



Review

A brief history of the global effort to develop a preventive HIV vaccine[☆]José Esparza^{*}

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ABSTRACT

Soon after HIV was discovered as the cause of AIDS in 1983–1984, there was an expectation that a preventive vaccine would be rapidly developed. In trying to achieve that goal, three successive scientific paradigms have been explored: induction of neutralizing antibodies, induction of cell mediated immunity, and exploration of combination approaches and novel concepts. Although major progress has been made in understanding the scientific basis for HIV vaccine development, efficacy trials have been critical in moving the field forward. In 2009, the field was reinvigorated with the modest results obtained from the RV144 trial conducted in Thailand. Here, we review those vaccine development efforts, with an emphasis on events that occurred during the earlier years. The goal is to provide younger generations of scientists with information and inspiration to continue the search for an HIV vaccine.

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1. Introduction

Soon after the human immunodeficiency virus (HIV) was isolated and confirmed as the cause of AIDS in 1983–1984 [1,2] it was widely expected that an effective vaccine would be rapidly developed. Thirty years have passed and we are still struggling to develop an elusive vaccine [3,4]. The goal of this review is to outline the major insights that have guided past efforts. Although the science underpinning the HIV vaccine effort is discussed, this is not intended to be a scientific review. It is rather an historical analysis informed by personal experience derived from more than 25 years of work on the subject, first at the World Health Organization (WHO), and currently at the Bill & Melinda Gates Foundation [5–14].

2. Initial optimism (1984–1986)

2.1. “A vaccine in two years”

In 1984 the US Secretary of Health and Human Services, Margaret Heckler, declared that “a vaccine (will be) ready for testing in approximately two years” [3]. Cautious optimism was also expressed by others. The US Assistant Secretary of Health, Edward Brandt, acknowledged that “nobody knows for certain how long it will take to develop the vaccine, although the general speculation is that it will be available for clinical testing within two or three years” [15]. Don Francis, then at the US Centers for Disease Control (CDC), in one of the first papers discussing prospects for a future HIV vaccine, expressed optimism based on the recent development of a vaccine for feline leukemia virus, another retrovirus causing an AIDS-like disease in cats [16]. However, no one knew at time that HIV/AIDS was much more complex than any other viral disease for which vaccines have been successfully developed [13].

2.2. The promise of modern biology

Although most current viral vaccines are based on either live-attenuated or whole-inactivated viruses, these approaches were generally considered unsafe for an HIV vaccine because of the danger of integration of the proviral DNA in the host chromosome [17]. New recombinant DNA technologies were booming during the mid-1980s, and these technologies were seen as the most practical approach to develop a safe and effective HIV vaccine. A recent precedent was hepatitis B for which a plasma derived vaccine had been licensed by the US Food and Drug Administration (FDA) in 1981. Although it was highly effective, concerns were raised that the source of the vaccine antigen was the plasma of individuals who could also be carrying the AIDS virus. The solution came when the surface antigen of the hepatitis B virus was successfully cloned and expressed in yeast, allowing for the manufacturing of a new recombinant hepatitis B vaccine which was licensed in 1986 [18,19].

Following the hepatitis B model, the HIV scientific community favored the development of a subunit vaccine based on genetically engineered antigens representing the outer envelope glycoproteins of HIV [20]. Perhaps the first attempt to organize a systematic search for an HIV vaccine occurred as early as 1984 when Robert

Gallo established an informal international collaborative network known as HIVAC, with an initial focus on envelope-based vaccines [21]. In 1986, Anthony Fauci, who had been appointed in 1984 as the Director of the National Institute of Allergy and Infectious Diseases (NIAID), reported that virus subunits, synthetic peptides and vaccinia-vectored vaccines were already being explored in small animals. Nevertheless, Fauci cautiously raised the issue of whether an effective vaccine would even be possible, recognizing the lack of information of what could constitute a protective immune response [22].

The initial vaccine effort was benefiting from rapid advances in the molecular biology of HIV that occurred in the following five years after its discovery. These advances include the identification of the major structural proteins of the virus [23] and the cloning and sequencing of the HIV genome [24]. Early information on the genetic variability of different HIV strains heralded one of the greatest challenges that would thwart HIV vaccine development for years [25]. Neutralizing antibodies were described in 1985 [26], although their protective efficacy was not evident since titers were similar among asymptomatic carriers and patients with the disease. Moreover, as early as 1988, it was recognized by the group of Jay Levy, from the University of California in San Francisco, that different HIV strains may belong to different neutralization subtypes [27].

Rapid progress was also made on the development of potential non-human primate (NHP) models for HIV infection and vaccine development. Experimental HIV infection of chimpanzees was achieved in 1984 [28,29] and in 1985 the first simian immunodeficiency virus (SIV) was isolated [30].

3. The first HIV vaccine trial in the world: 1986

The 1984 prediction of Secretary Heckler became a reality due to the audacity of Daniel Zagury, from the Pierre and Marie Curie University in Paris. In 1987, Zagury and collaborators reported that in November 1986 they initiated a phase I human trial in Zaire (now the Democratic Republic of Congo) [31]. Recognizing the potential variability of HIV, Zagury used a vaccinia vector expressing gp160, the envelope glycoprotein of the virus. With this approach Zagury hoped that the vaccine would induce both, antibodies capable of neutralizing virus strains closely related to the one included in the vaccine, as well as cytotoxic T lymphocytes (CTL) “that might overcome the limitations of type-specific responses to potential immunogens.” The paper described the results from the vaccination of Zagury himself, who reportedly was the first person to receive the vaccine. It also mentions that preliminary confirmation of those results came from additional immunization “of a small group of Zairians.” In 1988, Zagury and collaborators also reported the use of gp160 to boost the vaccinia-induced immune response [32]. Interestingly, this poxvirus-prime/protein-boost protocol was not significantly different from the one used in the modestly successful Thai RV144 trial 16 years later [33].

In addition, Zagury and French collaborators also explored, in 1990, the therapeutic potential of HIV vaccines, administering to HIV-infected patients formalin inactivated autologous cells that

had been previously infected *ex vivo* with a vaccinia vector expressing several HIV genes [34].

These pioneering efforts from Zagury, with both a preventive prime-boost approach and with a therapeutic vaccine, have almost but disappeared from the collective memory. The reasons are two-fold. In 1991, it was revealed in the media that the “small group of Zairians” who participated in the trial was in fact a group of young children. This led to accusations of unethical behavior from which Zagury was eventually exonerated. To make things worse, three of the volunteers who participated in the therapeutic vaccine trial, which was basically a compassionate trial in patients with advanced disease, died from possible complications related to disseminated infection with the vaccinia vector [35].

As Jon Cohen put it, “ultimately the trial went nowhere and was widely criticized for being run without the international community’s knowledge, involving children, and using unapproved materials for the vaccine” [3]. Cohen concluded that these trials “put AIDS vaccine researchers on notice that the ethics of the trials they stage, especially in poor countries, would receive intense scrutiny.”

4. Three overlapping “waves” of HIV vaccine paradigms and clinical trials

Despite many scientific uncertainties, a wide variety of candidate vaccines have been developed in the last 30 years, and the most promising have moved to clinical trials in humans.

The HIV vaccine pipeline has continuously evolved, passing through three main waves of vaccine development paradigms [9,11]: (1) induction of neutralizing antibodies, (2) induction of CTL responses, and (3) combinations of different immune responses (Fig. 1).

These vaccine waves have been overlapping and not strictly sequential. The overlap explains some of the disagreements that have plagued the field, with proponents of new paradigms challenging the previous ones, even before the field has had an opportunity to fully test them in properly conducted clinical trials.

An analysis of the clinical trials database from the International AIDS Vaccine Initiative (IAVI) indicates that a total of 218 clinical trials have been conducted since 1988, most of which have been categorized as early phase I safety/immunogenicity trials [36]. The majority of these trials involved different prime-boost combinations, followed in frequency by proteins or peptides, poxvirus vectors, DNA vaccines, adenovirus vectors, and other concepts. Only five vaccines have moved to phase IIb/III efficacy trials [37,38]. The majority of these trials (approximately 140) have been implemented in the United States, although, many have been done in other countries, including developing countries. Thailand has been the most active, having implemented two of the five efficacy trials have been conducted thus far [39,40].

5. First wave of HIV vaccine trials: induction of neutralizing antibodies (1988–2003)

We propose that this wave started with the first HIV vaccine trial conducted in the US in 1988, and ended in 2003 with the negative results from the efficacy trials of the VaxGen gp120 candidate vaccines [41,42].

5.1. Envelope glycoprotein vaccines

The initial HIV vaccines were based on the concept that neutralizing antibodies would be sufficient to confer protection against HIV infection. After all, most existing vaccines work through antibodies that block infection or interfere with systemic infection [43]. Most of those early HIV vaccines were based on the envelope glycoproteins of the virus (mainly gp120 or gp160), which are responsible for virus binding to the target cells, and serve as the main target for the neutralizing antibodies. The demonstration in 1987 that HIV envelope glycoproteins, either purified from virions or produced by recombinant technology, were safe and capable of inducing neutralizing antibodies in non-human primates (NHP), provided the scientific basis for the first clinical trials [44,45].

The first HIV vaccine trial conducted in the US evaluated a recombinant form of the envelope glycoprotein gp160 produced in a baculovirus-insect cell system. The product, named VaxSyn®, was manufactured by MicroGeneSys (Meriden, CT). The trial was approved by the FDA in September 1987 and the actual trial started in February 1988 [46]. The trial showed that the vaccine was safe and that it induced binding antibodies, although no significant neutralizing antibodies were generated [47].

Other envelope constructs rapidly followed through, especially gp120 and gp160 molecules produced in yeast or mammalian cell systems, with the main candidates manufactured by Chiron/Biocene (Emeryville, CA), Genentech (South San Francisco, CA), Immune AG (Vienna, Austria) and Pasteur Merieux/Connaught [48–53]. Between 1988 and 2003, at least 35 phase I trials were conducted with different envelope proteins. The results showed that, in general, the native glycosylated molecules produced in mammalian cells were safe and immunogenic, inducing binding and neutralizing antibodies. These vaccines also primed CD4+ cells, but were generally ineffective in inducing CD8+ CTL responses.

5.2. Vaccinia vectors and prime-boost regimes

The induction of CTL responses, which was already perceived as a desirable characteristic of an HIV vaccine, could be achieved through the use of live vectors, especially poxvirus vectors [54]. Live vectors were very appealing because they could present antigens to the immune system simulating natural infection. In 1986, two different groups reported the expression of the HIV envelope

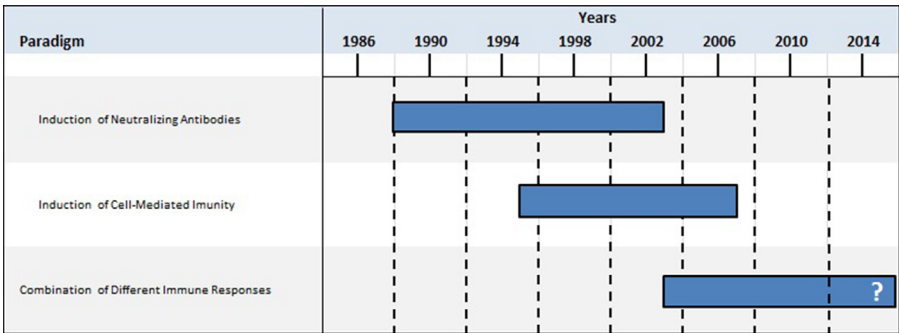


Fig. 1. Evolution of HIV vaccine paradigms and clinical trials.

gene in vaccinia vectors [55,56] and in November 1988 one of these constructs was the second candidate vaccine to enter clinical trials in the US. The vaccine, HIVAC-1e®, which expressed gp160, was manufactured by Bristol Myers-Squibb/Oncogen (Seattle, WA) and it had been found to be immunogenic in chimpanzees, although it failed to prevent infection after challenge [57]. In humans, the vaccine was found to induce strong T-cell responses, although these responses were transient and no antibodies against HIV were detected [58]. Another vaccinia vector produced by Therion Biologics Corporation (Cambridge, MA), expressing the *env* and *gag/pol* genes, entered clinical trials in 1994 [59].

An important early insight was that antibody responses could be enhanced by using a combined vaccine regime consisting of priming with a vaccinia recombinant expressing the HIV envelope and boosting with an envelope protein. Such a trial was formally initiated in the US in 1991, priming with HIVAC-1® and boosting with VaxSyn®, the two candidate vaccines that had been already evaluated separately in humans. This prime-boost approach had been previously used by Shiu-Lok Hu, then at Bristol Myers/Squibb, to protect macaques against SIV infection [60]. Prime-boost regimes were shown to greatly enhance humoral and cellular immune responses, including the induction of neutralizing antibodies [61,62]. Although the first vaccinia-prime/protein-boost trial was conducted by Zagury in 1988 [32], the above described prime-boost experiments provided ample scientific evidence that greatly influenced the field. From the 218 clinical trials conducted since 1988, 76 were conducted with different modalities of the prime-boost approach.

However, the use of the vaccinia virus as a vector had several practical concerns. On one hand, the immunogenicity of the vector is diminished in individuals who have been previously vaccinated against smallpox. But more importantly, the use of replicating vaccinia was contraindicated in immunosuppressed individuals, where its administration could cause severe disease [35,63]. In fact, a practical HIV vaccine for public health use should be safe in populations where HIV infection is prevalent. Consequently, attenuated poxvirus vectors were developed in the early 1990s by Jim Tartaglia and Enzo Paoletti, from Virogenetics Corporation (Troy, New York). These vectors are based on either a highly attenuated vaccinia virus (NYVAC) or on Canarypox (ALVAC®), an avian poxvirus which does not replicate in mammalian cells [54,64]. Another novel poxvirus vector strategy was the use of the modified vaccinia Ankara (MVA), a highly attenuated vaccinia virus originally developed in the mid-1970s which had been given to more than 120,000 people in Germany in the latest phases of the global smallpox eradication campaign. MVA grows to high titers in chicken embryo fibroblasts, but has virtually lost its ability to multiply in mammalian cells [65–67]. Over the last 20 years, 71 trials have been conducted with pox-virus based HIV vaccines [68].

An ALVAC®-vectored HIV vaccine expressing gp160 (vCP125, from Pasteur Merieux/Connaught, Lyon, France) entered clinical evaluation in 1993, and was used alone or in a prime/boost regime with an adjuvanted gp160 subunit [69]. The results showed that the ALVAC® vaccine significantly primed the neutralizing antibody response of the protein boost, also inducing CTL activity.

Second generation ALVAC® vectors (vCP205, vCP300, vCP1433, vCP1452, and vCP1521) were constructed to express not only the HIV envelope, but also *gag* and other HIV genes with the aim of inducing a broader cell-mediated immune response [70–72]. vCP205, which vectored multiple HIV genes (gp120, the anchor region of gp41, *gag* and protease) was the second vaccine tested in Africa, in Uganda in 1999 [73,74]. Although the vaccine tested in Uganda was based on clade B genes, while the circulating clades there are A and D, the trial was justified on the basis of extensive cross-clade cellular responses recently described [75]. And, of course, vCP1521 was the prime used in the Thai RV144 trial [33,71].

5.3. A focus on the V3 loop

Once it was established that neutralizing antibodies could be induced by the HIV envelope proteins, the next challenge was to identify specific neutralization epitopes in gp120 that could be used for vaccine design. This was achieved by animal immunization with gp120 segments produced in *E. coli* [76] and by identifying the peptides recognized by the neutralizing antibodies in HIV infected individuals [77]. Much work was done in this area by the team of Dani Bolognesi, from Duke University Medical School, and by 1990 there was general agreement in the field that the “Principal Neutralizing Determinant” (PND) of HIV mapped to the third hypervariable loop of gp120 (the V3 loop) [78,79]. In fact, Emilio Emini (who was then at Merck) showed in 1990 that mixing an HIV inoculum with an anti-V3 monoclonal antibody (mAb) abolished HIV infectivity for chimpanzees [80].

Immunization of guinea pigs with V3 peptides also induced neutralizing antibodies to HIV [81]. However, it was fully recognized at that time that the usefulness of V3 peptides in vaccine design could be limited by its extensive sequence variability. Another challenge is that the V3 loop is non-exposed on many primary isolates of the virus. Many years later, Susan Zolla-Pazner from New York University, argued that despite the sequence variability there was a significant conservation of the configuration of the V3 loop that could be exploited for the development of a broadly protective vaccine. She felt that the V3 conservation was related to its central role in the interaction of the virus envelope with the cell surface chemokine receptors [82,83].

In the early 1990s, concerns about the sequence variability of the V3 loop were balanced with the perception that it represented the principal neutralization determinant of HIV. The expectation existed that a limited repertoire of synthetic V3 peptides could meet the requirements for a broadly protective vaccine. With the leadership of Wayne Koff, who came from the NIH to work with United Biomedical Inc (UBI, Hauppauge, NY), the company developed an octameric synthetic V3 vaccine that entered clinical trials in 1993 [84]. This product was the first HIV vaccine to be tested in China, in 1993 [85], as well as in Thailand in 1994 [86]. The vaccine was found to be safe and to induce strain specific neutralizing antibodies [87]. A different V3 loop-based vaccine (TAB9) was tested in Cuba in 2001 [88].

Even though the V3 loop attracted considerable attention as a potential vaccine target, it was known since the early 1990s that some infected individuals made broadly neutralizing antibodies that were targeting conserved conformational epitopes in gp120, rather than the V3 loop [89]. Those observations reinforced the concept that conformational epitopes present in native envelope glycoproteins might protect against a broader range of HIV strains than a vaccine that presented exclusively linear determinants, such as those in the V3 loop.

5.4. Animal protection experiments

Different lines of research using NHP provided optimism to the field. In 1990, the groups of Phil Berman at Genentech and Marc Girard at the Pasteur Institute in Paris, found that immunizations of chimpanzees with candidate vaccines based on the HIV envelope glycoproteins conferred protection against an HIV challenge [90,91].

Although chimpanzees are the only NHP that can be readily infected with HIV, and they were extensively used in HIV vaccine research, it was becoming evident that a different animal model was needed. Chimpanzees are an endangered species, and their genetic proximity to humans made it ethically difficult to perform experiments, especially those involving challenges with virulent virus [92]. In addition, since chimpanzees were believed not to

develop disease after HIV infection, a pathogenic model where the effect of vaccination on both acquisition of infection and disease development, was highly desirable [93].

Consequently, many experimental vaccines were designed using SIV as a surrogate for HIV and tested in the macaque model [94]. The expectation was that such information could then be extrapolated to the design of HIV vaccines. The problem, of course, was that those vaccines were based on the wrong virus (SIV) and evaluated in the wrong animal (macaque monkeys). A breakthrough came around 1990, when teams in the US and Japan independently generated chimeric viruses constructed with the *gag* and *pol* genes of SIV and the *env* gene of HIV that are capable of infecting macaques [95,96]. Since then, these chimeric SIV/HIV viruses, known as SHIV, have been an important although imperfect tool, to study in macaques the protective effect of different immunogens based on the HIV envelope [97].

Unfortunately, most of the candidate vaccines tested in the SIV-SHIV/macaque models failed to protect against virus acquisition (often referred to as sterilizing immunity). However, many of these trials resulted in decreased virus load and protection against progression to disease in vaccinated animals that became infected after the challenge. Those observations led, as late as in 2007 to the reasonable conclusion that a vaccine capable of achieving sterilizing immunity might not be a realistic goal and that, perhaps, the best that could be done was to develop disease-modifying vaccines [98–100]. However, most of the experiments conducted at that time used large dose challenges, often administered intravenously, conditions that were very different from natural infection. Those large virus challenges could have overwhelmed any potential vaccine-induced protective immune response. To address those concerns, a more representative challenge model was proposed in 2004, using repeated low-dose challenges of virus administered intravaginally [101,102]. This challenge model has now become the standard and a number of experimental vaccines have shown to induce protection against virus acquisition in the macaque model [103,104].

5.5. *A faux pas: whole inactivated SIV vaccines*

Much excitement was created in 1989 when the groups led by Ron Desrosiers from the New England Regional Primate Research Center, Harvard Medical School, and Mickey Murphy-Corb from the Delta Primate Center at Tulane University, reported that adjuvanted whole inactivated SIV could protect macaques against a challenge with live SIV [105,106]. After all, whole inactivated vaccines have worked against many other viral diseases. Those findings were rapidly followed by the report that macaques could also be protected from SIV by immunization with inactivated preparations of cells that had been infected with SIV [107]. Suddenly, a critical control was included by Jim Stott and collaborators, from the National Institutes for Biological Standards and Control (NIBSC) in London. To the collective surprise and disappointment, it was found that immunization with similar preparations of uninfected cells also afforded partial protection against SIV [108]. Although it is known that envelope viruses pick up cellular antigens when budding from the cell membrane, a fact also established for HIV [109], never before have host cell components been shown to be responsible for protection [110]. An analysis of sera from protected monkeys demonstrated a positive correlation with antibodies to the HLA class I molecule [111]. Moreover, experimental immunization with HLA class I antigens protected macaques from SIV challenge, confirming that protection was due to xeno-immunization rather than to a virus specific immune response [112]. That essentially was the end of the road for the use of whole inactivated vaccines aimed at inducing viral-specific immune responses in healthy individuals [113].

However, in 1987 Jonas Salk, the developer of the first polio vaccine, proposed the use of inactivated HIV preparations as a therapeutic approach for the immunization of infected patients [114]. This led to the development of Remune®, an inactivated gp120-depleted immunogen, manufactured by the Immune Response Corporation (Carlsbad, CA). The company initiated large scale clinical trials in 1996, which were discontinued in 1998 [115].

Nevertheless, the monkey protection experiments described above stimulated more recent research aimed at inducing protective immunity using alloantigen-based vaccines, especially by Tom Lehner, from King's College London, and collaborators [116–118].

5.6. *The live-attenuated vaccine saga*

In the early 1990s Ron Desrosiers was studying the *in vivo* effect of the SIV accessory genes. In 1992, he reported that deletion of the *nef* gene resulted in an attenuated virus that, upon inoculation in macaques, resulted in protection against a challenge with wild-type pathogenic strains of SIV [119].

Interestingly, that finding was consistent with the reports from the Sydney Blood Bank cohort in which eight accidental recipients of blood containing a *nef*-deleted strain of HIV were not progressing to AIDS [120,121]. Those observations opened up the possibility of considering the development of a live-attenuated HIV vaccine [122–124]. By that time, the HIV vaccine field had suffered several setbacks and the possibility of a live-attenuated vaccine, although very risky and controversial, needed to be considered [125]. The discussion centered around two possibilities. The use of live-attenuated HIV strains for human trials or, alternatively, the use the SIV-macaque model to understand immune correlates of protection that could inform the development of safer vaccines for humans. As an act of desperation, or perhaps of altruism, a group of physicians proposed to volunteer for a trial using a live-attenuated HIV vaccine in 1997 [126].

The unacceptable risk of using attenuated strains of HIV as a vaccine was clearly demonstrated by the team of Ruth Ruprecht, from the Dana-Farber Cancer Institute at Harvard Medical School, who in 1999 reported that multiple deleted SIV mutants could actually cause AIDS in infant and adult monkeys [127–129]. Moreover, prolonged observation of the Sydney Blood Bank Cohort, showed immunological damage in some individuals chronically infected with the *nef*-deleted HIV strain [130]. Although those observations put an end to any consideration of using a live attenuated HIV in human, the live attenuated SIV model was intensively investigated by the IAVI Live Attenuate SIV Vaccine Consortium and others [131]. Recent results suggest that protection by live-attenuated virus might be mediated either by antibody-dependent cell-mediated cytotoxicity (ADCC) [132] or by persistence of T cells in the lymph nodes [133], findings that could inform future HIV vaccine development.

5.7. *Preparing for international vaccine trials*

Partially driven by the above monkey protection experiments, a sense of urgency developed in the early 1990s to prepare international sites to conduct efficacy trials of the HIV vaccines, which some expected to be available in the forthcoming years. In 1990, WHO formed its HIV Vaccine Advisory Committee, initially chaired by Hans Wigzell, from the Karolinska Institute. That same year, WHO began the process of identifying potential vaccine evaluation sites and initiated discussions with Brazil, Rwanda, Thailand and Brazil. These discussions led to the development of “National AIDS Vaccine Plans” [134,135]. An important component of site preparation was the development of ethical guidance for the conduct of HIV vaccine trials [136]. With the exception of Rwanda, where a genocidal war started in 1994, the three other countries

became pioneers in the international testing of HIV vaccines [39,40,73,74,137–140].

The major HIV vaccine player over the years has undoubtedly been the US National Institutes of Health (NIH). NIH established its Division of AIDS (DAIDS) in 1986 and the Office for AIDS Research (OAR) in 1988. The AIDS Vaccine Evaluation Group (AVEG), created in 1988, conducted most of the early phase I trials of HIV vaccines. The HIV Network for Prevention Trials (HIVNET) was formed in 1993 to conduct domestic and international multi-center trials to evaluate the safety and efficacy of promising HIV preventive interventions including, but not limited, to vaccines. To prepare for international vaccine trials, in 1993 the NIH launched the “Preparation for AIDS/HIV Vaccine Evaluation” (PAVE) projects, with international sites in Haiti, India, Kenya, Malawi, Rwanda, Thailand, Uganda, and Zimbabwe [141].

A 1993 review of the NIH AIDS activities led to some important changes, including the 1994 reorganization of the OAR [142], the publication of the influential Levine Report in 1996 [143] and the establishment (the same year) of the AIDS Vaccine Research Committee led by David Baltimore [144]. In 1999 the HIV Vaccine Trials Network (HVTN) was established under the leadership of Larry Corey to conduct all phases of clinical trials. The HVTN, which is headquartered at the Fred Hutchinson Cancer Research Center in Seattle, WA, currently conducts the large majority of the HIV vaccine trials through an international network of Vaccine Trial Units [145].

Other international HIV vaccine programs established in the 1980s included the Directed AIDS Research Programme of AIDS Research of the UK Medical Research Council, launched in 1987 under the leadership of Geoffrey Schild [146], and the French National Agency for AIDS Research (ANRS) that, who in 1992 initiated clinical trials of recombinant canarypox viruses (ALVAC) [147] and later of lipopeptide immunogens [148]. From 1986 ANRS and Pasteur Merieux convened in France the “Cent Gardes” Colloquia, where every year the latest HIV vaccine science was discussed. Italian investigators explored the potential vaccine use of Tat, a regulatory HIV protein [149], and Russia established more recently its own HIV vaccine development program [150].

The South African AIDS Vaccine Initiative (SAAVI) was established in 1999 under the leadership of Malegapuru William Makgoba, to coordinate and support the development and testing of HIV vaccines in South Africa. SAAVI focused on vaccines based around clade C strains, which are the most prevalent in southern Africa [151]. With the support from WHO, in 2000 the “African AIDS Vaccine Programme” (AAVP, now “Partnership”) was launched, with the goal of mobilizing support and advocating for a more Africa-focused HIV vaccine agenda [152]. More recently, a similar regional initiative has been launched for Asia (the AIDS Vaccine for Asia Network—AVAN) [153].

5.8. The emerging picture of HIV clades

Genomic sequencing of HIV isolates from many parts of the world began to reveal genotypic differences among different isolates, which were initially classified either as African or North-American strains. By 1991 it was clear from sequencing data of the *env* gene that there were at least five distinct lineages of the virus, usually referred to as genetic subtypes or clades. For the sake of simplicity, they were designated by the late Gerald Myers and Better Korber, from the Los Alamos National Laboratory, as A, B, C, D, and E subtypes [154]. Most of these subtypes were also subsequently established based on sequence of the *gag* gene [155].

In 1992, led by Saladin Osmanov, WHO formally established a network for HIV isolation and characterization. The network monitored HIV variability on a global basis, with a special focus on the four initially selected vaccine evaluation sites [156,157]. WHO

subsequently reported that by 2000 the largest incidence of HIV-1 infections was due to subtype C strains (47.2%), concentrated in southern Africa [158,159].

A pressing question at that time was if the HIV clades correlated with immunological subtypes or immunotypes. Initial data, comparing isolates from the US and Thailand, indicated that they belonged to two different immunotypes that correlated with viral clades B and E, respectively [160]. Those immunotypic differences suggested that a broadly effective vaccine may have to include strains from multiple clades (until that time all candidate vaccines were based on clade B strains). When more complex virus/antibody matrices were studied, the conclusion was that potential neutralization serotypes (or immunotypes) do not directly correlate with the genotype [161,162]. The potential existence of HIV immunotypes is currently been investigated using a comprehensive neutralization checkerboard using approximately 200 viruses and 200 plasma samples from chronically infected individuals (Montefiori DC, personal communication).

5.9. The virus neutralization surprise

As discussed before, many of the enveloped-based candidate vaccines tested in the 1980s and early 1990s were found to induce neutralizing antibodies in animal models and human trials. However, in 1994 it was found that those vaccine-induced antibodies were capable of neutralizing laboratory adapted strains of HIV, even though they could not neutralize isolates obtained from patients, usually referred to as primary, clinical or field isolates [163–167]. That observation had important implications for vaccine development since it was felt that a protective immune response should be effective against the clinically relevant transmitted virus.

To understand the above observation, we need to go back to 1988 when investigators in Sweden, led by Eva-Maria Fenyö and Birgitta Asjö, described biological differences among HIV strains that were classified as either slow/low or rapid/high according to their capacity to replicate *in vitro* [168,169]. That classification broadly corresponded with another classification proposed the same year by Dutch scientists. It was based on the ability to induce syncytium in activated peripheral mononuclear cells (PBMC), defining non-syncytium inducing (NSI) or syncytium inducing (SI) strains [170]. An additional viral phenotype was defined by the *in vitro* cell tropism, either as T-cell or macrophage tropic viruses, which also showed some correlation with the two other biological phenotypes discussed before [171,172]. Moreover, those biological features were found to correspond to the severity of HIV infection [173]. Progression to disease was associated with a shift from monocytopathic to T-cell-tropic virus population [174]. Of relevance to vaccine development, it was also found that virus strains transmitted between individuals are usually macrophage or dual tropic (being also slow/low and NSI) and cannot replicate in transformed T-cell lines [175].

The explanation for the biological phenotypes described in the previous paragraph was found in 1995/1996, with “the unexpected encounter of HIV and the chemokine system” [176–179]. Although it has been known since 1984 that the CD4 molecule is the main receptor for the virus [180,181], it was also known that the presence of CD4 in the cell surface was not sufficient for HIV infection. The missing factors were the co-receptors that HIV uses to infect the target cells, either CCR5 or CXCR4 or both. With that new understanding, HIV strains using the CCR5 co-receptor are classified as R5, and are usually macrophage-tropic and non-syncytia-inducing. On the other hand, viruses using the CXCR4 co-receptor are known as X4, and are usually T-tropic and syncytia-inducing [182].

However, the attractive possibility that the *in vitro* sensitivity of HIV isolates to neutralization was due to different co-receptor use (with X4 strains being more susceptible), which was found to be

untrue and most likely dependent on factors other than co-receptor usage [183–185].

In any case, the field reached the conclusion that vaccine-induced neutralizing antibodies should be able to neutralize a wide range of strains likely to be encountered in the real world, and not just a limited set of highly sensitive X4 laboratory strains. To facilitate the identification of vaccines with enhanced capacity for eliciting cross-neutralizing antibodies, a tiered system was proposed in which the overall potency and breadth of the responses is assessed with a panel of well characterized viruses with different sensitivities [186,187]. The question remains of what is the relevance of the current *in vitro* neutralization assays regarding potential vaccine protection in humans, and alternative neutralization assays are being currently explored [188,189]. Ultimately, the predictive value of any of these *in vitro* assays would only be known when correlates of protective immunity are identified in efficacy trials.

Answers to this “neutralizing antibody problem” [190] may come from a better understanding of the molecular structure of the HIV envelope protein. A first step in that direction was taken in 1998 by Peter Kwong, then at Columbia University and now at the NIH Vaccine Research Center (VRC), when he published the first crystal structure of gp120 [191,192]. Interestingly, despite dramatic biological differences between primary and laboratory-adapted HIV strains, their envelope core structure was found to be quite similar. This suggested that neutralization resistance is specified by quaternary interactions involving the variable loops [193]. More recent data from the laboratory of Joe Sodroski, at Harvard Medical School, indicates that HIV entry into cells as well as sensitivity to antibodies may depend on the “intrinsic reactivity” of the virus, which is defined as the ability of the envelope glycoproteins to negotiate transitions to lower stages of energy after stimulation [194]. The recent description of the subunit organization of the envelope trimer of HIV, by the Sodroski team, may provide additional clues to help designing the appropriate immunogens [195].

Current efforts to develop envelope based vaccines focus on the use of primary viruses isolated the weeks following clinical infection (referred to as transmitted/founder viruses). They have been shown to have biological properties that could be relevant for induction of protective immunity [196].

5.10. New energy in the field

The accumulation of new scientific knowledge during the 1990s led to renewed energy in the field, exemplified by three main events: the “Clinton challenge”, the launching of the AIDS Vaccine Advocacy Coalition (AVAC), and the establishment of IAVI.

On May 18, 1997, during a commencement speech at Morgan State University in Baltimore, US President Bill Clinton challenged the scientific community to develop an HIV vaccine within 10 years. He made of this challenge a national goal for science, comparing it to President Kennedy’s challenge 36 years earlier, to land a man on the moon before the end of the 1960s [197,198]. Since then, May 18 has become “HIV Vaccine Awareness Day”. To achieve the goal, President Clinton recommended a significant increase in the government budget for HIV vaccine research and announced the creation of the NIH Vaccine Research Center (VRC), with Gary Nabel appointed as its first Director [199]. Most scientists were against promising a vaccine within a specific period of time, but some argued that having such a goal would energize the field and convey the necessary sense of purpose and urgency. Although no promises were made, it is interesting to note that the first efficacy signal of an HIV vaccine (the RV144 trial) came 12 years later, in 2009, not too far from the 10 years challenge.

AVAC was founded in 1995, in large part due to the efforts of Bill Snow, a long time HIV vaccine advocate, to accelerate the

development of an HIV vaccine through education, policy analysis and advocacy. In 1998 AVAC published its first annual report, entitled “9 Years and Counting” alluding to President Clinton’s commitment to develop an HIV vaccine in 10 years [200]. In 2009, under the leadership of Mitchell Warren, AVAC broadened its mandate to become the “Global Advocacy for HIV Prevention”.

In 1996 IAVI was established under the leadership of Seth Berkley, based on a recommendation made by a group of experts convened by the Rockefeller Foundation in 1994 in Bellagio, Italy. In 1997, IAVI launched a “Call for Action” and in 1998 issued its first Scientific Blueprint for AIDS Vaccine Development. In 2011, Margie McGlynn, a former head of Merck’s vaccines and infectious diseases, became the second IAVI director after Seth Berkley assumed the leadership of the GAVI Alliance.

5.11. The first two efficacy trials: VaxGen

By 1994, two gp120 products emerged as potential candidates for an efficacy trial. Genentech and Chiron/Biocene have spent eight years testing envelope based vaccines in animal models and phase I/II clinical trials in humans. These vaccines had been shown to be safe and immunogenic in phase I clinical trials and to protect chimpanzees from an HIV challenge [90,201]. On the other hand, the recent finding that the antibodies induced by those vaccines failed to neutralize primary isolates of HIV threw a cloud of doubt over the scientific rationale for advancing their clinical evaluation. Moreover, the chimpanzee protection experiments were done with laboratory adapted strains of the virus and their relevance for human protection was not clear. In view of the urgency imposed by the epidemic, especially in developing countries, the dilemma that the scientific community confronted was between adopting a cautious strategy of more basic research *versus* a more aggressive approach based on “thoughtful empiricism” [202]. That dilemma was discussed in 1994 by two advisory groups of the NIAID: the HIV Vaccine Working Group and the AIDS Research Advisory Committee (ARAC). After considering the possibility of moving ahead with a scaled down efficacy trial, the NIAID finally decided not to support the proposed efficacy evaluation of the candidate vaccines [203].

The NIAID decision stimulated a broader discussion that led WHO to make in 1994 a recommendation encouraging efficacy testing of HIV vaccines in developing countries where the epidemic is most severe [204–207]. In addition, the negative decision of the NIAID did not discourage Don Francis, who had earlier moved from the CDC to Genentech, who decided to leave Genentech and start a new company, VaxGen (South San Francisco, CA). VaxGen sole purpose was conducting efficacy evaluation of the Genentech gp120 vaccines [208]. It took four additional years of preparations and discussions before VaxGen started its first efficacy trial in North America (VAX004) and Thailand (VAX003) [209–212]. Taking into account the recently gained knowledge about the genetic variability of HIV strains and the use of different co-receptors, the original candidate vaccines were redesigned as bivalent gp120 preparations (AIDS VAX® B/B or the North American trial and AIDS VAX® B/E for the Thai trial) derived from R5 and X4 strains [213]. The two efficacy trials were done from 1998 to 2003, involving 5417 volunteers in VAX004, (mostly men-who-have sex-with-men in North America) and 2545 volunteers in VAX003 (injecting drug users in Bangkok, Thailand) (Fig. 2).

The final results of these two trials were reported in 2003, indicating that the vaccines failed to confer protection against HIV [41,42]. A *post hoc* analysis suggested that in the North American trial (VAX004) women and African-Americans may have been somehow protected. This claim caused considerable confusion and controversy [214–217]. Many scientists who had expressed disagreement with the conduct of these trials, felt vindicated by the negative results. The claims of potential efficacy in some

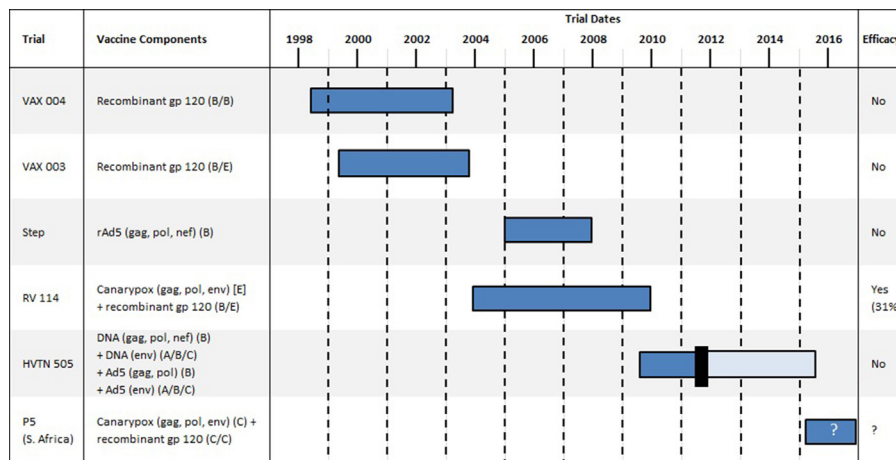


Fig. 2. Efficacy trials of HIV vaccines.

populations triggered a negative reaction from the scientific community that, in part, prevented or delayed a thorough scientific analysis of the data and samples obtained from these trials. Fortunately, samples from VAX003 and VAX004 were stored and recently analyzed. They were compared with results from the RV144, revealing important information that may be related to protection [218,219].

After these two trials, Don Francis, Phil Berman and other key players in the gp120 efficacy trials left VaxGen in 2004 to start Global Solutions for Infectious Diseases (GSID), a non-profit organization engaged in the development of vaccines and other products. Through the provision of AIDSVAX B/E, GSID was a partner in the RV144 trial.

6. Second wave of HIV vaccine trials: induction of CTL responses (1995–2007)

A second wave of HIV vaccine trials began with the recognition in the early 2000s of the critical importance of CD8+ T-cell responses in the control of HIV infection, especially due to the work of Bruce Walker from the Massachusetts General Hospital and Andrew McMichael from the University of Oxford [220–223]. This paradigm led to the development and refinement of live recombinant viral vectors, especially pox and adenovirus vectors, as well as DNA vaccines [224,225,68]. We suggest that this period was formally concluded in 2007 with the unexpected lack of efficacy in the STEP trial, which evaluated a cell-mediated immunity vaccine based on an adenovirus type 5 (Ad5) vector expressing HIV-1 *gag*, *pol* and *nef* [226].

6.1. Re-examining the global strategy

The negative results from the VaxGen gp120 trials catalyzed a rethinking in the field with a re-examination of the scientific basis for the development of an HIV vaccine, as well as the search of mechanisms for a more strategic and coordinated approach to solve the HIV vaccine challenge. In 2003, an international group of scientists proposed the creation of a global HIV Vaccine Enterprise as a new collaborative model of research to accelerate the global effort to develop an HIV vaccine [227]. Two years later, the Enterprise published its first Scientific Strategic Plan [228] identifying scientific priorities and making recommendations to address them. The Bill & Melinda Gates Foundation served as the interim secretariat of the Enterprise until early in 2008 when a permanent secretariat was established in New York under the leadership of

Alan Bernstein. The Enterprise secretariat continues functioning in New York, currently under the direction of Bill Snow [229–234].

Perhaps the two most direct responses to the Enterprise vision was the creation of the NIH-funded Center of HIV Vaccine Immunology (CHAVI) in 2005, directed by Barton Haynes from Duke University [235,236], and the 2006 launching of the Bill & Melinda Gates Foundation supported Collaboration for AIDS Vaccine Discovery (CAVD) [237]. Both programs are still in existence. The Enterprise also inspired the creation of Europrise, a Network of Excellence supported by the European Commission which operated between 2007 and 2012 [238], as well as the establishment of the Canadian HIV Vaccine Initiative (CHVI).

6.2. Lessons from natural history

It has been known since the early 1990s that infected people mount vigorous CTL responses to HIV [239,240] and these responses were considered as potential effectors of future HIV vaccines [223,241,242]. The 2000 decade saw much work trying to understand the dynamic of cellular immune responses in natural infection and in animal models [243–246]. Those studies provided strong evidence that CTLs were important in controlling virus replication in infected people, although not sufficient to completely eliminate the virus or suppress it indefinitely [247].

The “pessimistic expectation that the gp120 vaccine would not offer significant protection against primary HIV infection” was a powerful incentive to turn the attention to the cell mediated arm of the immune system, in particular to vaccines that can stimulate CD8+ T cells [248]. That was pursued mostly through the development of DNA immunization and the use of viral vectors.

6.3. The excitement of DNA vaccines

Several research groups reported some 20 years ago, in the early 1990s, that plasmid DNA delivered into the skin or muscle induced viral specific antibody responses [249–252]. This relatively simple technology was seen as a potential modern replacement of live-attenuated vaccines, capable of inducing a whole range of immune responses [253]. In the mid-1990s the potential of DNA vaccination was explored in the SIV/macaque model by different teams, especially those lead by David Weiner (from the University of Pennsylvania School of Medicine), Harriet Robinson (then at Emory University), and Shan Lu (from the University of Massachusetts Medical School). Those studies indicated that vaccination elicited persisting SIV-specific CTL although protection

against SIV challenges was not readily demonstrated in vaccinated animals only showing reductions in virus load [254–259].

However, it was rapidly found that the robust immunogenicity levels obtained with DNA immunization in small animals did not translate to NHP and to humans. To address that problem, different approaches were explored to enhance the immunogenicity of DNA vaccines, including: the use of routes of administration that combined priming with particles coated with DNA (gene-gun) followed by intramuscular and intradermal inoculation [260,261]; *in vivo* electroporation [262–265]; co-administration of DNA and different cytokines [266–269]; and boosting with different vectors or recombinant proteins [270–273].

Although at least four DNA vaccines have been approved in the area of veterinary medicine, none have yet been licensed for human use [274]. Nevertheless, according to the IAVI clinical trials database [36], 10 phase I clinical trials of HIV DNA vaccines have been conducted since 2001 [275,276]. Those early human trials showed disappointing levels of immune responses casting a doubt on the utility of DNA vaccines for human application [277].

Two studies published in 2008 provided evidence on the utility of DNA priming after boosting with either a vaccinia vector [278] or recombinant gp120 proteins [279]. Different approaches of DNA priming and heterologous boosting continue to be explored to date [280–284].

In fact, the latest efficacy trial of an HIV vaccine, HVTN 505, tested a DNA-primed candidate vaccine developed by the NIH VRC. It consisted of a prime-boost regimen involving three vaccinations with DNA encoding HIV clade B *gag*, *pol*, and *nef*, and *env* from HIV clades A, B, and C, followed by an Ad5 vector-based vaccine candidate encoding clade B *gag* and *pol*, and *env* from clades A, B, and C [285–287]. The trial, which was sponsored by the US NIH, was launched in 2009 with the final results originally expected by 2015 (Fig. 2). Unfortunately, the trial was stopped in April 2013, after an interim analysis revealed that the vaccine failed to prevent infection or to reduce virus load in vaccinated volunteers who became infected.

6.4. The rise of viral vectored vaccines: poxvirus and adenoviruses

Two viral vectors have been preferentially used for the development of HIV vaccines, poxviruses and adenoviruses.

Although poxvirus vectors were the first to be used for HIV vaccines, the emphasis during this wave shifted to adenovirus vectors. Work continued on the poxvirus front, mostly because of the work of three laboratories exploring different strains of the vaccinia virus: Gepi Pantaleo in Lausanne exploring novel vectors based on NYVAC, a highly attenuated vaccinia virus strain derived from the Copenhagen vaccine strain from which 18 open reading frames had been selectively deleted from the viral genome [68,288]; Tom Hanke in Oxford, working with MVA vectors [67,289]; and Yiming Shao in Beijing using the Tian Tan strain, used in China during the smallpox eradication campaign [290]. Genetic manipulation of the vectors and HIV gene inserts are being used to develop vaccinia vectors with improved innate and adaptive immunogenicity, especially when primed with DNA [278,291–293].

But, as mentioned before, the main paradigm explored during this phase was the use of adenovirus vectors, which were originally developed for the treatment of genetic disease and subsequently used as vaccine vectors [225,294]. Adenovirus vectors have been intensively explored as a vector for HIV vaccines, mostly with the goal of eliciting HIV-specific cellular immune responses [294–297]. The IAVI clinical trials database [36] lists a total of 42 clinical trials of different adenovirus vectors conducted since 2003, by themselves or in prime-boost combinations.

A major driving force of the adenovirus vector effort was the pharmaceutical company Merck & Co, Inc. (Whitehouse Station, NJ) which in 2001 publically announced results from their initial NHP protection experiments [298,299]. Their attention was focused on the use of a replication-defective adenovirus 5 (Ad5) vectors expressing the SIV *gag* gene, which in macaques was able to induce attenuation of viral infection after challenge [300]. Based on these results a candidate vaccine using a mixture of recombinant Ad5 vectors expressing the HIV *gag*, *pol* and *nef* genes from subtype B (known as MRKAd5® HIV-1gag/pol/nef) was tested in phase I clinical trials and found to be safe and immunogenic [301]. This was the vaccine used in the STEP and Phambili trials (see below).

An early concern related to the potential use of the Ad5 vector was that preexisting immunity to adenovirus 5, which is prevalent in human populations, especially in less developed countries [302], could impair the immunogenicity of the Ad5 vector. Although initial evidence from phase I clinical trials indicated that the MRKAd5® HIV-1gag/pol/nef was in fact capable of eliciting immune responses in participants who were both Ad5 seronegative and Ad5 seropositive, the field began to explore the use of other alternatives. Under the leadership of Dan Barouch from the Beth Israel Deaconess Medical Center in Boston and others, efforts are currently underway to develop novel vectors based on less prevalent human adenovirus types, or on simian adenoviruses for which no preexisting immunity exists in human populations [225,294,303–306]. In this regard it is important to emphasize that adenovirus vectors are complex biological entities and not simple physical carriers of foreign vaccine inserts. Different adenovirus serotypes have different biological properties that might influence their safety, immunogenicity and potential efficacy as vaccine vectors.

6.5. The STEP trial

The potential efficacy of the MRKAd5® HIV-1gag/pol/nef vaccine was tested in the STEP trial, with the co-sponsorship of the NIH/NIAID and Merck. Enrolling began in December 2004 where they ultimately recruited 3000 participants in Australia, Brazil, Canada, the Dominican Republic, Haiti, Jamaica, Peru, Puerto Rico and the United States. In all these countries HIV subtype B is prevalent (the same subtype as in the vaccine). The goal of this phase IIb “test-of-concept” study was to determine if the vaccine could prevent HIV infection, reduce the amount of HIV in those who do become infected, or both. In 2007 the same vaccine also entered phase IIb evaluation in South Africa (the Phambili trial), to explore if it would also be effective at preventing infection from HIV subtype C, which is prevalent in southern Africa. That was a testable possibility in view of the cross-clade reactivity of CMI responses against HIV.

However, in September 2007 both studies (STEP and Phambili) were halted because an interim review of the STEP trial revealed that the vaccine was not effective [307]. In a multivariate analysis of baseline risk factors, vaccination appeared associated with an increased risk of HIV acquisition in certain volunteers. When the final results of the trial were formally published in November 2008, the authors concluded that the type of cell-mediated immunity induced by this vaccine did not prevent HIV infection or reduce early viral load [226]. A *post hoc* analyses of the STEP trial confirmed that more HIV infections occurred in vaccinees vs. placebo recipients in men who had Ad5-neutralizing antibodies and/or were uncircumcised, although the vaccine-associated risk waned with time from vaccination [308].

The negative results from this trial came as a surprise to the scientific community who had high expectations with this CMI approach [309–314]. After these disappointing results, the NIAID held a scientific summit in March 2008 with the call to return

to basic science [315]. The meeting recommended broadening the research agenda to answer fundamental questions in HIV vaccine discovery through laboratory, nonhuman primate (NHP), and clinical research carefully prioritizing the effort to energize HIV vaccine discovery [316].

7. Third wave of HIV vaccine trials: combinations of different immune responses (from 2007)

We propose that the third and current wave of HIV vaccine research was initiated after the announcement of the disappointing results from the STEP trial. The scientific community realized, once again, that the development of an HIV vaccine is one of the most difficult challenges that biomedical science is confronting. This wave, that hopefully will take us to the development of an effective vaccine, should learn from past failures and systematically explore different alternatives, including novel concepts that do not fall within any of the current paradigms.

7.1. The RV144 surprise

The negative results from the STEP trial, announced in September 2007, were contrasted with the surprisingly positive (although modest) results of the RV144 trial announced two years later, in October 2009. RV144 tested a prime-boost recombinant vaccine against HIV among 16,402 adults in Thailand, from September 2003 to August 2009 (Fig. 2). The trial was an extraordinary collaboration of Thai scientists (particularly Supachai Rerks-Ngarm and Punnee Pitisuttithum) and US investigators (Nelson Michael and Jerome Kim, from the Military HIV Research Program), with support from the NIH and participation from Sanofi and GSK. The trial tested the protective efficacy of a “prime-boost” combination of two vaccines: ALVAC® (the vCP1521 canarypox vector manufactured by Sanofi Pasteur) and AIDSVAX® B/E vaccine (the Genentech gp120 proteins previously tested in VAX003 and VAXX04, supplied by GSK) (see Section 5.11).

The trial demonstrated a 31.2% efficacy in preventing HIV infection [33]. Although the initiation of this trial was marred by controversy [317,318], and the initial results were received with considerable skepticism [319], the field ultimately accepted the results. They were most especially accepted after the publication in May 2012 of an immune correlates analysis coordinated by Bart Haynes from Duke University [320]. This immune-correlates study generated the hypotheses that V1V2 antibodies may have contributed to protection against HIV-1 infection, whereas high levels of Env-specific IgA antibodies may have mitigated the effects of protective antibodies. This analysis failed to identify neutralizing antibodies as a potential correlate, turning the attention to a potential role of non-neutralizing antibodies, probably to those involved in mediating antibody-dependent cell-mediated cytotoxicity (ADCC) [132,321].

The immune correlate information, as well as information suggesting that vaccine efficacy in RV144 appeared to wane from 60% at 12 months to 31.2% at 3.5 years [322,323] is being considered in the design of candidate vaccines and clinical trials aiming to build on the modest efficacy achieved in the RV144 trial [324]. Discussions are under way to confirm and extend results from RV144 in other populations, including southern Africa (Fig. 2).

To some extent, the modest success obtained with the RV144 trial brought new attention to the importance of conducting clinical trials, especially efficacy trials, to complement the basic research effort [14]. As mentioned before, the RV144 trial also provided information suggesting that antibodies were important in conferring protection against acquisition of HIV infection. This, taken together with the failure of the CMI vaccine tested in the STEP trial,

turned the HIV vaccine paradigm pendulum back to the induction of antibodies. This ultimately may be needed to confer sterilizing protection in vaccinated individuals, or at least to prevent the establishment of chronic infection [325].

7.2. Broadly neutralizing antibodies and structure-based immunogen design

The Holy Grail of HIV vaccine research has been the development of immunogens capable of eliciting broadly neutralizing antibodies that can protect against the large number of immunological different strains that circulate globally [231], an approach that has been championed for many years by the IAVI Neutralizing Antibody Consortium under the leadership of Dennis Burton, from the Scripps Research Institute in La Jolla, and IAVI's Wayne Koff [190].

A major frustration in the field is that after many years of research with different versions of the HIV envelope glycoproteins, these products have failed to induce antibodies capable of neutralizing primary isolates of the virus. Nevertheless, it is known that some humans infected with HIV do develop antibodies that are capable of neutralizing different strains of the virus (broadly neutralizing antibodies), providing a hope that one day we would be able to induce those antibodies with a vaccine [326,327]. In fact, as early as 1992, broadly neutralizing monoclonal antibodies (bnmAb) targeting different HIV epitopes have been isolated and characterized [328–332], and in recent years there has been an explosion of new bnmAb against HIV, especially because of the work at Scripps, the VRC, the CAVD team of Robin Weiss and Antonio Lanzavecchia, and more recently, the laboratory of Michel Nussenzweig at The Rockefeller University [333–338]. The epitopes recognized by those bnmAbs are being explored as potential immunogens for vaccine development by different laboratories, particularly at Scripps and the VRC [190,339,340].

This structure-assisted approach to immunogen design has benefited from recent information on the molecular structure of the HIV envelope glycoprotein and the critical epitopes that are targeted by bnmAbs [341–344]. We have learned much of the unique characteristics of these bnmAbs, including polyreactivity for host antigens, extensive somatic hypermutation and long, variable heavy-chain third complementarity-determining regions [345]. However, a major challenge that the field is confronting is the dissociation between antigenicity (the ability of a molecule to be recognized by given monoclonal antibodies) and immunogenicity (the ability of those molecules to induce in animals or humans the corresponding antibodies). That challenge is being addressed by efforts that combine expertise in structural biology (to design the right type of immunogen) with B-cell biology (to ensure that the host immune response recognize the immunogen and drive the production of antibodies of the desired quality) [345,346].

8. Concluding comments

Three decades of HIV vaccine research has taken the field through a roller coaster of many failures and a few modest successes. In occasions, especially after results from failed efficacy trials are announced, there have been calls to give up the development of an HIV vaccine [309,347]. Those suggestions have been followed by well-argued rebuttals [325,348].

However, it is fair to say that results from efficacy trials have also been instrumental in changing the prevailing paradigms in HIV vaccine research. Every time results from efficacy trials of HIV candidate vaccines are announced, surprising information is obtained that in many cases, have forced the field to change the prevalent scientific paradigm [14]. The negative results from the gp120 VaxGen trials in 2003 [41,42] shifted the main efforts from vaccines aimed

at inducing neutralizing antibodies to vaccines that induce CTLs. Likewise, the failure of the STEP trials in 2007 [307,226] stimulated a re-examination of the scientific strategy for the development of an HIV vaccine, shifting the emphasis to basic research [315,316]. When we were still assimilating the lessons from the STEP trial, we learned in 2009 of the modest success of RV144 in Thailand [33]. This stimulated a new interest in addressing HIV vaccine development, not only through basic research, but also with well-planned clinical trials. The recent failure of HVTN 505 will undoubtedly trigger another round of soul searching.

It has been shown once and again that basic research has undeniable value, but that it alone will not produce a vaccine. The optimum balance between discovery and development is not easy to strike, but exclusive focus on either extreme of this spectrum is unlikely to represent the best strategy [13,14]. Human data trump everything we do *in vitro* or in animal models, and we need to be prepared to expect the unexpected and rapidly build on the results [14].

The current effort to rationally design an HIV vaccine needs to be pursued, although there are voices that warn that a strict reductionist approach may actually hamper the effort. Rational vaccine design efforts need to be complemented with strategically planned empirical immunogenicity and efficacy evaluations [349,350]. During this current third wave of HIV vaccine development, vaccines designed to engage both arms of the immune system, humoral and cellular, need to be pursued more strategically [351]. This is particularly important because evidence is increasingly showing that a humoral immune response to vaccines may be required to prevent acquisition of infection, while CTL may be the critical response in controlling virus replication in vaccinated subjects who become subsequently infected [352,353]. The results from the RV144 trial also brought new attention to the potential role of mucosal immunity, a topic that deserves much more attention in the future [232,354–357].

It is essential to explore innovative approaches and welcome, not dismiss, innovative approaches that may not conform to current scientific orthodoxy [14]. A case on point is the potential role of non-neutralizing antibodies that may confer protection through other mechanisms [321,358,359]. Another area for future exploration, although controversial, is the potential non-cytolytic effects of CD8+ cells [360–364]. To accelerate the clinical development of some of these new concepts, small and intensive experimental medicine trials, to rapidly obtain safety and immunogenicity data, need to be seriously considered as a complement to the most traditional progression through clinical trial phases used today [365].

It is concerning that after many years of intense research, the identified scientific challenges confronting HIV vaccine development have not qualitatively changed [5,7,11,366]. They were (and continue to be) related to the lack of natural protective immune responses in HIV infected persons that could be reproduced with a vaccine; the lack of information on immune correlates of protection; the limitation of primate experiments in predicting vaccine-induced protection in humans; and the significance of genetic and immunological variability of HIV strains in relation to potential vaccine efficacy. However, recent research is providing critical new insights on the evolutionary race between virus evolution and the protective antibody response elicited in infected individuals [367,368]. Novel vaccine design, with immunogens that target specific germline B cell receptors, might finally achieve the long-sought goal of inducing broadly neutralizing antibodies [369].

After roughly 30 years of HIV vaccine research, only five efficacy trials have been conducted [37,38] (Fig. 2) and only one (RV144) provided evidence of protective immunity, although it was at a very modest level. The field needs to learn, assimilate and accept lessons from history [14]. Perhaps the disappointment caused by the recent

failure of HVTN 505 will catalyze, again, a re-examination of the global HIV vaccine strategy just as it happened in 2003 after the failure of the VaxGen gp120 trials. This led to the establishment of the Global HIV Vaccine Enterprise, which now needs to be revitalized. Today, more than ever, we need a well-coordinated global effort with an open mind to re-examine the scientific challenges and opportunities. We must be ready to explore new research avenues and bring new partners and young minds to the search for an HIV vaccine. We should challenge the way we move products through the vaccine development pipeline, which more often than not, have ended up in disappointment after efficacy trials are conducted. We need to create incentives for the pharmaceutical industry, including developing country vaccine manufacturers. We need to enroll the full participation of people from developing countries, not only as vaccine testing sites, but as full partners who stand to benefit the most from an HIV vaccine. We need to have serious discussions with regulatory agencies to streamline a renewed effort to develop an HIV vaccine. And, very importantly, we need to convince national authorities, funders, and policy makers that the development of an HIV vaccine is not only a challenging scientific question, but rather a critical public health priority.

How soon we will have an HIV vaccine will depend on the decision we make today. The field needs to approach the challenge with a sense of urgency, recognizing that a vaccine would be essential for the ultimate control of the pandemic, especially in the poorest countries of the world, while complementing other prevention strategies [369].

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