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REVIEW

Building collaborative networks for HIV/AIDS vaccine development: the AVIP experience

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Abstract The need for an effective HIV/AIDS vaccine is imperative to halt a pandemic that involves more than 40 million individuals worldwide as of 2005 and is causing enormous socio-economic losses, especially in developing countries (DC). The overall failure of more than two decades of HIV vaccine research justifies the demands for a concerted effort for the rapid development of new and efficacious vaccines against HIV/AIDS. In this context, building international collaborative networks is a must for speeding up scientific research and optimizing the use of funding in a synergistic fashion, as resources for HIV/AIDS are limited and do not involve most of the biggest Pharmas that are more interested in drug discovery. The AIDS Vaccine Integrated Project (AVIP) consortium is an example of synergistic partnership of international European Union and DC experts with a common research goal. AVIP is a European Commission-funded (FP-6), consortium-based, 5-year program directed to the fast development of new HIV/AIDS vaccine candidates to be tested in phase I clinical trials in Europe for future advancement to phase II/III testing in DC. To ensure their rapid development, AVIP novel combined vaccines include both regulatory and structural HIV antigens, which have already been tested, as single components, in phase I clinical trials. In particular, such combination vaccines may be superior to earlier vaccine candidates, the vast majority of which are based only on either structural or regulatory HIV products. In fact, the generation of immune responses to both types of viral antigens expressed either early (regulatory products) or late (structural products) during the viral life cycle can maximize immune targeting of both primary or chronic viral infection. Further, the rational design of combined vaccines allows exploitation of immunomodulatory functions of HIV regulatory proteins, which can improve immunity against structural vaccine components. The building of the AVIP consortium and its scientific strategy will be reviewed in this paper as an example of the establishment of a consortium regulated by a specific intellectual property agreement.

Keywords Regulatory/structural HIV proteins · Rational vaccine design · Clinical trials · Developing countries · AVIP/VIAV

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HIV/AIDS vaccine development—a brief history

The need for a safe, effective and accessible vaccine against HIV/AIDS is one of the most urgent public health issues of this century. With more than 40 million people globally estimated to be living with HIV in 2005 and the HIV/AIDS pandemic progressing at the rate of five million new infections occurring each year (UNAIDS 2005), both therapeutic and preventive vaccines are required to hamper the human and socio-economic losses due to HIV/AIDS.

For over 20 years, research aimed at developing vaccines against HIV/AIDS led to disappointing results, yet taught us important lessons about designing new successful strategies to fight HIV/AIDS. Former vaccine candidates, intended to elicit antibody (Ab) responses against the HIV envelope (Env) protein, failed to provide protection against immunodeficiency viruses in both animal models and

humans (reviewed in [26]), mainly because of the Env variability and the heavy glycosylation that impede its accurate recognition by the immune system. On the other hand, further studies indicated that vaccine-generated cellular immunity against HIV, even though unable to prevent infection, could at least help containing viral replication and disease progression (reviewed in [26]). Nonetheless, the emergence of viral escape mutants that eventually overcame the benefits of vaccination raised the issue that cell-mediated immunity alone, although highly desirable, is not sufficient for a vaccine to be effective against HIV infection (reviewed in [22, 26]).

More recently, new insights into the potential of neutralizing Abs (nAbs) were provided by adoptive immunization studies in macaques (reviewed in [18, 26, 28, 32]) showing that virus-specific Abs can indeed be protective if at high titers and directed against broadly conserved, neutralization-sensitive epitopes of Env. In this regard, even though novel immunogens aimed at inducing such protective humoral responses are being developed, no approaches have as yet been completely successful. Taken together, these results suggest that an effective HIV/AIDS vaccine should elicit both cellular and Ab responses against broadly conserved viral determinants.

Previous general vaccine approaches aimed at providing sterilizing immunity, control of infection and/or the blockade of disease. If achieved, these conditions could greatly reduce virus transmission to healthy individuals and halt the HIV epidemic. This goal could be reached through rational vaccine design and the application of new concepts, which identify key and unconventional vaccine targets according to viral protein structure/function, role in virus life cycle and pathogenesis, conservation among different subtypes, and induction of cross-clade immune responses.

As part of a successful strategy for the development of effective vaccine candidates against HIV/AIDS, a well coordinated concerted effort became necessary. An example of harmonized HIV vaccine research effort is represented, in the context of the European Commission 6th Framework Program, by the 5-year AIDS Vaccine Integrated Project (AVIP), which is being conducted by a consortium of 16 partners from seven countries, with the mission of developing novel HIV/AIDS vaccine candidates to be tested in preclinical and clinical studies.

AVIP

AVIP background and rationale

The failure of classical vaccine strategies based on HIV structural antigens (Env, and/or Gag, and Pol) in preventing infection against HIV, simian immunodeficiency virus (SIV), or the chimeric simian/human immunodeficiency virus (SHIV) (reviewed in [26]), together with the promise represented by less traditional vaccines based on HIV regulatory gene products (Tat, Rev, and Nef), which proved effective in containing virus replication and halting disease onset and progression (reviewed in [19, 26]), constituted the rationale for the design of vaccine approaches combining both structural and regulatory viral proteins. Specifically, novel vaccine modalities including both nonstructural and structural antigens were developed to generate broad cell-mediated and Ab immune responses capable of blocking both early events leading to the establishment of primary infection and later events, such as HIV reactivation and the development of severe immunodeficiency heralding progression to disease (reviewed in [19, 26]).

Such innovative vaccines might be superior to earlier strategies exploiting only one or the other antigen type as a single component. In fact, combined vaccines should generate immune responses to both viral products expressed early (regulatory proteins) and late (structural proteins) during the viral life cycle, thus maximizing immune targeting of viral infection. Not only can combined vaccines elicit broader immunity, but they also can allow the exploitation of the known immunomodulatory functions of HIV regulatory proteins [17, 21, 26], which, in turn, can improve immune responses against the structural vaccine component.

In fact, regulatory Tat, Rev, and Nef proteins share properties that make them very promising vaccine targets. They are the first HIV proteins expressed in infected cells, even before virus integration [26, 45], and are essential for effective virus replication [5, 26, 39, 46]. These regulatory proteins exert multiple immunoregulatory functions, either directly or indirectly, through which they facilitate target cell recruitment and activation, and promote HIV replication and spreading (reviewed in [19, 26]).

Particularly interesting for vaccine development, Tat, Rev, and Nef proteins are greatly conserved in their immunodominant regions and can induce broad cross-clade immunity [9, 12, 15, 26, 47]. Consistent with this hypothesis, recent studies showed that sera of individuals from sub-Saharan Africa mainly infected with HIV clades A, C, and D cross-react with clade B Tat [9, 26]. This is of relevance and suggests that an HIV vaccine based on clade B regulatory gene products may induce responses that cross-recognize HIV strains belonging to virus clades that are prevalent in the most affected regions of the world, and may, therefore, be broadly effective.

Of note, in their extracellular form, HIV regulatory Tat and Nef proteins exert immunomodulatory functions, as indicated in vitro by the promotion of dendritic cell maturation and activation [17, 26, 36]. Moreover, results from recent vaccine preclinical studies in the murine model

showed that co-administration of Tat (DNA plasmid, recombinant adenoviral vector, or native protein) with Gag increases Th-1 and cytotoxic T-lymphocyte (CTL) responses against the latter ([26, 48], Gavioli et al., submitted; Guzman et al., in preparation; and Caputo et al., unpublished data). Co-immunization of mice with Tat and Env or Gag also resulted in enhanced recognition of the structural antigen, as indicated by the detection of responses specific for new epitopes that were not recognized upon immunization with the structural protein alone (Gavioli et al., submitted). The recent finding that Tat is capable of changing the composition of the proteasome catalytic subunit [21, 26] provides an explanation for the capacity of Tat to modulate CTL epitope hierarchy, and underscores the importance of basic science in applied research.

In addition to the control of HIV-induced disease by cell-mediated immune responses to Tat, Rev, and Nef [1, 2, 13, 26], a strong correlation was found between the presence of anti-Tat- and Nef-specific Abs and nonprogression to AIDS, in both cross-sectional and longitudinal studies [14, 26, 37]. This suggests that the induction of Abs neutralizing the effects of extracellular Tat and Nef should be sought after in HIV vaccine development. Of note, immunization of HIV-infected individuals with DNA encoding Tat, Rev, and Nef generated new CTL responses in all vaccinees [10, 11, 26], suggesting that therapeutic vaccination with such antigens is also feasible.

Vaccination with the Env structural proteins responsible for the binding and entry of the virus may result in the induction of nAbs capable of protecting from infection (sterilizing immunity). However, results from preclinical and clinical trials, including the first phase III trial (AIDSVAX by VaxGen), in which no protection from primary infection in Caucasians was observed, have been largely disappointing. This can be accounted for by the inability of such vaccines to elicit protective nAbs, mostly due to Env variability [25], which hampers recognition of relevant epitopes by nAbs. Heavy glycosylation, conformational masking, and immunodominant variable loops are additional factors that contribute to hiding neutralization-sensitive epitopes (reviewed in [8, 26]). To overcome this problem, AVIP relies on the use of a modified Env protein that is deleted in the V2 loop (ΔV2 Env). This modification permits the exposure of conserved epitopes to increase the breadth of nAb responses generated upon vaccination, and therefore can circumvent clade-related issues and allow the development of a broadly effective vaccine against HIV/AIDS [6].

In developing a combined vaccine, it is especially important to optimize formulations and immunization schedules because of the risk that immunodominant structural viral antigens may reduce the response against small regulatory proteins, as suggested by preclinical studies evaluating vaccination with Gag, Env, Tat, Rev, and Nef [3, 26, 30, 31, 44] and by the pattern of immunity developed during natural infection [1, 26, 33]. Therefore, as part of the development of combined vaccines, novel vaccine design, formulation, and vaccination protocols are also being carefully evaluated within AVIP to ensure induction of balanced responses to all antigens.

The main goal of AVIP is, therefore, to develop novel combined vaccines and to test them in preclinical studies and in phase I clinical trials in Europe, to further develop the most promising candidates for future advanced clinical testing (phase II/III trials) in DC. Specifically, preventive and therapeutic phase I clinical trials will be conducted in Europe with four novel vaccine candidates consisting of combinations of both regulatory and structural HIV antigens.

Building up the AVIP consortium

AVIP is based on a consortium consisting of 16 international partners with complementary expertise from six European countries (Finland, France, Germany, Italy, Sweden, and United Kingdom) and one African country (South Africa). A common feature among AVIP participants is their long-term experience in HIV/AIDS research and, particularly, in vaccine development. The partnership encompasses all fields necessary for vaccine development, namely, basic science, process development/bioengineering, production and testing, preclinical and clinical-applied research, epidemiology, and clinical trials. AVIP participants are also very focused on training and technology transfer both in Europe and DC, as well as in innovation activities and in the industrial exploitation of scientific results and products. In particular, industrial exploitation of AVIP products and the consequent rapid transfer of AVIP successful candidate(s) into the global market are guaranteed by the presence in the consortium of members from the vaccine industry with a strong focus on HIV/AIDS vaccine development.

As AVIP is a translational, bench-to-bed research program, all of the above is required for the fulfillment of the project, which is being pursued through the thorough integration of research and technology development (RTD), innovation, demonstration, and training activities within the project.

AVIP scientific strategy

The rational design and identification of the most promising vaccines ready to be placed in a clinical trial setting was the first step towards defining the AVIP program. Next, as AIDS vaccines are mostly needed for DC, preparing sites in these nations for advanced clinical testing also became

central to the project. The whole program was assembled taking into account social, ethical and training aspects, which are essential to shaping the optimal setting for vaccine trials that meet the highest standards, but also to ensure a joint effort from both EU countries and DC. The choice of candidate vaccines was based on the existing experience of AVIP partners and on vaccine strategies that were already in the process of being developed in their own laboratories and clinical centers. These strategies are based on the rational combination of structural and/or regulatory genes/proteins. Therefore, several combinations of viral gene products, as well as different delivery systems, are being tested in preclinical studies, in the context of the AVIP program, to select the best candidates to enter phase I trials.

In particular, four different vaccine candidates have been selected by the AVIP consortium. The first two are based on the combination of the same structural gene product (ΔV2 Env) with two different regulatory proteins, Tat or Nef. The other two vaccines are complex vaccine formulations involving the use of blocks encompassing multiple structural and nonstructural antigens [7]. One is a pure DNA vaccine, which is based on a single-vector coding for selected antigens and epitopes, namely, full-length Rev, Tat, Nef, p17 and p24, and the DNA from Pol and Env encoding more than 20 T-cell epitopes (hereafter referred to as Multi-HIV antigens/epitopes). The other complex vaccine is composed of an HIV multigene (*nef*, *rev*, *tat*, *gag*, *rt*, *env*) administered as individual DNA constructs or proteins according to a prime-boost protocol in which GM-CSF is used as an adjuvant (hereafter referred to as HIV multigene [7]).

One of the selection criteria of these four vaccine approaches is that each component has already completed or is undergoing phase I clinical studies and, therefore, has been approved for human use by both regulatory and ethical committees; in fact, clinical sites for phase I trials with the single components have already been set up in Estonia, Finland, Germany, Italy, Sweden, and the United Kingdom. Further, for these antigens, GMP process development, toxicology data, and efficacy data in animal models of HIV infection are available. Comparative analysis of the results from both preclinical and clinical testing of these vaccines will be key for the selection of vaccine candidates for phase II/III trials in DC.

Of the four vaccines selected for evaluation by the AVIP consortium, the first three (Tat,±ΔV2 Env, Nef±ΔV2 Env, and Multi-HIV antigens/epitopes) will undergo phase I preventive trials, whereas the HIV multigene will be tested as a therapeutic vaccine in phase I studies in seropositive individuals. A brief description of each of the four vaccine approaches is given below.

Tat±ΔV2 Env

In this vaccine candidate, the early regulatory viral antigen, the native Tat protein, is combined with a V2-loop-deleted Env structural glycoprotein. The ΔV2 Env immunogen has a trimeric form that, due to deletion of the V2 region, exposes cryptic conserved sites for neutralization. On the other hand, the Tat protein, in a native form, is capable of eliciting cell-mediated immune responses to contain viral replication and also serves as an immunomodulator (reviewed in [19, 26]). Thus, a broad range of humoral and cell-mediated immune responses, including the production of nAbs, is expected to be elicited by the Tat±ΔV2 Env vaccine. These responses should have the potential to act synergistically to prevent or to reduce virus entry via anti-Env responses, and virus spread via anti-Tat and/or anti-Env responses.

The vaccine will be administered in HIV-negative people and the criteria for an advancement of this approach beyond phase I are its safety and the demonstration that the combined vaccine induces stronger and broader immunogenic responses against both antigens, compared to those induced by each antigen separately. Potency and breadth of virus neutralization will be measured against the vaccine strain and the minimum criteria for Env-specific responses will be the detection of nAbs in at least 50% of vaccinated individuals.

Nef±ΔV2 Env

This vaccine is composed of the *nef* gene inserted into the Modified vaccinia virus Ankara (MVA) in combination with the ΔV2 Env protein. As for the Tat/ΔV2 Env approach, the Nef/ΔV2 Env vaccine will be administered in HIV-negative volunteers, seeking to induce mostly anti-Nef cellular immunity and anti-Env humoral immunity, in particular nAbs, to prevent (or reduce) virus entry and to control virus replication.

Criteria for advancement of this vaccine beyond phase I will be proven safety and broader and more potent immune responses against components of the combined vaccine, as compared to those obtained upon vaccination with the single antigens. As for the Tat/ΔV2 Env vaccine, minimum criteria of success for Env-specific responses will be the induction of nAbs against the vaccine strain (i.e. homologous neutralization) in at least 50% of the trial participants.

Multi-HIV antigens/epitopes

This DNA-based vaccine consists of a vector expressing a fusion protein of 120 kDa comprising full-length regulatory proteins Rev, Nef and Tat, p17 and p24 proteins of Gag, and T-helper and CTL epitope stretches

from the Pol and Env proteins of HIV-1 A, B, C and FGH clades arranged in tandem [27]. The vaccine will be administered to HIV-negative individuals. Minimum criteria for the advancement of this vaccine approach beyond phase I, will be the induction of CD4+ and CD8+ T-cell responses to more than one viral antigen in at least 50% of the trial participants.

HIV multigene

The vaccine contains a combination of several plasmid DNA constructs, encoding the regulatory proteins Rev and RT, and the structural proteins/epitopes of Gag and Env for subtypes A, B and C of HIV-1. The inclusion of the GM-CSF adjuvant gives rise to high nAb titers in preclinical studies [7, 38]. The aim of this strategy is to provide the same broad protection mechanism as that elicited by a live attenuated vaccine. The vaccine will be delivered to HIV seropositive individuals through a Biojector or directly into the skin, which will reduce the dose of DNA to be used. It is expected to induce T-cell responses against the encoded HIV antigens in at least 50% of the vaccinees.

AVIP scientific plan

The AVIP program encompasses four main objectives:

1. Conducting phase I preventive and therapeutic trials in Europe with novel vaccine candidates based on the combination of at least one regulatory HIV antigen (Tat and/or Rev and/or Nef) with a structural antigen (Env and/or Gag/Pol). Vaccine candidates include four advanced vaccines composed of individual antigens that already underwent or are undergoing phase I trials and have demonstrated efficacy in animal models. Vaccine formulations and the vaccination protocols are being optimized in preclinical studies before entering phase I trials.
2. Transferring technology to DC, as well as performing epidemiological, immunological, and virological preparatory studies for future phase II/III trials to move rapidly into the clinical platform upon completion of phase I studies in EU.
3. Implementing training in RTD and demonstration activities in EU and DC through the AVIP international school.
4. Involving the community and preparing joined community advisory boards (CAB) in EU and DC for the ethical information of the volunteers, counseling, quality of life and risk assessment evaluation.

AVIP structure

To ensure an optimal project organization and implementation, the AVIP 5-year program has been divided in seven integrated and complementary workpackages (WPs) (Fig. 1), namely:

WP1. Preclinical studies in rodents and non-human primates for selection of best formulations and vaccination protocols amongst AVIP vaccine candidates to be tested in phase I clinical trials

WP2. Good Manufacturing Practice (GMP) production and toxicology assessment of vaccines, dossier preparation to obtain approval for human use

WP3. Preventive vaccine phase I clinical trials

WP4. Therapeutic vaccine phase I clinical trials

WP5. Immunological field studies to prepare for future phase II/III trials in DC

WP6. AVIP reagents repository (EVA Programme)

WP7. Coordination of science, training, business and administrative management.

WP1 includes testing in preclinical models for selecting the best vaccine candidate formulation, administration route, and immunization schedule, based on safety and immunogenicity data for advancement to phase I clinical trials. Efficacy studies will also be carried out in both non-human primate and novel mouse models of HIV infection. WP2 comprises all activities required for GMP vaccine production, formulation, testing, dossier preparation and approval for human use, whereas WP3 and WP4 concern, respectively, the conduct of preventive and therapeutic phase I trials in EU. Feasibility studies and capacity-building activities are being performed as part of WP5 in DC to prepare for future advanced clinical testing (see below for further details). The EVA Programme (WP6) supports and contributes to each WP by providing standardized reagents and a centralized repository facility (see also below). Training activities are being carried out in all WPs and are managed by the AVIP International School within WP7, which also includes coordination and supervision of science, business, and administrative management (see below).

AVIP consortium management

The presence in the AVIP program of partners with different expertise and the complexity of the objective to be achieved require a complex organization. For this reason, one of the six WPs through which the AVIP program is being implemented is entirely dedicated to management activities. The scientific management structure of AVIP is composed of six principal investigators (PIs) and six co-PIs of each WP, all highly qualified scientists, most of whom have been involved in vaccine development and

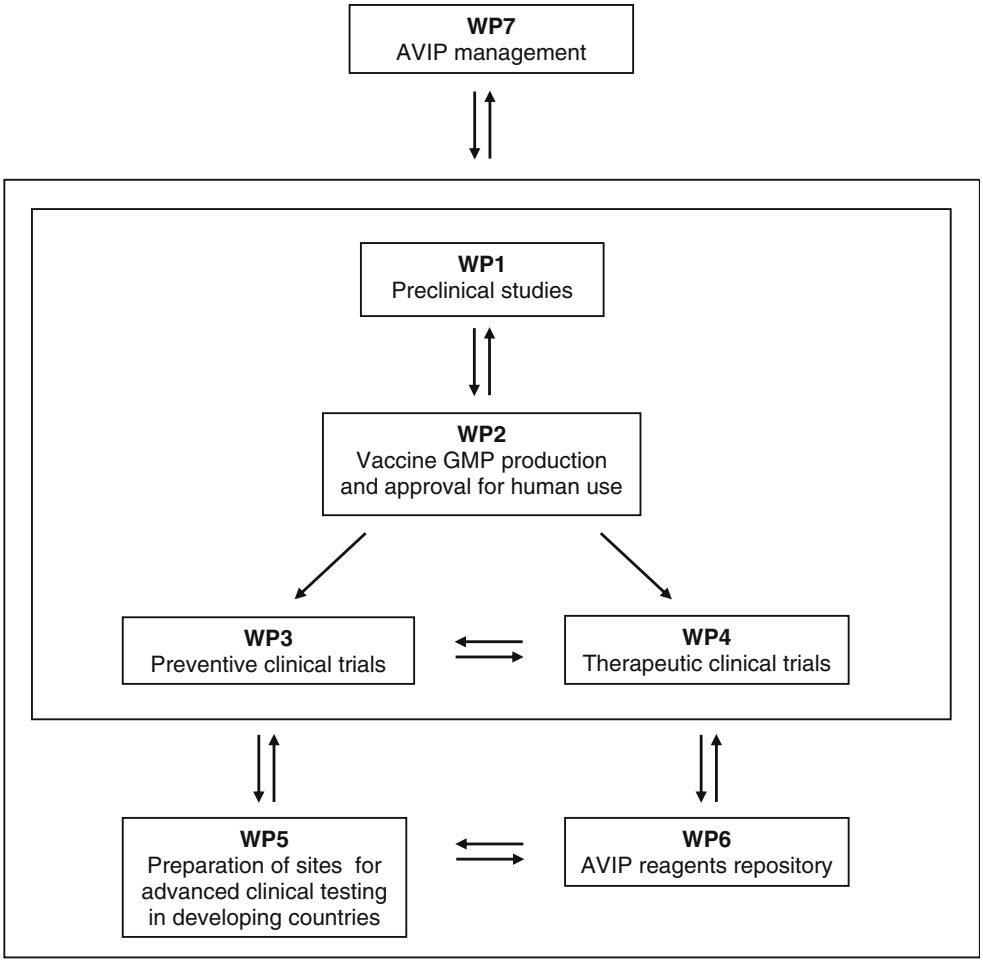

Fig. 1 AVIP integrated and complementary workpackages

testing for many years, and all with a long-term experience in management.

These investigators, together with a business manager, constitute the AVIP Steering Committee (SC), which represents the governing body of AVIP. The implementation of AVIP activities and the flow of information and interaction among different WPs are ensured by the management structure of AVIP (WP7). Through the SC, the coordinator and the co-coordinator of AVIP make sure that the project activities are properly and timely implemented, that the information flow is constant within the consortium, and that WPs are conducted in an integrated manner. The business manager oversees the overall legal, contractual, intellectual property actions, and technology transfer activities of AVIP. Two project managers oversee project reports, administrative issues, and dissemination plans.

The SC is supported in its activities by a monitoring committee (MC), which supervises the proper scientific and technological interactions among the different WPs, and by an advisory board (AB) consisting of world experts in the different aspects of the AVIP program. The AB provides guidelines and evaluations of AVIP activities throughout. Both MC and AB oversee the scientific, technological, innovative, regulatory, clinical, and training activities related to the project, with particular regard to ethical and society-related issues, including community involvement and gender issues. Finally, an ad hoc administrative and business structure provides the proper support for the execution of AVIP decisions. The AB meets once a year, or as required by the SC, whereas the MC meets every 6 months, keeps records of its meetings, and makes independent reports to the SC. The management of AVIP is graphically summarized in Fig. 2.

Promotion of public awareness concerning the goals, activities and results of the AVIP Consortium is ensured by an internet web site (http://www.avip-eu.org), which has been developed for disseminating AVIP results and for internal consortium management. Scientific publications and communications at national and international meetings constitute an additional opportunity for AVIP dissemination plans in the scientific and industrial community. Particular

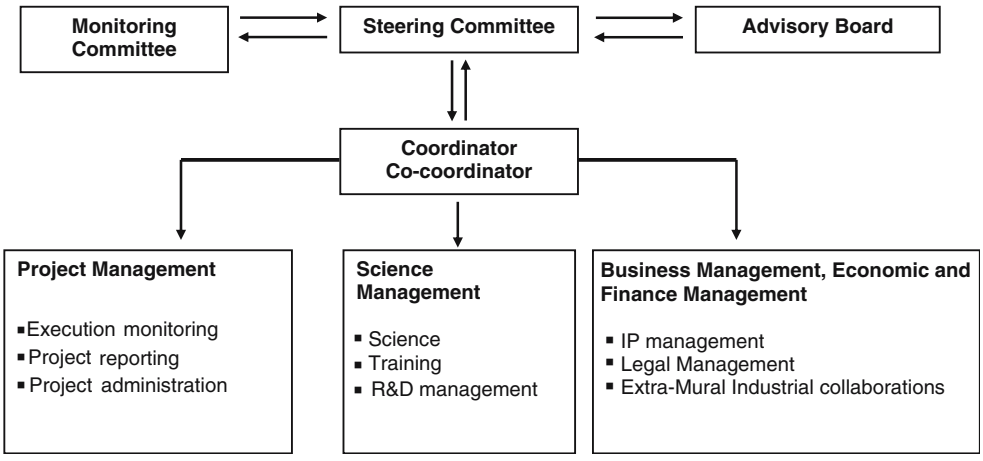

Fig. 2 A graphical summary of the management of AVIP

attention is also being devoted by AVIP SC to the exploitation of innovation and intellectual property coming from AVIP scientific and technological activities.

Selection criteria for vaccine candidate advancement to phase I and phase II/III clinical testing

AVIP encompasses two different strategies for the development of combined vaccine candidates, including both structural and regulatory HIV antigens: a minimalistic and a maximalistic approach. The former relies only on two HIV proteins (one structural and one regulatory), the latter mimics, to some extent, live attenuated vaccines comprising as many HIV antigens as needed. In both cases, the endpoint for an effective vaccine is providing protective immunity, defined as protection against HIV infection and/or protection against disease onset and progression.

The purpose of the minimalistic combined vaccine is the generation of anti-Tat or Nef CTL responses and Abs, as well as broadly neutralizing anti-Env Abs. In fact, the rationale of the minimalistic vaccine approach includes the concept that upon HIV infection, the first cells targeted by the virus in the mucosa, Langerhans cells and macrophages, require the contact with specific T-cells for spreading the virus to non-infected cells [26, 40]. In particular, the expression of early regulatory proteins like Tat and Nef in these antigen-presenting cells has been reported to mediate chemotaxis for HIV target cells directly and/or indirectly, by inducing chemokine expression, thus contributing to the establishment and spreading of infection [4, 17, 29, 42]. As a consequence, vaccine-induced immunity interfering with this local process could halt the infection at its early stages.

The maximalistic combined vaccine approach consists of immunization with multiple HIV genes. As protection provided by live attenuated vaccines is not yet completely understood, maximalistic combined vaccines include all possible HIV genes known to be antigenic and to induce both broad T-cell and Ab responses.

Criteria for choosing the AVIP vaccine candidates to be advanced to phase I clinical testing will be the results of preclinical immunogenicity and efficacy studies in mice and monkeys. With regard to efficacy, data obtained in mouse and monkey preclinical models of infection will provide additional information for further ranking the vaccine candidates. At the end of the project, advancement to phase II/III testing will be decided on the basis of the results obtained in phase I trials. Candidate vaccines that will have induced vaccine-specific immunity in greater than 50% of the vaccinees, at multiple time points, will be considered for further clinical development.

In particular, for prophylactic vaccine candidates, the criteria for advancement to phase II/III are: 1) generation of cross-reactive cellular immune responses and/or nAbs against HIV strains that are predominant in the country where the trial will be conducted; 2) induction of CD4 T-helper cell activity and memory functions for HIV-specific CTL, to contain the initial spread of infection. For therapeutic vaccine candidates, selection criteria are the presence of newly induced virus antigen-specific CD4 and CD8 T-cell immunity, i.e., expansion of the epitope repertoire, or favorable changes in the antigen-specific T-cell phenotypes. If necessary, more stringent or supplementary selection criteria will be employed to choose the most promising among the AVIP vaccine candidates.

Of note, standard operational procedures (SOPs) have been established and will be followed throughout the vaccine program to permit comparison of experimental results and fair ranking of the different vaccine candidates for selection. Moreover, a major effort of AVIP is dedicated to the harmonization between clinical and laboratory platforms to ensure proper circulation of reagents and specimens and to guarantee the quality and comparability of results.

AVIP capacity building in DC and its future relevance

South Africa is one of the sub-Saharan countries most hit by the HIV epidemic, with an HIV prevalence rate of 29.5% among women attending antenatal clinics in 2004 (National HIV and syphilis antenatal sero-prevalence survey in South Africa; South African Department of Health, Directorate Health Systems Research, Research Coordination and Epidemiology). To achieve the long-term goal of developing an anti-HIV vaccine that is appropriate for use in South Africa and in other DC, advanced vaccine clinical trials (phase II and phase III) must be performed.

To this aim, studies to evaluate the feasibility of vaccine trials in selected South African sites must be performed in advance. An important activity in the AVIP program concerns two main objectives, one being to build up the capacity of South African sites for advanced clinical testing, and the other one being to perform epidemiological, immunological, and virological/molecular studies to evaluate the feasibility of future phase II/III clinical trials with the AVIP vaccine candidates. The vaccine to be tested in phase II/III trials in South Africa will be chosen from the four AVIP vaccines after head-to-head comparison of the results obtained with each vaccine in phase I trials in Europe. The major issues inherent to these two objectives are shortly reviewed below.

Capacity building at the South African sites

The conduct of HIV vaccine clinical trials in Africa poses great challenges; therefore, one of the goals of AVIP is to favor the creation in South Africa of networks of relevant stakeholders, investigators, communities, politicians, governments, and regulatory authorities to discuss and reach a consensus about the trial conduct. In addition, a number of logistic, ethical, political, and community issues will be addressed before establishing HIV clinical trial sites to ensure transparency of research and good researcher–community interactions [16, 26].

One of the goals of AVIP is to build up or strengthen the clinical and laboratory infrastructures necessary to conduct vaccine trials. In South Africa, this activity has already begun and has been facilitated by the presence of already experienced clinical and laboratory staff due to previous involvement of sites in different South African provinces with already existing research structures in the conduct of other HIV vaccine clinical trials. Within AVIP, standardization of techniques and procedures, and training for clinical and laboratory personnel in these sites is being achieved by staff exchange programs and exchange of SOPs between the sites and the AVIP scientific members on the basis of the International Conference on Harmonisation (ICH) guidelines for good clinical practice (GCP) training.

An important challenge is to establish a volunteer recruitment strategy for vaccine clinical trials. To this aim, information to the local population concerning both HIV transmission modalities and the aims of the trial is an essential step to recruit and maintain informed suitable volunteers from the site population for both preventive and therapeutic clinical vaccine trials and to guarantee their follow-up over reasonable periods of time.

Prescreening of volunteers is started by the adult voluntary counseling and testing (VCT) centers. All eligible individuals are invited to enter a prescreening protocol, where formal information on the HIV vaccines, their need to be tested in human trials, and the modalities of trial conduct are given through vaccine discussion groups (VDG) in which each individual is invited to participate. In addition, VDG can assess the individuals' health and risk behavior, as well as the willingness of each potential volunteer to participate in clinical vaccine trials. This mechanism ensures that the trial is conducted in communities that thoroughly understand the issues around HIV vaccine research and feel actively involved in all aspects of the trial. This is essential for forming, strengthening, and maintaining relationships between HIV vaccine researchers and the communities and, eventually, for the good outcome of the trial and for the future involvement of the community in other vaccine trials [26]. Beyond VDG, other strategies will be put in place. Among these, information for volunteers can be accomplished by representative structures that are able to mediate between the researchers and the community. Such structures can be identified in the Community Advisory Boards (CAB) that can provide a very effective way to ensure that community and volunteers are fully informed when making the decision to participate in HIV vaccine research.

Estimating prevalence and incidence of HIV infection in selected trial sites

Knowing prevalence and incidence of HIV infection in the target site or in a target population is essential to correctly plan vaccine trials. For example, populations with high prevalence of HIV infection but with low incidence of HIV infection may not be suitable targets for phase III preventive clinical vaccine trials. Incidence data are especially important for purposes of clinical care and prevention, in particular, to monitor the impact of preventive interventions [20, 26]. Whereas prevalence estimates are more easily obtained through programmed serosurveys, incidence data are more difficult to produce. These data can be obtained by forming and maintaining for several years large cohorts of seronegative individuals representative of the population targeted for vaccine clinical trials, who will undergo periodic testing for HIV Abs to identify new

seroconversions. However, although cohort enrollment with repeated measurements to detect new HIV infections remains the gold standard to assess HIV incidence in communities, newer, cheaper, and more reliable methodologies for identifying recent HIV infections are being developed, as phase III HIV vaccine trials become a reality [23, 35, 41]. AVIP will take advantage of such methods, whose applicability in DC is under study in other programs linked to AVIP.

Immune cross-recognition of candidate vaccine antigens and molecular virological studies

The predominant HIV subtype in the developed world (clade B) is not representative of the local predominant subtypes in other regions of the world. In South Africa (and Southern Africa in general), clade C still accounts for the vast majority of infections [26, 34]. In addition, it is becoming increasingly evident that a relevant proportion of HIV infections in the world is sustained by inter-clade HIV recombinants. However, the majority of vaccine development research has been focused (and still is focused) on clade B [26, 43]. In the context of the AVIP Program, AVIP vaccine antigens, which include both structural and regulatory gene products, are also mostly from clade B strains. To facilitate the evaluation of potential vaccine candidates in phase II/III trials in South Africa, it is important to determine the degree of cross-clade recognition of the antigens incorporated in the AVIP vaccines by sera of HIV-infected individuals from areas with different viral subtype predominance.

Finally, continuous evaluation of the circulating virus to determine the HIV subtype dynamics and the emergence of new recombinants is also being performed in the framework of other projects. These studies will provide a clearer picture of HIV genetic and antigenic heterogeneity in the candidate sites for advanced clinical testing.

Future relevance

The AVIP program in South Africa constitutes the basis for future advanced clinical research (phase II/III vaccine trials) in Africa. In fact, activities in South Africa will permit the buildup of new potential clinical trial sites and/or the strengthening of the existing ones. In addition, the AVIP activities will contribute to create potential collaborations between sites of different African countries. In fact, projects other than AVIP, conducted by AVIP members in cooperation with different African countries, may synergize with AVIP and constitute the basis for collaboration between several African countries in clinical vaccine research. In this regard, studies similar to those reported in AVIP have been already put in place (or are starting) in Uganda, Swaziland, Tanzania, Rwanda, and Burundi (see also "AVIP links to other projects" below).

AVIP links to other projects

Some of the most important institutes and clinical centers in Europe and in South Africa constitute the AVIP consortium. AVIP researchers have very considerable experience in both basic and applied science and in clinical trials and are involved in several HIV vaccine projects, which they conduct in parallel with AVIP and to which AVIP has been linked.

In particular, clear synergies arise from the exploitation of a vaccine candidate based on HIV Tat and Env, which is included in another FP6 European project (MUVAPRED), also involving some of the AVIP scientists, aimed at developing mucosal HIV and TB vaccines. As a consequence of this, the same Tat-Env combined vaccine candidate will be evaluated after systemic (AVIP) or mucosal (MUVAPRED) administration. Although the vaccine protocol design in the two projects differs in terms of route of administration, adjuvant, formulation, schedule of vaccination, and challenge virus in the monkey model, the importance of this synergy is evident. In fact, the information resulting from both AVIP and MUVAPRED on this vaccine candidate will be complementary and will help our understanding of the impact of the immunization route on the vaccine immunogenicity and efficacy.

The EU program on HIV Immunization Study (HIVIS) aims at a prophylactic vaccination with seven different DNA plasmids covering subtypes A-E of HIV, followed by an MVA recombinant vaccine boost. The preliminary phase of this vaccination program has shown that it is possible to use many plasmids and acquire immune responses against most of them in humans. In this study, the concept of the human use of GM-CSF as an adjuvant is also being tested.

The Very Innovative AIDS Vaccine (VIAV) is also tightly linked to AVIP. In fact, VIAV concerns the molecular and preclinical development in small animal models of vaccines based on the combination of HIV Tat and Env and can directly fuel AVIP with novel vaccine candidates for further development in monkeys and possibly in humans.

AVIP also benefits from synergies with aid agencies and with several non-governmental organizations. Specifically, the AVIP consortium possesses links with ongoing national and international programs, including the national programs of the participating countries, such as the Italian AIDS Program, the Italian Concerted Action on HIV/AIDS Vaccine development (ICAV), the Swedish International Development Cooperation Agency (SAREC/SIDA), and the bilateral programs with DC (Sweden/Tanzania, Italy/South Africa/Uganda/Swaziland, Italy/Rwanda/Burundi,

UK/Uganda). ICAV is a program included in the Italian National Program on AIDS and is coordinated by the Italian Istituto Superiore di Sanità (ISS). Some of the researchers included in the AVIP consortium are also present in ICAV, which provides funding to a scientific network devoted to a bench-to-bed translational program that ranges from basic science to clinical trials. ICAV is also aimed at developing an effective vaccine against HIV/AIDS. Besides involving many Italian key researchers, ICAV is connected with European and American partners, who are expert in different areas of vaccine research. In particular, ICAV is connected to the Italy/USA vaccine program through the collaboration established between NIH (USA) and ISS (Italy). This program stems from a bilateral cooperation agreement between Italy and USA, formally signed in 1998 and renewed in 2003, that is aimed at developing new strategies for HIV/AIDS vaccines.

Other synergies arise from the collaboration of AVIP researchers with African countries, in particular, from projects and programs that are being funded by the ISS, through the Italian National Program on AIDS (Uganda and South Africa), by the Italian Ministry of Foreign Affairs (South Africa, Uganda, Rwanda, Burundi and Swaziland), and by the South Africa AIDS Vaccine Initiative (SAAVI) (South Africa). In addition, AVIP benefits from other collaboration programs such as the EU project HIV Immunization Study (HIVIS) involving Sweden, Tanzania, South Africa, Germany, and several vaccine programs in South Africa that are sponsored by the International AIDS Vaccine Initiative (IAVI) and the NIH through the HIV Vaccine Trial Network (HVTN) and the collaboration between the Pediatrics Research Unit (University of Tampere) and the Ministry of Health of Finland, and the Lungwena clinical trial site in Malawi.

Finally, other links with the EU Project HIV PRIME BOOST THERVAC (Sweden, Belgium, Holland), the EU Project HIVComTher (Netherlands, Spain, Italy, Germany, Belgium), and the EUREKA project HIVVAC between Finland and Sweden have been recently established to support GMP and good laboratory practice (GLP) production of HIV vaccines for preclinical and clinical trials within AVIP.

Activities supporting AVIP

AVIP repository activities

The RTD and demonstration activities within AVIP are supported by the Programme EVA Centralised Facility for AIDS Reagents (CFAR) at the National Institute for Biological Standards and Control (NIBSC) in the UK, which supplies standardized reagents and a centralized repository facility.

Although it is yet unknown which HIV/AIDS vaccine will ultimately prove efficacious, the availability of high-quality HIV and SIV recombinant proteins is important for analyzing both humoral and cellular immune responses. Acquiring high-quality recombinant proteins, especially those expressed in eukaryotic expression systems, has previously proven very difficult with limited supplies and variable quality, but the EVA Program has been able to establish an extensive range of high-quality reagents, which is being built upon to support AVIP. Both HIV and SIV structural and regulatory gene products are being provided by EVA Program to AVIP participants. Batches of individual proteins can be reserved for specific purposes to help minimize assay variability. The EVA CFAR has an extensive collection of peptides representing individual HIV and SIV epitopes, as well as overlapping peptide series covering many of the structural and regulatory gene products.

The CFAR collaborates closely with the WHO-UNAIDS Virus Network, a program aimed at monitoring the global distribution and prevalence of HIV-1 subtypes especially in countries where AIDS vaccine trials may take place. The Centralised Facility houses the central repository for the Network and holds a wide range of donor peripheral blood mononuclear cells (PBMCs) and field isolates representing all the main subtypes and regions. Human serum/plasma samples and cloned genes have been generated through the Network.

A range of humoral and cellular assays such as virus neutralization, EIA, viral load, ELISpot, and intracellular cytokine assays are being performed by AVIP laboratories, and CFAR is working closely with them in identifying and providing suitable means of ensuring the comparability of assays performed across the Network.

An advantage of having the Centralised Facility is that AVIP participants are also encouraged to donate reagents, even unique materials developed in their own laboratories, to the CFAR for safe storage and efficient distribution to other AVIP participants. This sharing of key reagents is a central component in the AVIP and is an important means of demonstrating integration of the various participating laboratories. CFAR has extensive experience of shipping perishable and biohazardous reagents on dry ice in compliance with IATA regulations and is using its expertise in ensuring the safe transfer of materials to and between AVIP laboratories.

The EVA CFAR has worked with DC through the WHO-UNAIDS Virus Network and has an extensive repository of samples from Africa and Asia. It is, therefore, supporting AVIP through the provision of clade-specific reagents, standards, and know-how. The support of the already

established EVA Program, in terms of reagents providing repository and distribution functions, constitutes an optimal basis for the standardization of techniques and procedures, and for supporting all AVIP RTD and demonstration activities.

AVIP International School

The training in both EU and DC is a focus of each specific WP. To this aim, an AVIP International School has been established by networking existing EU programs and by developing a new educational program on clinical trials development in South Africa. The AVIP International School also benefits from key interactions with the European Molecular Biology Organization (EMBO), which has outstanding training programs for both EU and DC.

The training includes exchange of personnel between developed and DC. As AVIP participants have a constellation of complementary skills (e.g., virology, molecular biology, genetics, microbiology, pathogenesis, immunology, small animals and nonhuman primate model systems, vaccinology, downstream processing/bioengineering, QA/QC, GMP production, clinical trials, medicine, immunomonitoring) and work side-by-side with industrial partners and international agencies, the AVIP international school network provides the ideal setting for training in all aspects required for vaccine design, development, production, and testing. Training of technical, scientific, and clinical personnel is being conducted through multilevel integrated activities. The training program includes technology transfer between European countries and to DC, which is essential to ensure correct results and appropriate standardization. The school functions as a two-way process, in which African personnel will visit their European counterparts, and the European partners will visit the African sites.

Conclusions

The lessons taught by the AVIP experience

The AVIP program shows that international experts from around the world can come together and work with a common goal in mind, in an organized, synergistic manner. The development of new collaborations, the implementation of regular communication among partners, and the preparation of common SOPs all contribute to building up a big multi-component operative unit encompassing numerous institutions and countries for the development of an effective HIV/AIDS vaccine.

AVIP partners are targeting resources toward a very focused agenda to exploit the synergies existing among the numerous and diversified programs to the maximum potential. In fact, AVIP relies on a complex but highly rational web of experts in the field, which, with the core funding from the European Commission, are also mobilizing the highest volume of aggregate resources in Europe.

The AVIP program comprises a unique level of critical mass of vaccine-related science, products and RTD resources, which is compatible with the objective to be reached and with a probability of success that is undoubtedly higher than the historical average for AIDS vaccine research efforts in the European Union. The AVIP consortium consists of 15 among the centers of excellence in AIDS research (including a large industrial partner), and is making an RTD effort benefiting from a scientific base of more than 400 scientists and an overall RTD budget of more than 72 million euros, of which, more than 20 million euros is funding from the European Commission and co-funding from AVIP partners, and more than 52 million represents other aggregated RTD resources dedicated to AIDS vaccine research.

AVIP in the context of the global HIV/AIDS vaccine effort

Overall, the AVIP program has the potential of leading to significant improvement of living conditions in both developing and developed countries. The development and evaluation of promising HIV/AIDS vaccines in preclinical and phase I clinical trials for future phase II/III testing by a consortium of numerous international experts, such as the AVIP consortium, will guarantee the best possible outcome. The AVIP initiative is allowing implementation of a major concerted action aimed at the fast development of an HIV/AIDS vaccine based on rational design combining both structural and regulatory viral proteins. Such an effort well fits the spirit of the Global HIV Vaccine Enterprise, a virtual consortium built to accelerate HIV vaccine development [24].

Of note, the network and collaborations established within AVIP will also constitute a well-tested working platform for the implementation of future research projects that will thrive on the experience and work already completed, including the capacity building performed in DC. In this regard, AVIP has a real chance to contribute to the improvement of human life expectancy and health of young people who are the major work force of countries affected by the HIV epidemic, thus leading to the reduction of the tremendous social and economic loss due to the spreading of AIDS.

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