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Synthetic biology approach for the development of conditionally replicating HIV-1 vaccine

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Abstract

While the combined antiretroviral therapy has resulted in a significant decrease in HIV-1 related morbidity and mortality, the HIV-1 pandemic has not been substantially averted. To curtail the 2.4 million new infections each year, a prophylactic HIV-1 vaccine is urgently needed. This review first summarizes four major completed clinical efficacy trials of prophylactic HIV-1 vaccine and their outcomes. Next, it discusses several other approaches that have not yet advanced to clinical efficacy trials, but provided valuable insights into vaccine design. Among them, live-attenuated vaccines (LAVs) provided excellent protection in a non-human primate model. However, safety concerns have precluded the current version of LAVs from clinical application. As the major component of this review, two synthetic biology approaches for improving the safety of HIV-1 LAVs through controlling HIV-1 replication are discussed. Particular focus is on a novel approach that uses unnatural amino acid-mediated suppression of amber nonsense codon to generate conditionally replicating HIV-1 variants. The objective is to attract more attention towards this promising research field and to provoke creative designs and innovative utilization of the two control strategies.

Keywords

HIV-1 vaccine; amber suppression; unnatural amino acid; live-attenuated vaccine; virus engineering

Introduction

Human immunodeficiency virus type 1 (HIV-1) has caused one of the greatest global public health crises. It is estimated that the HIV/AIDS pandemic has already claimed 40 million human lives, 35 million people are living with HIV-1 infection and approximately 2.4

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million people are newly infected with HIV-1 worldwide each year.[1] A safe and effective HIV-1 vaccine is urgently needed to prevent HIV-1 transmission and to curtail the HIV-1 pandemic. Nevertheless, the development of such vaccines may still have a long way to go.

Up to now, four major types of HIV-1 vaccines have been evaluated in clinical Phase IIB or Phase III efficacy trials (Table 1).[2,3] The first one is a protein subunit HIV-1 vaccine based on monomeric Env protein. However, this vaccine failed to elicit protective humoral immune responses. To address this issue, the focus of vaccine research shifted to the second vaccine development strategy – elicitation of cellular immune response by using adenovirus type 5 vector encoding *gag*, *pol*, and *nef* genes of HIV-1. This approach, exemplified by the 'Merck STEP trial', also failed.[4] The third vaccine strategy employs a DNA prime followed by a recombinant adenovirus vector boost, aiming at generating both cellular and humoral responses, but it failed, too. The fourth vaccine strategy uses a canarypox vector prime followed by a protein subunit boost, i.e. the HIV-1 vaccine trial (RV144) conducted in Thailand in 2009.[5] This vaccine approach was designed to elicit both cellular and humoral immune responses and to enhance the immune response by several prime and boost. The results showed that a poxvirus-protein prime-boost provided about 31% protection against HIV-1 acquisition.[5] While the modest protection may not be significant enough for clinical uses, the landmark RV144 trial implicated that an HIV-1 vaccine was possible, and brought renewed energy to the field. With a successful initial clinical trial, a large clinical Phase III trial (HVTN 702) will be conducted in South Africa to determine whether the regimen is safe, tolerable, and effective at preventing HIV-1 infection.

The following discussions first summarize the lessons from these vaccine trials and then state-of-art HIV-1 live-attenuated vaccines (LAVs) are discussed. Among all the HIV-1 vaccine modalities developed thus far, HIV-1 LAVs, such as *nef*-deleted LAV, confer the best protection against HIV-1 acquisition in simian immunodeficiency virus (SIV)/rhesus macaque model of HIV-1 infection.[6] However, owing to safety concerns, current versions of HIV-1 LAVs cannot enter clinical trials. Given their strong protective efficacy in animal models, HIV-1 LAVs are still of great interest. This review mainly discusses strategies aiming to improve the safety of HIV-1 LAVs, particularly the strategy based on controlling HIV-1 replication through the unnatural amino acid-mediated suppression of amber nonsense codon.

Four HIV-1 Vaccine Strategies from Completed Phase IIB or III Clinical Trials

HIV-1 vaccine based on protein subunits

When researchers started the development of HIV-1 vaccine in the late 1980s, vaccine paradigms were brought from successful vaccine strategies against other pathogens. Several clinical trials focused on multiple recombinant envelope proteins derived from different HIV-1 strains. VAX004 and VAX003 employed recombinant bivalent subtype B/B and B/E GP120 and went as far as Phase III clinical trials.[7,8] These early approaches were able to successfully induce binding antibodies, but had limited success in eliciting neutralizing antibodies against HIV-1. In addition, the antibodies produced lacked the breadth of neutralization to other HIV-1 strains.[9,10] The failure to induce neutralizing antibody

responses led to poor or even no efficacy of these vaccines in early trials. With the goal of developing an efficacious HIV-1 vaccine, alternative strategies were pursued.

HIV-1 vaccine based on recombinant adenovirus vectors

In the 2000s, a vaccine strategy for eliciting cytotoxic T lymphocytes (CTLs) response was emphasized. The rationale was that CTL response would be effective against different HIV-1 strains by targeting the conserved region of different HIV-1. Thus eliciting CTL response became the major trend of vaccine design afterwards. In this strategy, HIV-1 genes were inserted into viral vectors in order to deliver these antigens into MHC class I antigen-presenting route. In the initial clinical trial (HVTN502 or STEP trial), *gag*, *pol,* and/or *nef* genes of HIV-1 subtype B were used as antigens and inserted into a recombinant adeno 5 (Ad5) vector. This vaccine contained no Env antigen, and the main focus was on inducing the CTL response in order to lyse infected cells post viral entry. Although the vaccine did not replicate well *in vivo*,[11] it conferred effective immunogenicity and was capable of reducing viremia after infection, especially in the non-human primate study.[12] However, the human clinical trials failed to elicit protection, instead enhanced virus acquisition was observed in comparison with the control group.[11,13,14] One plausible explanation is that the pre-existing immunity to Ad5 increased the availability of HIV-1 target cells at the portal of virus entry.[15] Another possible reason is that the CTL responses targeted the variable regions rather than the conserved regions, even though durable and considerable CTL responses recognizing both HIV-1 clade B and C were obtained.[16]

HIV-1 vaccine based on DNA prime and adenovirus vector boost

To overcome the breadth issue of CTL responses and to combine the humoral immunity responses, a third strategy was developed. It employed HIV-1 DNA prime (*envA*, *envB*, *envC*, *gagB*, *polB*, *nefB*) and boosted by a recombinant Ad5 vector harboring *envA*, *envB*, *envC*, and *gag-polB*. This novel prime-boost strategy was demonstrated to be protective against the low-dose challenge in non-human primate study.[17] However, the human clinical trials, including HVTN204 and HVTN505, showed no efficacy, no decrease in viral load, and little neutralizing antibody activities.[18,19] Certain data indicated that most of the binding antibodies were cross-reactive to an *E. coli* antigen,[20] which may explain why this strategy was unsuccessful. In addition, some other studies indicated that the failure might be caused by a viral escape mechanism derived from neutralizing antibody resistance.[21]

Canarypox vector prime and protein subunit boost HIV-1 vaccine

A fourth vaccine strategy was developed between 2004 and 2009, which employed the same prime and boost concept as that of strategy three. This vaccine used a canarypox vector for priming and viral proteins for boosting. By employing this strategy, the RV144 Thai HIV-1 vaccine trial (referred to as Thai Trial vaccine hereafter) showed modest but significant protection against HIV-1 acquisition.[5] This HIV-1 vaccine employed ALVAC expressing Env from HIV-1 clade E and Gag and Pol from HIV-1 clade B as the prime, followed by AIDSVAX GP120 from clade B and E as the boost and alum as the adjuvant. For the first time in the history of HIV-1 vaccine development, the Thai Trial vaccine demonstrated that a vaccine could be possible to protect people from HIV-1 infection. The Thai Trial vaccine had 60.5% efficacy after one year and had more than 31% efficacy three and half years post

vaccination. The RV144 Thai Trial is one of the most visible achievements so far in HIV-1 vaccine development. All other HIV-1 vaccines that entered clinical efficacy trials either ended up lacking efficacy or even enhanced HIV-1 transmission. Currently, the protection of the Thai Trial vaccine is correlated with antibodies of the V1V2 region of gp120 (in particular the IgG1 and IgG3 subclass)-mediated antibody-dependent cell-mediated cytotoxicity (ADCC).[22] However, the exact protective mechanisms of Thai Trial vaccine are still not fully understood.

Although the level of protection elicited by Thai Trial vaccine is modest and not sufficient for clinical use, it laid the foundation for further improvement of its protection. Based on the RV144 Thai Trial vaccine, a few other HIV-1 vaccine forms, such as HVTN097, HVTN100, and HVTN702,[23,24] were constructed and examined. A better understand of the underlying mechanisms of Thai Trial vaccine-induced protection is therefore critical for further improving its efficacy. With the lessons learned from above clinical trials and the identification and characterization of HIV-1 broadly neutralizing antibodies, designs of novel and efficacious HIV vaccines are expected.

Other strategies

Beyond the above four clinically evaluated concepts, great efforts have been continuously made to develop a range of different approaches (Table 1), which could probably shed light on the development of preventive HIV-1 vaccines. Among them, protein subunits that exhibit improved immunogenic properties are of great interests. For example, Env gp140, which represents a better mimicry of the native trimeric insoluble Env gp160 than the traditional Env gp120, was able to elicit moderate systemic and mucosal antibodies in clinical trials.[25,26] In addition to protein subunits, other potential immunogens, such as peptide and lipopeptides, have been proven to elicit HIV-1 specific CD8 and CD4 T-cell responses in clinical trials.[27,28] To generate protective antibodies against diverse HIV-1 strains, mosaic immunogens have been designed by *in silico* tools that identify and optimize the coverage of global HIV-1 epitopes.[29,30] In non-human primates, expanded cellular and humoral immune responses have been observed.[29,30] The generation of HIV-1 immunogens that can elicit broadly neutralizing antibodies (bNAbs) is also more promising than ever before. Several approaches have been pursued to elicit bNAbs. One approach aims to design immunogens that could better mimic bNAb epitopes.[31,32] Another approach uses B-cell lineage vaccine strategy to design specific immunogens that target the desired precursors of bNAb-producing cells, allowing the maturation of B-cells through uncommon pathways.[33] In a third approach, passive administration of bNAbs, such as VRC01, has provided complete protection in rhesus macaques[34] and proven to be safe in human.[35]

Live-attenuated vaccines (LAVs)

By mimicking naturally occurring infections, LAVs have successfully prevented a series of viral diseases, such as measles, mumps, yellow fever, and chickenpox by eliciting broad and long-lasting protective immune responses.[36] Researchers have developed a number of SIV based live-attenuated vaccines.[6,37] The attenuation is generally achieved by the deletion of accessory gene(s) from the viral genome, either individually or in combination.[38–41] The majority of monkeys that are vaccinated with such deletion mutants of SIV are able to

efficiently protect against pathogenic SIV challenge. One notable example was the *nef*-deleted SIV mutants, which provided the vaccinated monkeys with a strong protection (the average protection rate was 95% versus the average of all the other type of vaccines 7%) from homologous and heterologous SIV challenge.[42,43] Although these *nef*-deleted SIV mutants could protect some vaccinated monkeys from challenge, they led to the establishment of a life-long and persistent viral infection and caused AIDS in some animals especially the infant – monkeys.[44–46] Moreover, restoration of deletion in *nef* has been revealed by genetic analysis. This raised significant safety concerns that the LAVs could revert back into virulent forms and cause dis ease over time in vaccinated animals.[47–49]

Multiple deletions in both accessory genes and regulatory elements on the virus genome can be used to increase vaccine safety by decreasing viral replication capacity, but often led to the loss of vaccine efficacy.[42] In fact, a reported evidence showed an inverse correlation between the attenuation of virus replication capacity and the degree of protection.[42] In addition, an HIV-1 variant with deletions in the accessory regulatory protein-encoding genes, including *vpr*, *nef* , and LTR sequences, could still regain substantial replication capacity through viral evolution in long-term cell culture infections.[50]

The above results highlight the genetic instability and evolutionary capacity of attenuated SIV/HIV-1 strains. An uncontrolled replication of attenuated virus can lead it to regain virulence, which poses a serious safety risk for any future experimentation with HIV-1 LAVs in humans. As an alternative strategy, a virus that can execute only a single round of replication has been used as a vaccine.[51–54] However, because of the limited replication, such a single-cycle virus vaccine is less potent and does not provide the necessary duration for the induction of protective immunity against HIV-1. Therefore, novel strategies are needed to improve the safety of HIV-1 LAVs. With unsuccessful attempts based on other vaccine modalities,[55] HIV-1 LAVs represent a promising approach if the safety concerns can be resolved.

Development of HIV-1 Vaccine Using Conditionally Replicating Virus

Research efforts have been made to construct conditionally replicating HIV-1 or SIV variants.[56–65] One representative example is the use of doxycycline (dox)-inducible gene expression system (the Tet-On system[66]), which allows the virus to replicate in the presence of dox (Table 1).[61] The other strategy to control HIV-1 replication entails the manipulation of essential HIV-1 protein biosynthesis through unnatural amino acid (unAA)-mediated suppression of genome-encoded amber nonsense codon (Table 1).

Doxycycline-dependent HIV-1

Bekhout *et al.* constructed a number of HIV-rtTA and SIV-rtTA variants (Fig. 1(A)) in which the transcription activation can be switched on and off using a doxycycline (dox)-inducible gene expression system.[62] In their constructs, the natural Tat-TAR transcription control element was replaced with the dox-inducible Tet-On gene expression system.

In HIV-1, the LTR (long terminal repeat) acts as the viral promoter. The expression of viral genes is dependent on the Tat (trans-activator of transcription; a transactivation factor)

protein and the TAR (trans-activating response element) region of the LTR. Tat induces transcriptional elongation by directly interacting with the bulge of TAR region and by recruiting cellular transcriptional co-activators, such as the positive transcription elongation complex (P-TEFb). In the absence of Tat, RNA polymerases initiating transcription mostly stall near the promoter and only short RNAs are produced. In order to abolish the Tat-TAR transcription control mechanism, mutations were introduced into both Tat and TAR.[62] Specifically, Tat was inactivated by the Tyr26Ala mutation that hinders activation of the HIV-1 LTR promoter. Several mutations were introduced into the bulge and loop regions of TAR, which prevented TAR from binding to Tat and subsequent transcription activation.

In order to restore HIV-1 gene expression in a dox-dependent manner, a set of dox-associated control elements, including the tet-operator (*tetO*) sequence and the reverse tetracycline– controlled transactivator (rtTA) protein-encoding gene, were inserted into the viral genome. Multiple copies of tetO were inserted between NFκB and SP1 transcription factor binding sites in the U3 promoter of the 5′-LTR. The *nef* gene was replaced by the reverse rtTA gene.[62] With the administration of dox, the dox-bound rtTA protein was able to bind exclusively to the tetO and initiated viral gene expression.[62] On the other hand, the removal of dox resulted in the inactive form of rtTA and switched off viral gene expression.[62]

To further improve viral replication in the presence of dox, the Nef protein was restored in an HIV-rtTA-Ub-nef variant using the Ub fusion protein system.[62] This modification led to the expression of the complete proteome of HIV-1. In comparison with the Nef-lacking HIV-rtTA variants, HIV-rtTA-Ub-nef replicated more efficiently in primary T cells and HIS mice. The conditionally replicating HIV variants could also be further optimized by using the safety-lock rtTA variants as well as the improved tetO elements. The use of this optimized rtTA–tetO system can potentially reduce the chance of conditional-live HIV-1 variants from losing the dox-control.[63,64]

While the tetO-rtTA system is powerful at generating conditionally replicating viruses, the long-term *in vivo* evolution of tetO–rtTA along with the acquired mutations within the virus genome could cause problems. This might lead to inactivated or constitutively active transcription of the vaccine virus, which exposes the vaccination hosts to the danger of receiving no protection or life-long infections.

Amber suppression-dependent HIV-1

Amber suppression is a generally applicable method to incorporate unnatural amino acids (unAAs) with desirable functions into a protein of interest in bacteria, yeast, mammalian cells, and – even in animals.[67–69] However, few applications were reported to incorporate unAAs into proteins in live viruses, which is probably due to the complicated life-cycle of human viruses.[65] Chen and co-workers first demonstrated a successful incorporation of unAAs into a surface protein of hepatitis D without compromising its viability or infectivity.[70] Rather than manipulating the structural protein of viruses, we are interested in developing an amber suppression-dependent approach to stringently turn on/off the replication of HIV-1 strains (Fig. 1(B)), which can be potentially used as LAVs. This approach (Fig. 1(B), (C)) entails the manipulation of essential HIV-1 protein biosynthesis

through amber suppression that is precisely controlled by three mutually dependent exogenous regulatory components, a unique amber suppressor $tRNA_{CUA}$ (component 1) that can decode a blank codon (e.g. amber nonsense codon[67–69] or a quadruplet codon[71,72]) and a special aminoacyl-tRNA synthetase (aaRS, component 2) that charges the suppressor $tRNA_{CUA}$ with an unnatural amino acid (unAA; component 3).

Introduction to the approach—In the amber suppression strategy, TAG amber nonsense codons were introduced into the essential viral genes of HIV-1. An orthogonal aaRS-$tRNA_{CUA}$ pair that specifically recognizes an unAA was included during virus replication. In the absence of the unAA, the full-length essential HIV-1 proteins could not be synthesized and led to no production of infectious HIV-1. On the other hand, the amber nonsense codons could be decoded and rendered the assembly and replication of HIV-1 in the presence of unAA. The key control components of the amber suppression system include an aaRS that specifically recognizes the small molecule switch, an unAA of one's choice, and charges it onto an amber suppressor $tRNA_{CUA}$. To examine the feasibility of using this approach in HIV-1 vaccine development, it was first demonstrated that an overexpression of aaRS and $tRNA_{CUA}$ from an exogenously provided plasmid exhibited no apparent influence on the assembly of wild-type HIV-1 virus (pSUMA; the infectious molecular clone of a founder/transmitter HIV-1 virus, catalog #11748, NIH AIDS Reagent Program) by *in vitro* infectivity assays.[65] Next, it was demonstrated that unAA was absolutely required for the assembly of live HIV-1 variants containing amber mutations in their genomes.[65]

The Choice of Amber Mutation Sites on HIV-1 Genome

HIV-1 has a highly compact and efficient genome. The HIV-1 genome contains three major viral structural protein-encoding genes *gag*, *pol* and *env*, two essential regulatory elements *tat* and *rev*, and four accessory regulatory protein-encoding genes, *nef* , *vpr*, *vif* and *vpu*. The *gag* (group-specific antigen) gene encodes the precursor Gag protein that is subsequently processed by the HIV protease (PR) into matrix protein p17 (MA), capsid protein p24 (CA), spacer peptide 1 (SP1), nucleocapsid protein (NC), spacer peptide 2 (SP2), and P6 protein. The *pol* gene encodes important viral enzymes, including reverse transcriptase (RT), RNase H, integrase (IN), and protease. The *env* gene encodes gp160 that is post-translationally cleaved to gp120 and gp41. In addition to the coding genes, HIV-1 also contains several essential genomic structural elements such as long terminal repeat (LTR) and trans-activating response element (TAR). Furthermore, HIV-1 uses a sophisticated splicing system to produce over 40 different mRNA species, both completely and incompletely spliced.

Thus, it is critical to choose proper amber mutation sites on the HIV-1 genome so that the assembly and function of HIV-1 are not affected by amber mutations. While a large amount of genetic information of HIV is available, the choice of amber mutation sites mostly relied on a trial and error approach. Multiple HIV-1 mutants were initially constructed that contained amber mutations at a range of different sites, including SUMA-Tyr132 (amber mutation at position Tyr132 of *gag*), SUMA-Ala119 (amber mutation at position Ala119 of *gag*), SUMA-Tyr59 (amber mutation at position Tyr59 of *pol*), and SUMA-Leu365 (amber mutation at position Leu365 of *pol*). Among these four mutants, only the SUMA-Tyr59

mutant was confirmed to contain a proper amber mutation that did not affect viral assembly and function.[65]

The Choice of UNAA

Another important consideration in applying amber suppression strategy to HIV-1 vaccine development is the choice of unAA. First, the introduction of an unAA should not affect the function of HIV-1 proteins. Ideally, the structure and chemical property of unAA should resemble the natural amino acid that it replaces. For example, Tyr132 in SUMA-Tyr132 should be substituted with a close analog of tyrosine. To this end, three tyrosine analogs were examined, including *p*-acetylphenylalanine (AcF), *p*-iodophenylalanine (IodoF), and *p*-azidophenylalanine (AzF).[65] Although IodoF is structurally very similar to Tyr, it could not be functionally introduced into Gag protein to replace Tyr132. Presumably, the hydrophobic iodo substituent might interfere with the translation of Gag, which led to the translational termination at position Tyr132 and the production of truncated proteins. On the other hand, AcF could be efficiently introduced at the position of Tyr132 of Gag. However, the assembled HIV-1 mutants did not show any infectivity. In this case, replacing Tyr with AcF probably interfered with proteolytic processing of Gag or abolished the function of p24 (a processed product from Gag). Finally, AzF was shown to be a proper unAA for the construction of amber suppression-dependent HIV-1 mutants.[65]

The fidelity of unAA incorporation is critical for the generation of safe and conditionally replicating HIV-1 vaccine using the amber suppression strategy. Ideally, the full-length essential HIV-1 proteins should only be synthesized in the presence of the unAA of one's choice. This requires that the special aaRS-$tRNA_{CUA}$ pair does not recognize any of the endogenous natural amino acids in the host. To this end, the fidelity of the $tRNA_{CUA}$-AzFRS (an evolved aaRS that specifically charge the $tRNA_{CUA}$ with AzF[73]) pair was thoroughly examined. This was conducted by co-transfecting plasmid pAzFRS (a plasmid containing AzFRS and $tRNA_{CUA}$) with a reporter plasmid encoding an enhanced green fluorescent protein (EGFP) with an amber nonsense codon at position 40 (pEGFP-TAG40).[65] The full length EGFP was observed only in the presence of 1mM AzF. On the other hand, no fluorescent signal was detected in the absence of AzF. The site-specific incorporation of AzF at position 40 was further confirmed by tandem mass spectrometry.[65] Therefore, the AzFRS-$tRNA_{CUA}$ pair can be used to incorporate AzF into proteins in response to amber nonsense codon with excellent fidelity. Besides fidelity, the efficiency of the $tRNA_{CUA}$-aaRS pair is also important. Optimization of the $tRNA_{CUA}$-AzFRS pair can be performed using a previously reported strategy by fine tuning the interaction between $tRNA_{CUA}$ and AzFRS.[74]

Construction and testing of amber suppression-dependent HIV-1 variants

To demonstrate the feasibility of amber suppression-dependent virus replication, the assembly and function of SUMA-Tyr59 mutant were examined in the presence and in the absence of AzF (Fig. 2). The AzFRS-$tRNA_{CUA}$ pair was provided exogenously on a plasmid. The experimental results showed that live and functional SUMA-Tyr59 mutant could be assembled in the presence of AzF. On the other hand, no detectable functional virus

was produced in the absence of AzF. The infectivity of SUMA-AzF59 is roughly 1.7% of the wild-type SUMA according to the tissue culture infectious dose 50 (TCID50) values.

Based on theoretical calculations, if we take the HIV mutation rate as approximately 3×10^{-5} per nucleotide base per cycle of replication[75] and the virus life cycle as one day, the possibility of mutating amber codon(s) back to sense codon(s) is approximately 1%, 0.01%, and 0.0001%, respectively, in the human life span (100 years) when one, two, and three amber codons are used. Therefore, introducing one amber mutation into the HIV genome may not sufficiently prevent the virus regaining virulence through mutations. Two or more amber mutations are needed in the vaccine development. To this end, a HIV mutant (SUMA-Trp36Gln127) was constructed to contain two amber mutations in the matrix protein section of Gag. To our delight, similar infectivity was observed with the SUMA-Trp36Gln127 mutant in comparison with HIV-1 mutant containing only single amber mutation. As a control, no live SUMA-Trp36Gln127 virus was produced in the absence of AzF during viral assembly in 293T cells according to the infection assay (Fig. 2).

In the above experiments, the AzFRS-$tRNA_{CUA}$ pair was provided exogenously on a plasmid, and cannot be inherited by the progeny virus. Once injected in the vaccination host, the assembled live viruses can only survive one-cycle since the host does not have either the AzFRS-$tRNA_{CUA}$ pair or the unAA. As previously reported, transient replication might not be sufficient to elicit potent protective immune responses,[76] HIV-1 mutants that are capable of multi-cycle infection of host cells are needed as HIV-1 LAV. To address this problem, an HIV-1 mutant that contains a genomic copy of the aaRS-$tRNA_{CUA}$ pairs was recently constructed (the manuscript is currently under revision) and examined. In this case, the regulatory components are always co-expressed with the viral proteins to ensure the replication capacity of attenuated HIV-1. The ability to control multi-cycle HIV-1 replication in mammalian cells represents an important step towards the generation of a safe and efficacious vaccine to control the worldwide HIV-1 epidemic.

Conclusion

In this review, we first summarized four Phase IIB or III clinical trials of HIV-1 vaccines (Table 1). Although only the RV144 Thai vaccine trial demonstrated a moderate protection, the results from all of those strategies have provided valuable information and lessons for future vaccine development. Since none of the prior vaccine strategies yielded an effective HIV-1 vaccine in clinical applications, new and more effective approaches are highly desirable, such as the use of conditionally replicating HIV-1 strains (Table 1). The current frontiers of the development of conditionally replicating HIV-1 mutants were discussed, including the dox-dependent and the amber suppression-dependent systems. While the dox-dependent strategy has been extensively reviewed,[62] this article represents the first overview of the technical details and perspectives of the newly developed amber suppression-dependent system. In addition, a closer comparison of the two systems is provided. While both of them could potentially be used to increase the safety of HIV-1 LAVs, the two approaches differ in their level (transcription or translation) of control. The dox-dependent strategy controls HIV-1 replication at the DNA transcription level. One individual event of losing the dox-dependent control would lead to the synthesis of one copy of mRNA, which

serves as the template for the synthesis of multiple copies of protein. On the other hand, the amber suppression-dependent approach controls virus replication at the protein translation level, one undesirable read-through of amber codons would lead to the synthesis of one copy of protein. In this sense, the amber suppression-dependent approach may give a lower level of basal replication of HIV-1 in comparison with the dox-dependent strategy. It may also be possible to further improve the safety of conditionally replicating HIV-1 variants by a combination of the above two strategies and/or the inclusion of additional control mechanisms. The intention of this review is to attract more attention towards this promising research field and to provoke creative designs and innovative utilization of the two control strategies.

In summary, the two control strategies for the construction of conditionally replicating HIV-1 variants laid a solid foundation for the future development of replication-competent but controllable HIV-1 LAVs. There are still considerable technical challenges that need to be overcome to yield an effective and safe HIV-1 vaccine. Nevertheless, the two conditionally replicating HIV-1 strategies represent elegant combinations of chemical/synthetic biology and virology, which is likely to open up new avenues in HIV-1 vaccine research. In addition, these control strategies can also be potentially applied to the development of novel LAVs against other pathogens or to improve the safety of vaccines currently being used.

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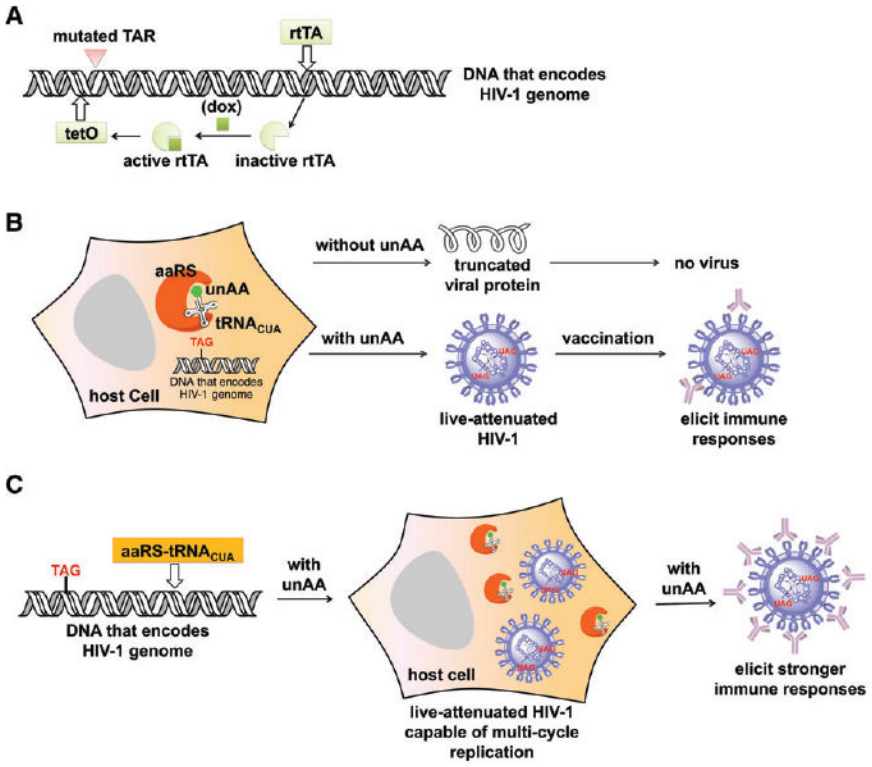

Figure 1.

Synthetic biology approaches for the development of conditionally replicating HIV-1 variants. (A) Doxycycline-dependent HIV-1. (B) First generation of amber suppression-dependent HIV-1. In this design, HIV-1 mutant can only complete a single infection cycle in the host in the absence of exogenously provided $tRNA_{CUA}$-aaRS pair. (C) Second generation of amber suppression-dependent HIV-1. In this design, HIV-1 mutant with a genomic copy of the $tRNA_{CUA}$-aaRS pair can complete multiple infection cycles in the host.

Abbreviations: TAR, trans-activating response element; rtTA, reverse tetracycline-controlled transactivator; dox, doxycycline; tetO, tetracycline operator; aaRS, aminoacyl-tRNA synthetase; unAA, unnatural amino acid.

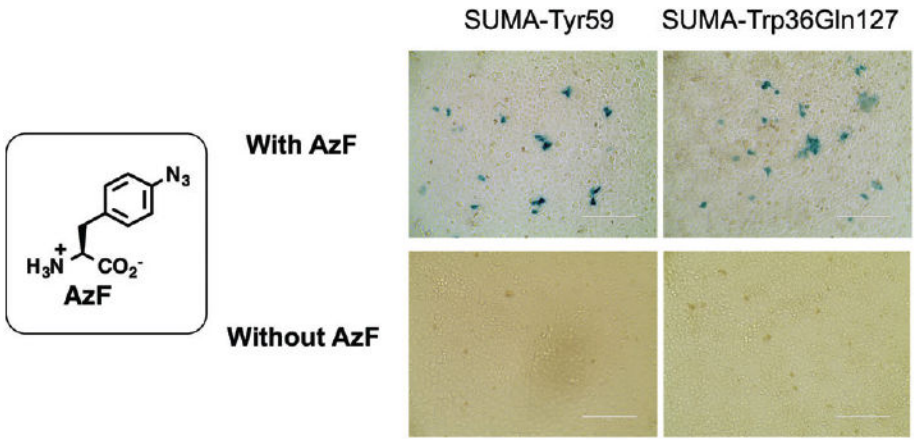

Figure 2.

Infection assays with HIV-1 mutants in the presence and the absence of AzF. SUMA-Tyr59 contains an amber mutation at position Tyr59 of *pol*. SUMA-Trp36Gln127 contains two amber mutations in matrix protein domain of Gag. The assay was conducted with TZM-bl cells, which harbors a Tat-driven bacterial lacZ gene. A positive X-gal staining (to detect the expression of Tat) indicates that HIV-1 virus is active. AzF, *p*-azidophenylalanine.

Table 1

Summary of HIV-1 vaccine strategies

Vaccine strategies	Description	Outcome
Completed clinical efficacy trials		
Protein subunits	Express bivalent HIV-1 Env gp120	No efficacy[7–10]
Recombinant adenovirus vector	Express HIV-1 Gag/Pol/Nef	No efficacy, increased risk[11–15]
DNA Prime-Adenovirus vector boost	DNA prime expressing HIV-1 Gag, Pol, Nef, Env, boost with adenovirus vector expressing Gag, Pol, Env	No efficacy[17–21]
Canarypox vector prime and protein subunit boost	Canarypox vector expressing Env, Gag, Pro, boost with Env gp120	31% protection[5,22–24]
Representative strategies undergoing clinical trials		
Protein subunits with improved immunogenic properties	Employ soluble Env gp140	Elicit moderate systemic and mucosal antibodies[25,26]
Other immunogens	Use peptides and lipopeptides	Elicit specific T-cell responses[27,28]
Broadly neutralizing antibodies	Generate or passively administer broadly neutralizing antibodies	Provide complete protection in non-human primates[29–33]
Novel strategies by employing synthetic biology tools		
Doxycycline-dependent HIV-1	Doxycycline-dependent expression of HIV-1 proteome	Observe protection in SIV-rtTA vaccinated animals[56–64]
Amber suppression-dependent HIV-1 (single-cycle)	Unnatural amino acid-mediated translation of HIV-1 proteome using exogenously provided aaRS-$tRNA_{CUA}$ pair	Single-cycle replication of HIV-1 *in vitro*[65]
Amber suppression-dependent HIV-1 (multi-cycle)	Unnatural amino acid-mediated translation of HIV-1 proteome using genomic copy of aaRS-$tRNA_{CUA}$ pair	Multi-cycle replication of HIV-1 *in vitro*[a]

[a]From a manuscript that is currently under revision.