGGCAGCGGCGGCGGCAGCAACAACAACACCCGCAAAAGCAΨΨCGCAΨΨCAGGGC

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37; uridine replaced with pseudouridine)	GGCGGCAGCGGCGGCGGCAGCAAACAGAGCAGCGGCGGCGAΨCCGGAAAΨΨGGCGGCGGCAGCGGCG GCGGCAGCACCΨGGΨΨΨAACAGCACCΨGGAGCACCGAAGGCAGCAACAACACCGAAGGCAGCGAΨG GCGGCGGCAGCGGCGGCGGCAGCAAACAGΨΨΨAΨΨAACAΨGΨGGCAGGAAGΨGGGCAAAGCGAΨG ΨAΨGCGCCGCCGGGCGGCGGCAGCGGCGGCGGCAGCCΨGGGCGΨGGCGCCGACCAAAGCGAAACGCC GCGΨGGΨGCAGCGCGAAAAAGGCGGCGGCAGCGGCGGCGGCAGCGAAGCGCAGCAGCAΨCΨGCΨGC AGCΨGACCGΨGΨGGGGCAΨΨAAAGGCGGCGGCAGCGGCGGCGGCAGCCAGGAAAAAAACGAACAGG AACΨGCΨGGAACΨGGAΨAAAΨGGGCGAGCCΨGGGCGGCGGCAGCGGCGGCGGCAGCAAAAGCCΨGG AACAGAΨΨΨGGAACAACAΨGACCΨGGGGCGGCGGCAGCGGCGGCGGCAGCAAAGCGΨAΨGAΨACCG AAGΨGCAΨAACGΨGΨGGGGCGGCGGCAGCGGCGGCGGCAGCAΨΨCCGAΨΨGAΨAACGAΨACCACC AGCΨAΨGGCGGCGGCAGCGGCGGCGGCAGCAGCACCCAGCΨGΨΨΨAACAGCACCΨGGGGCGGCGGC AGCGGCGGCGGCAGCACCGCGGΨGCCGΨGGAACGCGAGCΨGGGGCGGCGGCAGCGGCGGCGGCAGCC GCGΨGAAAGAAAAAΨAΨCAGCAΨCΨGΨGGGGCGGCGGCAGCGGCGGCGGCAGCACCACCGCGGΨGC CGΨGGAACGCGAGCΨGGGGCGGCGGCAGCGGCGGCGGCAGCGCGCGCAΨΨCΨGGCGGΨGGAACGCΨ AΨGGCGGCGGCAGCGGCGGCGGCAGCAΨΨAAACAGCΨGCAGGCGCGCAΨΨCΨGGGCGGCGGCAGCG GCGGCGGCAGCAAAΨGGAGCAAACGCGΨGACCGGCΨGGCCGACCGGCGGCGGCAGCGGCGGCGGCAG CCCGCAGGΨGCCGCΨGCGCCCGAΨGACCΨAΨGGCGGCGGCAGCGGCGGCGGCAGCCΨGAAAGAAAA AGGCGGCCΨGGAΨGGCCΨGAΨΨΨAΨAGCCAGAAACGCGGCGGCGGCAGCGGCGGCGGCAGCGGCΨA ΨΨΨΨCCGGAΨΨGGCAGAACΨAΨACCCCGGGCCCGGGCAΨΨCGCΨAΨGGCGGCGGCAGCGGCGGCGG CAGCCCGGAAAAAAΨΨGAAGAAGCGAACAAAGGCGAAAACGGCGGCGGCAGCGGCGGCGGCAGCCA GCAΨGGCAΨGGAΨGAΨCCGGAACGCGGCGGCGGCAGCGGCGGCGGCAGCCAΨCΨGGCGΨΨΨCAGCA ΨΨAΨGCGCGCGAACΨGCAΨCCGGAAΨAΨGGCGGCGGCAGCGGCGGCGGCAGCAΨΨCGCΨAΨCCGCΨ GACCΨΨΨGGCΨGGGGCGGCGGCAGCGGCGGCGGCAGCGGCAΨΨCGCΨAΨCCGCΨGACCΨΨΨGGCΨ GGGGCGGCGGCAGCGGCGGCGGCAGCAAACGCCAGGAΨAΨΨCΨGGAΨCΨGΨGGGGCGGCGGCAGCG GCGGCGGCAGCCAΨACCCAGGGCΨAΨΨΨΨCCGGAΨΨGGGGCGGCGGCAGCGGCGGCGGCAGCCΨGG ΨGCCGGΨGGAACCGGAAAAAAΨΨGGCGGCGGCAGCGGCGGCGGCAGCAΨGCGCCGCGCGGAACCGG CGGAACΨGGGCGGCGGCAGCGGCGGCGGCAGCGAACGCGAΨAAAAAAAAACΨGΨAΨAACGAAACCΨ GGΨAΨAGCGGCGGCGGCAGCGGCGGCGGCAGCGAAAACAGCAGCGAΨAGCAAAACCGCGAACAAAA GCAAAGAΨAACGGCGGCGGCAGCGGCGGCGGCAGCGAAAGCΨGCGAΨGAACAΨΨAΨΨGGGGCGGCG GCAGCGGCGGCGGCAGCACCAACΨAΨAGCGGCΨΨΨGAACCGAAAΨGCAGCAAAGΨGGΨGGCGAGCA CCGGCGGCGGCAGCGGCGGCGGCAGCGΨGAAACAΨCCGCGCΨAΨAAAGGCACCAACGAΨACCCGCAA CGGCGGCGGCAGCGGCGGCGGCAGCGCGGCGCAGGGCAAAGGCAGCGAΨGGCGGCGGCAGCGGCGGC GGCAGCCΨGAACΨGGAΨΨGAAAACAAAACCGGCCGCGGCCAGAAACAGCAΨGGCGGCGGCAGCGGC GGCGGCAGCGCGACCCΨGAACCGCACCAACAΨΨACCΨΨΨAGCGCGGAAGΨGGCGGAACΨGΨAΨCGC CΨGGAACΨGGGCGAΨΨAΨGGCGGCGGCAGCGGCGGCGGCAGCΨΨΨGCGCCGACCCCGGAAAAACGC ΨAΨAGCAGCGAΨCAΨGGCCGCCCGGGCGGCGGCAGCGGCGGCGGCAGCCAΨAΨΨCGCCAGAΨΨGΨG AACACCΨGGGGCGGCGGCAGCGGCGGCGGCAGCAAACGCGGCGΨGΨΨΨGΨGCΨGGGCΨΨΨGGCGGC GGCAGCGGCGGCGGCAGCCAΨAGCCAGCCGAΨΨAACCGCCGCCCGCGCCAGGCGΨGGGGCGGCGGCA GCGGCGGCGGCAGCGGCAGCGAΨCCGGAAGΨGGAAΨAΨAΨGΨGGGGCGGCGGCAGCGGCGGCGGCA GCCGCGCGGAAAACCGCACCΨAΨAΨΨΨAΨΨGGGGCGGCGGCAGCGGCGGCGGCAGCCGCAΨGAΨGG AAACCCAGACCAGCACCΨGGGGCGGCGGCAGCGGCGGCGGCAGCAAAGGCAGCGAΨCCGGAAGΨGGA AΨAΨAΨGΨGGGGCGGCGGCAGCGGCGGCGGCAGCΨΨΨCGCΨAΨΨGCGCGCCGCCGGGCΨAΨGGCG

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	GCGGCAGCGGCGGCGGCAGCΨAΨAΨΨΨAΨΨGGCAΨGGCCGCGAΨAACGGCGGCGGCAGCGGCGGCG GCAGCΨGGΨΨΨGGCΨΨΨAACGGCACCCGCGCGGGCGGCGGCAGCGGCGGCGGCAGCCAGGGCGGCC ΨGGAAGGCAΨGΨAΨΨAΨAGCGAACGCCGCCAΨGGCGGCGGCAGCGGCGGCGGCAGCAΨΨGΨGAGC GGCΨGGCAGAACΨAΨACCCAΨGGCCCGGGCAΨΨCGCΨAΨCCGGGCGGCGGCAGCGGCGGCGGCAGC CCGGCGGCGACCCGCGAAGAAGAAGAAGGCGGCGGCAGCGGCGGCGGCAGCAΨΨAGCAGCΨGGGAΨ GAΨAΨΨCAΨGGCGGCGGCGGCAGCGGCGGCGGCAGCAΨΨCGCΨAΨCCGAAAΨAΨΨΨΨGGCΨGGGG CGGCGGCAGCGGCGGCGGCAGCCGCCGCCAΨCGCAΨΨCΨGGAΨACCΨAΨGGCGGCGGCAGCGGCGGC GGCAGCAACCGCΨΨΨCCGGAAGAAΨΨΨGGCΨAΨGGCGGCGGCAGCGGCGGCGGCAGCGCGCGCΨΨΨ CCGGAAGAAΨΨΨGGCΨAΨGGCGGCGGCAGCGGCGGCGGCAGCAGCAGCΨGGGAΨGAΨAΨΨCAΨAΨ ΨΨGGGGCGGCGGCAGCGGCGGCGGCAGCGGCCGCΨAΨCCGAAAΨAΨΨΨΨGGCΨGGGGCGGCGGCAG CGGCGGCGGCAGCΨΨΨCGCΨΨΨCCGGAAGAAΨΨΨGGCΨAΨGGCGGCGGCAGCGGCGGCGGCAGCΨ ΨΨGAAAACGAAGAAGGCAΨΨGΨGAGCGGCGGCGGCAGCGGCGGCGGCAGCΨAΨGGCCGCCΨGAGCG GCGAACGCCGC
3′-UTR (268)	The 3′ untranslated region comprises two sequence elements derived from the amino-terminal enhancer of split (AES) mRNA and the mitochondrial encoded 12S ribosomal RNA to confer RNA stability and high total protein expression: GCTAGCTGCCCCTTTCCCGTCCTGGGTACCCCGAGTCTCCCCCGACCTCGGGTCCCAGGTATGCTCCCACC TCCACCTGCCCCACTCACCACCTCTGCTAGTTCCAGACACCTCCCAAGCACGCAGCAATGCAGCTCAAAA CGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAAACGAAAGTTTAACT AAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACACCCTGGAGCTAGC
poly(A) (110)	A 110-nucleotide poly(A)-tail consisting of a stretch of 30 adenosine residues, followed by a 10-nucleotide linker sequence and another 70 adenosine residues: AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCATATGACTAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Lipid Nanoparticle (LNP)

The delivery of mRNA vaccine has been validated, particularly using lipid nanoparticles (LNPs), designed to include an ionizable cationic lipid for effective delivery, a non-cationic lipid for structural stability, and a PEGylated lipid to extend circulation time in the bloodstream. Lyophilization of the LNP is included to enhance stability and shelf-life, making the vaccine suitable for distribution and storage [32]. Lyophilization of LNP formulation extends the storage life of the product. It allows storage at higher temperatures, making it an essential consideration for the distribution of the HIV vaccine that is direly needed in many countries where storage conditions are non-compatible with non-lyophilized products [33]. The delivery of LNPs is continuously evolving, but the developers continue to face intellectual property issues that need resolution [34]; however, technologies are available to deliver LNPs with little IP constraints.

5. Conclusions

The mRNA vaccine platform is safe, does not infect the body or integrate into the genome, and does not carry the risk of infection or mutation. Its immune effect is better as it can be stabilized by various modifications and encapsulation, yielding strong humoral and cellular immune responses. It is also most convenient to produce at a low cost, making the mRNA vaccine a significant humanitarian assistance. Our experience teaches us that a new vaccine can be brought to clinical

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testing within six months with a high potential of receiving IND approval due to the innate safety of these vaccines [35–37].

The application of the mRNA vaccine to prevent HIV infection has been a challenge, and several such vaccines are under development and trial; however, none of these vaccines have chosen the broad approach presented in this paper. The proposed mRNA vaccine will prevent acute infections as well as latent recurring infections due to the Nef proteins; choosing both HIV-1 and HIV-2, we broaden the scope for global protection against HIV infections.

6. Patents

The authors have filed a US patent on the composition reported in this paper.

Author Contributions: SKN: concept, research, and writing; MM: research, development, and review.

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Conflicts of Interest: The authors are developers of mRNA vaccines (www.therarna.com) and the data presented in this article may be part of US patents pending.

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