HIV-1 prophylactic vaccines: state of the art

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Abstract

The quest for an effective HIV-1 vaccine began early in the course of the HIV pandemic. Over time, the paradigm has evolved from B cell- towards T cell-based vaccines. Results from initial Phase II/III trials have been disappointing; however, while modest, the unexpected results of the Phase II/III RV144 trial in Thailand have re-energised the field. Indeed a clear correlation was demonstrated in this trial between protection and immunological biomarkers, namely non-neutralising antibodies against the V1V2 region.

Recent data obtained from cohorts of recently HIV-1-infected individuals have enabled exploration of the role of neutralising antibodies and their potential use in HIV-1 prevention. Results from non-human primate models using a cytomegalovirus vector have also shown the potential for a prophylactic HIV vaccine to induce effective T cell responses. Finally, the development of new vaccine vectors and trial strategies has also allowed progress in the field. Therefore, HIV-1 vaccine research remains a dynamic field that has also been stimulated by the recent positive results of pre-exposure prophylaxis strategies with antiretrovirals.

Introduction

It has been over 30 years since HIV-1 was first identified as the causative agent for AIDS. More than 60 million people worldwide have been infected with the virus, mostly in the developing world, and nearly half of these individuals have died. While there is clear improvement in the evolution of the AIDS pandemic especially with the increased access to antiretroviral therapy (ART), there remains an urgent need to strengthen preventative measures such as health education, treatment of sexually transmitted diseases, circumcision, vaccines, topical microbicides and therapeutic interventions such as rapid treatment initiation or 'test and treat' strategies. However, in geographical areas where the prevalence of HIV-1 in antenatal cohorts is as high as 50%, the task of treating all infected individuals is a daunting prospect and may well be beyond the scope of public health services. Moreover, even in the presence of comprehensive treatment services, sexual transmission will most probably continue to occur. This is clearly demonstrated in Europe where the estimated incidence of HIV-1 infection in men who have sex with men (MSM) is around 1–2% per year [1]. A safe and effective HIV-1 vaccine will undoubtedly be the best strategy for achieving the ultimate control of the AIDS pandemic.

Despite extensive research over the past decades and the development of more than 30 HIV-1 vaccine candidates, which have induced various degrees of immunological response during Phase I/II trials in humans or non-human primate (NHP) models, no effective prophylactic HIV-1 vaccine is available so far [2]. Only a few Phase IIB/III trials have been conducted with vaccine candidates, and unfortunately, the majority of them have shown either no protection or an increased risk for HIV-1 acquisition.

However, the results of the RV144 trial, conducted in Thailand and which combined two immunogens that had failed when used separately, has shown for the first time a modest protective effect in the vaccine arm with a heterologous prime-boost strategy [3]. This trial has raised new hopes for the possibility of developing a prophylactic HIV-1 vaccine. Immunological data from this trial in terms of correlates of protection will continue to represent an important step towards the discovery of an effective prophylactic vaccine.

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HIV-1 vaccine stumbling blocks: antigenic diversity and natural protection

HIV-1 vaccine development has been hampered by the fact that correlates of protection against the virus are still imperfectly characterised. The initial stage of the infection is associated with robust cellular and humoral immune responses, but these fail to clear or totally control the ongoing chronic viral replication in the majority of HIV-1-infected individuals. Studies aimed at analysing the determinants of protection among high-risk HIV-1-seronegative individuals have failed to provide evidence for immunological responses that might be induced with immunisation [4]. Nevertheless, there is a broad scientific consensus that a successful vaccine to prevent HIV-1 transmission will need to elicit both HIV-1-specific T cell and neutralising antibody responses [5,6]. Rather than being ineffective *per se*, B cell responses against HIV-1, and probably T cell responses as well, seem to occur too late. Indeed, almost all infected individuals develop strain-specific antibodies that can neutralise autologous, but not heterologous viruses. Broadly neutralising antibodies (bNAbs) can also be found in some individuals after several years of infection [7]. Finally, while not protective, HIV-1-specific T cell responses may allow control of viral replication in HIV-1 elite controllers [8].

There is wide diversity in HIV-1 subtypes [9] and as the aim of a prophylactic vaccine is to bring global protection against all strains, the vaccine approach must be able to deal with this diversity. Antibody-mediated neutralisation studies from several laboratories have all shown that genetic subtypes are not predictive of the neutralisation serotypes [10]. Studies of the cytotoxic T lymphocyte (CTL) reactivity in infected subjects and vaccinees suggest that cross-subtype CTL reactivity is common [11]. Cross-reactive CTLs have been described following immunisation with canarypox prime regimens [12]. Thus, it remains important to find ways to more rigorously assess cross-reactivity in Phase I/II HIV vaccine trials, as this may be a key determinant in the selection of immunogens to take forward into large-scale field trials.

Historical perspectives on HIV vaccine development

Most vaccines that have been explored have been based on either live-attenuated HIV-1 or whole inactivated viruses. The interest in live-attenuated HIV-1 vaccines has come from two different reports. First was the observation that the infection of macaques with a *Nef*-deleted, i.e. attenuated simian immunodeficiency virus (SIV) strain, may protect from subsequent challenges with a wild-type pathogenic strain [13] and second was the long-term non-progression of individuals accidentally infected in Australia with a *Nef*-deleted strain of HIV-1 [14]. While immunisation with live-attenuated vaccine clearly brings robust protection against SIV challenges in macaques [15] and represents a good model to study immune correlates of protection [16], major concerns regarding safety issues have been raised that preclude its use in humans [17].

The use of inactivated viruses associated with adjuvants initially brought great excitement as vaccinated macaques seemed to be protected against an SIV challenge [18]. However, this protection was later attributed to antibodies specific for cell proteins, including human leukocyte antigens (HLA) class I and II, which were incorporated into virions during vaccine and challenge virus preparation [19,20].

It is interesting to mention that the evolution of the HIV-1 vaccine field can be followed with the results of Phase IIb/III trials (Table 1). Relying on the success of the hepatitis B (HBV) vaccine, initial HIV-1 immunogens were based on the concept that a recombinant envelope glycoprotein will reproduce what is obtained with classical vaccines, i.e. protective neutralising antibodies (NAbs). HIV-1-based envelope glycoproteins were shown to induce neutralisation in NHP and their use as a sole vaccine candidate has been studied in several Phase I and in two Phase IIb/III trials (Vax 003 [21], Vax 004 [22]; Table 1). While vaccine-induced antibodies were present in all vaccinees in both trials, they were not associated with protection against HIV-1 acquisition. Indeed while strong NAb responses were seen against a subset of tier 1 viruses (Table 2), only sporadic and weak responses were seen against tier 2 viruses; moreover, the antibody response waned rapidly in both trials [23,24].

The second wave of HIV-1 vaccine trials aimed to induce strong HIV-1 specific CD8 T cells. The rationale for this approach was based on several findings: (1) evidence that in the SIV macaque model and in humans, CD8 T cells played a pivotal role in virological control [26]; (2) strong linkage between certain HLA class I alleles and viral control [27]; and (3) the hypothesis that even if a T cell-based vaccine was not found to be protective, it could impact on the epidemic through its effect on viral load [28]. Two Phase IIb/III trials were performed using a poxvirus-based vaccine candidate developed by Merck: the Step and Phambili trials (Table 1). Both trials failed to show any protection or an impact of the CD8 T cell vaccine-induced responses on viral load [29–31]. In both trials results have shown not only futility but also an increased risk of HIV-1 acquisition in men, a finding confirmed

Table 1. HIV vaccine Phase II/III trials

Trial	Date	Vaccine components	Country	Populations	Main immunological target	Infection rates
Vax004	1998–2002	Recombinant gp120 (B/B)	United States Canada Netherlands	5403 HRSTs	Neutralising antibody	6.7% in vaccinees 7.0% in placebo recipients NS
Vax 003	1999–2002	Recombinant gp120 (B/E)	Thailand	2546 IDU	Neutralising antibody	8.4% in vaccinees 8.3% in placebo recipients NS
Step	2004–2007	rAd5 (gag, pol, nef) (B)	North America Caribbean South America Australia	3000 HRST	CD8 T cell responses	4.6% in vaccinees 3.1% in placebo recipients *P*=0.07*
Phambili	2007	rAd5 (gag, pol, nef) (B)	South Africa	801 HRST**	CD8 T cell responses	8.4% in vaccinees 7% in placebo recipients *P*=NS
RV144 (Thai trial)	2003–2009	**Prime:** canarypox (gag, pol, env E) **Boost:** recombinant gp120 (B/E)	Thailand	16,402 General population	Neutralising antibody	0.192% in vaccinees 0.279% in placebo recipients *P*=0.04; VE=31%***
HVTN 505	2009–2013	**Prime:** DNA (gag, pol, nef, B) + DNA (env A/B/C) **Boost:** Ad5 (gag, pol, B) + Ad5 (env A/B/C)	United States	2496 HRST	CD8 T cell responses	2.7% in vaccinees 2.1% in placebo recipients *P*=NS

HRST: high risk for sexual transmission; IDU: intravenous drug users; IR: infection rate ; VE: vaccine efficacy.
* Analysis in men in the Step trial (all but one infection occurred in men); ** enrolment of 3000 patients was originally planned but the trial was stopped after the results of the Step trial; *** Modified intention-to-treat analysis.

Table 2. Tier neutralisation [25]

In order to standardise the neutralisation assay among all the laboratories involved in HIV vaccines around the world, a GLP assay has been created based on a three-tier algorithm that allows comparison between different immunogens. In this algorithm:

Tier 1 neutralisation potency is defined as a serum able to neutralise homologous virus strains represented in the vaccine and a small number of heterologous viruses that are known to be highly sensitive to antibody-mediated neutralisation. This tier is currently viewed as a triage stage.

Tier 2 neutralisation potency is defined as a Tier 1 serum also able to neutralise viruses that are matched in genetic subtype to the vaccine strain, taken from a virus panels of 12 viruses from each major genetic subtype (A, B, C, D, E and A/G)

Tier 3 neutralisation potency is defined as a Tier 2 serum able to neutralise six viruses from each of the heterotypic clades (i.e. not included in the vaccine strains)

in a recall study of Phambili participants [32]. The failure of this vaccine strategy was blamed on the inefficiency of analogous strategies in the rhesus macaque challenge model. Indeed, vaccine protection studies that have used challenges with a chimeric simian-human immunodeficiency virus (SHIV) in macaques did not predict the results of the human trials, as the results in macaques were associated, in contrast to the Step and Phambili trials, with a clear reduction in viral load. It was thought that a prime-boost strategy (see later) combining a DNA vaccine with the adenovirus serotype 5 (Ad5) of the Step trial might induce protection in the macaque model [33]. Unfortunately, the HVTN 505 trial that tested the efficacy of a DNA prime-recombinant adenovirus type 5 boost (DNA/rAd5) vaccine regimen, while showing the presence of a strong T cell response, has also brought disappointing clinical results (Table 1) [34].

The negative results from these T cell-based vaccines are in contrast to those from the RV144 trial initiated in 2003, which has explored a prime-boost combination with two immunogens, ALVAC and AIDSVAX B/E (Table 1)[3]. This heterologous prime-boost combination was shown to lower the rate of HIV-1 infection acquisition by 31.2% after 3 years of follow-up and, more importantly, by 61% after 1 year from the time of last vaccination. A large number of studies and important efforts are attempting to improve our understanding of the mechanisms of protection elicited by this immunisation strategy. Levels of vaccine-induced IgG Abs recognising the V1V2 regions from multiple HIV-1 subtypes were shown to correlate negatively with the risk for HIV-1 acquisition [35], which is also clearly influenced by certain FCGR2C polymorphisms [36]. It indicates that (a) a prime-boost combination is potentially effective and (b) the protective effect from the vaccine wanes over time. Therefore, the aims of the post-RV144 trials are to develop strategies that may improve the level of vaccine efficacy, such as augmenting the overall protection (above the 61% efficacy rate observed 1 year post immunisation) and inducing durable protection. The results of the RV144 trial have also led to the rapid development of secondary trials to further decipher immune correlates of protection both in human and NHP models.

Novel T and B cell-based vaccine strategies

The outcome of the RV144 trial strongly supports the conclusion that the development of an effective HIV-1 vaccine will require both humoral and cellular immune responses; however, strategies using only B or T cell-based vaccines may bring important information.

Researchers have focused on the description of the natural occurrence of bNAbs and their impact on HIV-1 acquisition through passive immunisation in animal models. We now have access to a more detailed description of the epitopes recognised by the main naturally occurring bNAbs (Table 3). It is important to note that the number of newly described bNAbs has increased from four prior to 2009 to more than a hundred in 2015. They appear to share unusual characteristics, which include polyreactivity for host antigens, extensive somatic hypermutations, long variable heavy-chain third complementary-determining regions, and target one of four sites of vulnerability on the virus envelope glycoproteins. B cell development is characterised by several steps that are regulated by the expression of membrane immunoglobulins, and IgM expression leads to selection against Abs with long complementary determining region 3 (CDR3s).

Moreover, B cells with long hydrophobic heavy chain complementary determining region 3 (HCDR3) are usually eliminated at the naïve B cell stage. While polyreactivity characterises up to 40% of pre-selected antibodies, this property clearly decreases among post-selection antibodies. Finally, the frequency of hypermutations of HIV-1 bNAbs greatly exceeds what is observed in other viral infections. Therefore, the induction of bNAb seems to use an usual pathway [2]. Several studies have described the natural occurrence of such Abs showing a necessary parallel evolution of the viral envelope and antibody maturation [38,39]. These findings have led to the hypothesis that bNAbs could be generated through the sequential use of various envelope proteins. Indeed, as wild-type gp120 proteins seem to lack detectable affinity for the predicted germline precursors of most bNAbs, making them poor immunogens for a prime, researchers have used computation-guided *in vitro* screening to create a germline-targeting gp120 immunogen that is able to bind bNAbs and their germline precursors [40]. Reverse antigen vaccination could be a long and complicated process and some authors have focused on the use of bNAbs through passive immunisation. These types of immunisation studies have been performed in the mouse model but also in NHP, using an SHIV challenge. bNAbs have repeatedly shown in these experiments an *in vivo* protective effect correlated with their *in vitro* neutralisation potency (Table 3) [37]. Apart from their use in the vaccine field, bNAbs are potentially interesting compounds in terms of other preventative strategies such as microbicides or as therapeutic agents [41,42]. Finally, the induction of bNAbs may not be the only way forward, as indeed the RV144 trial has demonstrated that non-neutralising antibodies may also be protective. This type of antibody is easier to induce by envelope monomers, as well as gp41 stumps that have shed gp120 in contrast to bNAbs, which require a functional trimeric envelope. These may act in several non-exclusive ways: antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated viral inhibition, antibody-dependent cellular

Table 3. Epitopes recognised by the main naturally occurring bNAbs

Antibody	Specificity	Isotype	Polyreactivity	Use in passive immunisation experiments in macaques
2F5	gp41 MPER	IgG3	Yes	No
4E10	gp41 MPER	IgG3	Yes	No
2G12	Env, glycans	IgG1	Fungal carbohydrates	Yes
1b12	CD4 binding site	IgG1	Yes	Yes
VRC01	CD4 binding site	IgG1	No	Yes
HJ16	Core/CD4 binding site	IgG1	N/A	No
PG9	V2V3 loop	IgG1	No	Yes
PG16	V2V3 loop	IgG1	No	No

MPER: membrane-proximal external region.
Adapted from [2,37].

phagocytosis, complement activation and viral capture [43]. The first report on passive immunisation with these antibodies has shown that they are not protective but that they may control viral load [44]. Recent results with bNAbs have demonstrated that their protective effect is not restricted to their neutralising activity, but that it may also lie with other aspects of their activity as mentioned above [45]. Nevertheless, whatever the expected biological impact is, the development of an HIV-1 vaccine candidate directed solely towards the induction of protective antibodies will probably take many years.

The failure of the STEP, Phambili and HVTN 505 trials has questioned the validity of a T cell-based prophylactic HIV-1 vaccine (Table 1). Most heterologous (DNA/Ad5, DNA/NYVAC and DNA/MVA) T cell-based vaccine candidates have shown to be immunogenic with approximately 80–90% of responders, and inducing both CD4 and CD8 T cell responses, which were polyfunctional and durable [46]. However the type of T cell response that correlates with protection remains to be determined [47].

The recent success of a cytomegalovirus (CMV)-based candidate vaccine has renewed interest in the T cell field. Louis Picker's team has developed a CMV vector (RhCMV) expressing SIV genes that induces strong and persistent effector memory CTL responses in rhesus monkeys. This candidate vaccine protects 50% of macaques after intra-rectal or intra-vaginal challenges and interestingly enough these animals are infected but subsequently clear the virus soon after the peak of viraemia during acute infection. Subsequent removal of CD8 T cells *in vivo* was not associated with rebound of viraemia [48,49]. This vaccine candidate has elicited an atypical CD8 T cell response with an exceptionally broad central memory phenotype when compared to responses generated by conventional T cell-based candidate vaccines, and for two-thirds of them restricted by MHC class II [50]. The RhCMV vector includes an equivalent of the US11 gene of the human CMV that is required for the suppression of the classical MHC class I response and the deletion of three CMV genes encoding molecules that determine viral tropism [50]. The results are promising, while several questions remain to be answered. A Phase I trial in humans is planned for 2016.

New leads in the choice of T cell epitopes

A major issue when building a T cell-based HIV-1 vaccine candidate is the choice of epitopes. These should be conserved among HIV-1 strains, able to induce immunodominant CTL responses and associated with favourable clinical outcomes during natural infection. During primary HIV-1 infection, the efficiency of the CTL response is correlated with the high number of recognised gag epitopes and a low number of Env epitopes [51]. During chronic infection an increased number of recognised Env epitopes tends to be correlated with lower CD4 T cell counts. Moreover, if Env epitopes are immunodominant, as it was the case in HVTN 505, they may compete with more conserved epitopes expressed on the same cell. These results therefore strongly argue against the inclusion of Env epitopes into a T cell-based vaccine [47]. In contrast gag seems to be the best HIV-1 protein to include in a T cell-based vaccine as it induces strong immunodominant responses. However, authors have questioned this choice by arguing that rather than relying on results obtained in some individuals with known HLA class I alleles, the choice of epitopes should be based on information on CTL responses from comprehensive analyses collected from a cohort with a large HLA class I heterogeneity. Finally, a T cell-based vaccine should induce immunodominant CTL responses toward conserved regions of HIV-1, thereby overcoming natural immunodominance. Therefore, redirecting the immune response toward conserved regions of HIV-1 will be a major issue for a T cell-based vaccine [51].

A mosaic approach may deal with HIV-1 diversity. Mosaic vaccines include two or three bioinformatically integrated regions of natural HIV-1 sequences that contain a maximum of potential T cell epitopes, optimised for perfect coverage of a nine-amino acid segment [52]. The resulting immunogens therefore cover the sequence diversity of thousands of peptides. Mosaic antigens have been shown to induce broader immune responses than single immunogens [53]. A recent report has shown that a rectal challenge performed with a heterologous, difficult-to-neutralise hybrid SHIV in monkeys vaccinated with a mosaic (gag, pol, env) defective adeno/MVA prime-boost combination did not result in complete protection but reduced the risk of infection per intrarectal challenge by about 90% [54]. Moreover, it was associated with a more favourable outcome in five of 12 control animals, as none of the vaccinated macaques died from simian AIDS by 9 months post-challenge. The importance of these results was reinforced by the fact that the gag and pol proteins encoded by the strain used for the challenge did not cross-react with the gene sequences included in the vaccine [54]. Human Phase I/II clinical trials using mosaic antigens in humans are on their way in the US.

Vectorology

While B cell and CD4 T cell responses are easily induced by all currently available vaccines, CD8 T cells require endogenous protein expression, which is achievable with live-attenuated vaccines that unfortunately cannot be used for prophylactic use against HIV-1 infection (see above). Therefore, intense efforts are aimed at developing new vaccine candidates that can circumvent this problem. Different kinds of tools are used to allow the presentation of HIV-1 epitopes to CD8 T cells: (1) recombinant vectors: (2) DNA vaccines; (3) lipopeptides; and (4) dendritic cell targeting.

To date, dozens of different types of vectors, such as replication-defective ones, have been used for this purpose, including viral (almost all DNA and RNA viruses), bacterial (BCG, *Salmonella, Listeria monocytogenes*), parasitic (*Leishmania*), and plant plastid vectors [55]. The most studied are poxvirus and adenovirus vectors (Table 4).

They offer several advantages as they can carry foreign genes with uptake by and/or infect antigen-presenting cells (APCs), be altered so as not to carry pathogenic genes, have a good level of expression of the inserted genes, and are not associated with a risk of integration. However, they can induce rapid anti-vector immunity or be associated with pre-existing anti-vector immunity, which has shown some unwanted effects as in the case of the Step trial [29]. Moreover, the cross-presentation and direct presentation mechanisms may be suboptimal with replication-deficient viral vectors and the amount of HIV-1 antigenic material available for processing limited as a result of the abortive infection of target cells. In order to deal with these issues, new types of such vectors are currently in development, using less sero-prevalent serotypes (Ad26) or chimpanzee virus (ChAd) for adenoviral vectors or replication-competent viral vectors for poxvirus (NYVAC-KC). A large number of vaccine vectors are currently under evaluation in clinical trials in terms of their safety and immunogenicity [56].

The first DNA vaccines were developed more than 20 years ago [57] and are currently used in veterinary vaccinology. This simple technology offers several advantages: an excellent safety profile, easy manufacturing, great stability and induction of B and T cell responses. However, the strong immunogenicity obtained in small animal models has led to disappointing results in NHP and humans. Further improvements, including sequence optimisation and novel delivery methods, have greatly increased their efficiency [58].

Table 4. Viral vectors used in HIV vaccine trials

Type	Virus	Replication	Use in clinical trial
Poxviruses	Canarypox (ALVAC)	Deficient	Phase III (RV 144)
	Modified Vaccine Ankara (MVA)	Deficient	Phase II
	NYVAC	Deficient	Phase II
	NYVAC-KC	Competent	NHP studies only
Adenoviruses	Adenovirus 5	Deficient	Phase IIb (Step, Phambili, HVTN 505)
	Adenovirus 26	Deficient	Phase I
	Adenovirus 35	Deficient	Phase I
	Chimp adenovirus	Deficient	Phase II
Cytomegalovirus	Rh cytomegalovirus	Competent	NHP studies only

However, real progress has come from their use as prime-boost strategies with viral vectors.

In contrast to live-attenuated vaccines that can induce a persistent immunological response after a single injection, other vaccines need prime-boost vaccination to induce this type of immunity. With currently available vaccines, prime-boost strategies mainly consist of injections of the same vaccine. In the case of HIV-1 vaccine candidates, heterologous prime-boost strategies using DNA and viral vectors have been developed. They have several advantages. The first is circumvention of the problem of pre-existing or vaccine-induced immunity against the viral vector. The second advantage is an increase in the intensity of T cell responses, which was the basis for the development of the HVTN 505 trial after the failure of the Step trial [33]. This problem is crucial for adenovirus vectors but seems to be less important for poxvirus vectors. The third is the ability to bring T cell help with an epitope shared by the prime and the boost vaccines to the B cell response to a unique epitope of the boost, which was the rationale of the design of the RV144 trial. The development of optimal heterologous prime-boost strategies is under consideration and questions such as how to use these vaccines in combination and timing of injections are still being debated.

A novel strategy developed in vaccinology is to target directly the key cells of the immune responses, i.e. dendritic cells (DC). This type of strategy has mainly been used in the context of HIV-1 therapeutic trials, using *ex vivo* matured DC pulsed with HIV-1 antigens [59]. New strategies aimed at using antibodies coupled with HIV-1 peptides to target antigens towards different DC subsets, or the same DC via different receptors, thereby enabling antigen delivery concomitantly with unique DC activation signals, have been initially described by Steinman *et al.* [60] and are currently in development in our institution [61–63].

In terms of the vaccines for HIV prevention, the type of adjuvant is also a key issue. Indeed, appropriate adjuvants are clearly needed for non-live attenuated vaccines in order to induce innate immune responses allowing proper dendritic cell maturation [64]. Recent results obtained in a macaque model that mimics the RV144 trial have shown that a change of adjuvant may increase the candidate vaccine immunogenicity while abrogating its protective effect [65].

New concepts for clinical trials

The results described above in HIV-1 vaccine development indicate that there is an urgent need to define biological correlates of protection. It will therefore be important to use a different type of terminology to describe 'correlates of protection'. In this context, Qin *et al.* have suggested three different types of concepts for immune correlates, using the following nomenclature: 'correlate of risk', 'level 1 surrogate of protection' and 'level 2 surrogate of protection' and a general framework for assessing these three levels of immune correlates in vaccine efficacy trials [66]. Moreover, recent advances in systems biology will also allow faster characterisation of more robust biological signatures linked to clinical protection [67]. Recent results from the RV144 trial will also help to develop an experimental medicine approach suggesting biological parameters (magnitude of IgG responses against V1/V2 loop, ADCC responses, frequency of *Env*-specific CD4 T cell responses) that maybe used as go/no go criteria for a down-selection process of vaccine candidates. This is one of the main objectives of the recent European Commission funded EHVA (European HIV Vaccine Alliance) consortium (Table 5).

Table 5. Go/no go criterion to support licensure study. Studies must simultaneously meet all four main variables

Variable measured at month 6.5	Rationale
Env Ab response rate (≥2 of 3)**	Adequate Ab take to vaccine Env
Env Ab magnitude (≥2 of 3)*	Non-inferior Ab magnitude *vs.* RV144
Env CD4 response rate (1 of 1)*	Non-inferior CD4 T cell take *vs.* RV144
Env V1V2 response rate (≥1 of 3)**	Adequate to predict achieving VE=50% for 2 years if V1V2 Ab is an immune correlate

* Based on variables 1–3 (Insert binding Abs); ** Require that the same two Env inserts pass on 'take'.
Adapted from Tartaglia J. Substantiating and extending upon the results of RV144. An update on HIV vaccines: prospects for the future. *Les Cent Gardes Conference*. October 2014. Veyrier du Lac, France.

One of the other main obstacles for the development of an effective HIV-1 vaccine is the length of time needed to advance projects. The classical clinical development schemes with several Phase I and II trials is slow, and efforts should be made to accelerate these processes. Use of the adaptive trial concept may help to solve this issue. This type of design allows the inclusion of participants into several parallel arms that may be modulated in response to the data acquired during the study. It does, however, require rapid access to biological and clinical results [68]. This type of trial is currently under development [69]. Finally, we may anticipate that, in a global prevention 'tool kit approach', the recent results from the PreP trials [70,71] will profoundly change the way we perform and define the next Phase II/III vaccine trial endpoints [72].

Conclusions

While we still do not have an effective prophylactic HIV-1 vaccine, results from the RV144 trial and several novel vaccine candidates in the NHP model have galvanised the field. The RV144 trial outcome may be considered modest; however, the intense level of scientific research performed after this trial has allowed the discovery of new correlates of protection and generated new studies in order to further our understanding of the mechanisms of protection. Previous Phase III trials and the RV144 trial together with the development of new technologies and strategies in terms of clinical trials have also deeply altered HIV-1 vaccine research.

However, several questions remain to be addressed with urgency, in particular the duration of immune responses generated with current strategies. It will be important in the future to develop new animal models such as humanised mice, to better define immune correlates of protection and test hypotheses in the context of human clinical trials, using as diverse as possible a portfolio of vaccine concepts in order to allow the discovery of a protective HIV-1 vaccine and control the global HIV-1 pandemic.

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