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Microbes and Infection 7 (2005) 1445–1452

Microbes and Infection

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Forum in immunology

Rational vaccine strategies against AIDS: background and rationale

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Available online 22 September 2005

Abstract

New vaccine candidates exploiting the rational combination of regulatory and structural HIV gene products are being developed within the program of the AIDS Vaccine Integrated Project (AVIP) and will be tested in comparative preclinical and clinical trials with the ultimate goal of selecting proper candidates for advanced clinical testing in developing countries.
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Keywords: Combined-vaccine; HIV regulatory proteins; HIV structural proteins

1. Background and rationale

WHO/UNAIDS estimates indicate that 36–44 million men, women, and children are currently living with HIV or suffer from AIDS worldwide, with about 14,000 new infections occurring daily. More than 90% of these infections occur in developing countries (DC). Life expectancy in the most affected countries has declined by 10–20 years, wiping out decades of hard-won gains in health improvement. In South Africa, the national HIV prevalence rate is 27.9% for pregnant women attending antenatal clinics in the public sector and, based on this figure, an estimated 5.6 million people were living with HIV in South Africa at the end of 2003. The proportion of infected women is also increasing in Eastern and Western Europe, and in the United States, as a result of the overwhelming heterosexual transmission. Thus, the social and economic impact of HIV/AIDS pandemic is unprecedented, and may lead to political instability, misguided market reforms and famine. Antiretroviral treatment, which halts disease progression and prolongs survival of HIV-infected persons, is not yet easily applicable in the developing world. The therapy regimens are difficult to comply with, and the efforts of DC to modify the behavior and culture of their populations through intervention programs of the National Public Health Plans have had only a modest, although appreciable, impact. Therefore, prevention of transmission through the development of a vaccine against HIV/AIDS may conceivably be the most effective way to stop the spread of HIV infection.

Over the past two decades, the search for an AIDS vaccine has focused on the use of HIV Env as the immunogen. Since Env is responsible for virus binding and entry into cells, the rationale for developing an Env-based vaccine was to generate neutralizing antibodies 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 has been observed, have been largely disappointing. This can be accounted to the inability of such vaccines to elicit protective neutralizing antibodies due to the Env variability [1] that hampers recognition of relevant, mostly conformational, epitopes by neutralizing antibodies, and to the heavy glycosylation of the gp120 Env subunit that also contributes in masking neutralizing epitopes (reviewed in [2]). Clearly, an alternative rational vaccine approach is required for the further development of an efficacious vaccine against HIV/AIDS.

Recently, new strategies have been developed aimed at blocking virus replication and disease onset in the absence of sterilizing immunity. Control of virus replication may modify the virus–host interaction, favoring the host immune response and providing protection to disease progression and virus transmission to healthy individuals. Thus, such strategies may be used for both preventive and therapeutic vaccine strategies. In particular, they should utilize viral products i) exert-

Abbreviations: Ab, antibody; AVIP, AIDS Vaccine Integrated Project; CAB, community advisory board; DC, developing countries; ΔV2 Env, V2-deleted Env; GMP, good manufacturing practice; WP, workpackage.

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doi:10.1016/j.micinf.2005.07.024

ing key functions in the early virus life cycle, in infectivity or in pathogenicity, ii) capable of inducing a broad immunity, iii) and conserved across HIV clades. These viral products include the nonstructural proteins Tat, Rev and Nef.

In particular, the HIV regulatory proteins Tat, Rev and Nef share properties that make them very promising vaccine targets. They are the first HIV proteins expressed upon infection, even prior to virus integration [3], and are essential for virus replication [3–5]. These regulatory proteins exert multiple immunoregulatory effects, either directly or indirectly, aimed at facilitating target cell recruitment and activation, further promoting HIV replication and spreading (reviewed in [6]). In addition, Tat and Nef are also found extracellularly and in this form they exert effects on different cell types [4,7–13], including chemotactic activity for HIV target cells [14].

Importantly, Tat, Rev and Nef proteins are highly conserved in their immunodominant regions encompassing B and T (including CTL) epitopes, a very desirable feature for the generation of a vaccine that has to circumvent major intra and inter-clade variability [15–18]. In particular, recent studies indicate that there is a remarkable degree of cross-recognition of clade B Tat by sera from Ugandan and South African individuals infected with virus strains mainly belonging to the clades A, C and D [15]. This is of relevance, since these clades are prevalent in the sub-Saharan Africa, by far the most affected area in the world.

Of outmost importance, cellular immune responses to Tat, Rev and Nef have proven important in controlling disease onset and progression [16,19,20]. Consistently, a strong correlation between the presence of anti-Tat antibodies and non-progression to AIDS has been found in both cross-sectional and longitudinal studies [21]. This suggests that the induction of antibodies neutralizing the effects of extracellular regulatory proteins can be a desirable feature of a vaccine. Interestingly, immunization in humans with the three regulatory genes induced new CTL responses in all vaccinees [22,23].

Another feature of regulatory gene products, which is key to a novel vaccine design, is that they possess immunomodulatory properties, as indicated by the promotion, in vitro, of dendritic cell maturation and activation by Tat or Nef [24,25], and by the modulation, in vitro and in vivo, of CTL epitope hierarchy by Tat through effects on the proteasome catalytic subunit composition [26]. In particular, results from very recent vaccine preclinical studies in the murine model indicate that co-administration of Tat (DNA plasmid, recombinant adenoviral vector, or native protein) with Gag leads to enhancement of Th-1 and CTL responses against this structural protein, whereas responses to Tat are maintained (Robert-Guroff and Ensoli, submitted; Guzman et al., in preparation; Caputo et al., unpublished data). Of note, co-immunization of mice with Tat and Env or Gag also resulted in increased antigen recognition, as indicated by the appearance of responses to epitopes not recognized upon immunization with the structural protein alone (Gavioli et al., unpublished data).

With regard to the HIV structural protein Env, novel variants are currently available, which include modifications (i.e. V2 loop-deletion) that permit exposure of conserved epitopes in order to increase the breadth of the neutralizing antibody (Ab) responses. Such new antigens have great potential for the design of an effective HIV/AIDS vaccine.

In particular, preclinical studies have shown that V2-deleted Env (ΔV2 Env) proteins are immunogenic and able to induce cross-clade neutralizing Ab in rabbits in a DNA-prime/protein-boost regimen, as well as containment of infection in monkey challenge experiments [27–32]. The availability of these modified antigens represents a substantial advance in the area of Env immmunogen design, since numerous studies, including the VaxGen Phase III clinical trials, have shown that the monomeric form of Env is likely ineffective in inducing cross-reactive or protective neutralizing Ab responses [33,34]. In contrast, several studies have so far indicated that HIV-1 Env oligomers, consisting of gp120 and the ectodomain of gp41, are superior to the monomeric gp120 for eliciting strong humoral responses directed toward conformational epitopes [35–37] as well as neutralizing Ab against both T cell-line adapted (X4) and selected primary isolates (R5 and X4) of HIV-1 [37–39]. Moreover, as HIV employs various means to minimize the immunogenicity of the Env protein by shielding the important conserved sites on the molecule by extensive glycosylation and external polypeptide ("variable") loops (reviewed in [40]), strategies to overcome this problem are further required.

To enhance the immunogenicity of Env for the induction of broadly reactive neutralizing Ab, trimeric Env derived from the subtype B HIV-1_{SF162} with a 30 amino acid deletion in the V2 loop (o-gp140 $\Delta V2_{SF162}$) was produced and evaluated in vivo [29,31]. Studies performed in rabbits and rhesus macaques have demonstrated that the trimeric ΔV2 Env from HIV-1_{SF162} is more effective at eliciting neutralizing Ab than the non-deleted form of trimeric Env [27]. It is important to note that sera from animals immunized with the ΔV2 Env are capable of neutralizing several heterologous subtype B primary isolates. Using a DNA-prime/protein-boost immunization methodology in rhesus macaques, protective neutralizing Ab were elicited that, in the absence of CD8+ cells, reduced plasma viral load levels during acute infection by the high replication-competent, R5-using $SHIV_{SF162P4}$ virus [28,29]. Some of the protected macaques were followed for longer than 2 years during which time they remained negative for plasma viral RNA, with stable numbers of CD4+ and CD8+ T lymphocytes, no signs of disease and no evidence for the emergence of escape viruses (not shown). During this period, anti-HIV Env Ab titers remained stable, and CTL antiviral responses against SIV Gag (primarily) and HIV Env proteins were measurable during chronic infection in these animals. These results demonstrate the usefulness of high-quality trimeric ΔV2 Env proteins as important components in the development of an effective HIV vaccine.

Taken together, this body of epidemiological and experimental evidence regarding regulatory and structural HIV gene products represents the rationale to develop and to evaluate in preclinical and clinical trials a new generation of vaccines based on their rational combination.

2. The AVIP project

These novel combined-vaccine strategies are currently under evaluation within the European AIDS Vaccine Integrated Project (AVIP). In this program, several combinations of structural and nonstructural gene products, as well as different delivery systems, are being tested in preclinical studies to select the best candidates to enter phase I trials. The polyvalent vaccine is, to date, one of the most promising approaches for an effective vaccine, as also indicated by preclinical studies.

In particular, the optimization of formulations and immunization schedules is highly important, especially in view of the risk that structural, immunodominant viral products, such as Gag and Env, may diminish the response against small regulatory proteins. This potential complication was suggested by preclinical studies that included Gag, Env, Tat, Rev and Nef [41–44] and by the pattern of immune responses detected in the course of natural infections [19,45]. Therefore, the combination of regulatory and structural gene products requires a rational vaccine design, appropriate formulations of the antigens, and/or immunization protocols to ensure induction of balanced responses to all antigens. The rational combination of regulatory and structural genes (or their products) should ensure immune responses to both early and late gene products, and the generation of broadly protective immunity.

2.1. Objectives and scientific structure of AVIP

The general aim of AVIP is to generate novel HIV-1 vaccine candidates to be tested in phase I trials in Europe within a 5-year program. To achieve this goal, four novel vaccines have been selected from a larger pool based on two criteria: the combination of HIV regulatory (Tat and/or Rev, and/or Nef) with structural (Env and/or Gag/Pol) genes/products, and the advanced stage of development of the single components, including efficacy studies in both monkeys and new murine models, good manufacturing practice (GMP) process development, and phase I studies. Training, education and technology transfer activities are also within the general scope of the program and are viewed as preparatory to advanced clinical trials with selected AVIP vaccine candidates that will eventually be performed in DC. Therefore, AVIP major objectives are:

- To develop innovative HIV vaccines based on the rational combination of existing vaccine antigens that have already been tested in preclinical animal models and in phase I studies in humans.
- To conduct phase I trials in Europe with four novel combined-vaccines in both HIV-infected and low-risk healthy individuals. The candidates will be first tested in preclinical models to optimize the formulations and the vaccination protocols. Specifically, the preclinical studies are aimed at comparing safety and immunogenicity in mice and monkeys to select the best vaccine formulations and protocols for human trials, and at evaluating preclinical efficacy in two novel mouse models and in monkeys.
- To implement capacity building and to transfer technology to DC, and to perform cross-clade immunological and virological preparatory studies in order to move rapidly with selected vaccines into phase II/III trials in DC upon completion of phase I studies in the EU.
- To carry out training in EU countries and DC. To this end, the AVIP International School has been established.
- To ensure community involvement both in EU countries and DC in order to guarantee the correct ethical information of the volunteers and counseling, and to ensure that quality of life evaluation and risk assessment are carried out.

Due to the complexity of the program, special attention has been paid to its scientific organization, which includes seven workpackages (WPs) dedicated to the accomplishment of the aims described above. The specific objectives of the WPs are listed below:

- WP1. Preclinical studies (mice, monkeys) aimed at comparing the safety, immunogenicity and efficacy of the vaccine candidates and at selecting the best formulations and vaccination protocols for phase I human trials.
- WP2. Antigen production/GMP production/toxicology, dossier preparation and regulation (approval for human use).
- WP3. Phase I trials in healthy individuals and extended follow-up.
- WP4. Phase I trials in HIV-infected individuals and extended follow-up.
- WP5. Clinical and laboratory studies for the development of phase II/III HIV vaccine trials in South Africa.
- WP6. European Vaccine against AIDS (EVA) Program to support all research and technology development and demonstration activities.
- WP7. Coordination of science, training, business and administrative management.

The AVIP program is being implemented by a Consortium of 15 partners expert in the field of HIV/AIDS vaccine from Italy, Sweden, France, Germany, Finland, United Kingdom and South Africa.

2.1.1. Development of novel vaccines and preclinical/clinical testing

To ensure the success of the program (i.e. completion of phase I trials within 5 years), priority has been given to those vaccine candidates whose components have already completed or are undergoing phase I studies and, therefore, have been approved for human use by both regulatory and ethical committees. Further, for these antigens GMP process development, toxicology data, and protection data in animal efficacy models are available. Therefore, the combined-vaccine candidates in an advanced stage of development will ensure completion of four phase I clinical trials in both seronegative and seropositive subjects within the program in a comparative fashion and in the most cost-effective way. It is impor-

Table 1
Preclinical and clinical data of the vaccine candidates to be tested in phase I trials within the AVIP project: previous work and development stage of single antigenic components

Combined-vaccine candidates (clade)	Single components (clade)	Mouse safety and immuno-genicity	Mouse efficacy	Monkey safety and immuno-genicity	Monkey efficacy[a]	GMP develop-ment	Approval for human use	Clinical trials
1. HIV Tat ± ΔV2 Env (B, C)	HIV Tat (B)	D **	ND ***	D	D	D	D	Ongoing (HIV–**** and HIV+*****)
	HIV ΔV2 Env (B)	D	ND	D	D	D	D	Ongoing (HIV–)
2. HIV Nef ± ΔV2 Env (B, C)	MVA-HIV Nef (B)	D	D	D	D	D	D	Completed (HIV+)
	HIV ΔV2 Env (B)	D	ND	D	D	D	D	Ongoing (HIV–)
3. Multi HIV vaccine antigens and epitopes*	GTU-MultiHIV B clade[b]	D	D	ND	ND	D	D	Completed (HIV– and HIV+)
	Auxo-GTU antigens and epitopes (A, B, C, FGH)	D	D	ND	ND	D	Ongoing	To be started (HIV– and HIV+)
4. HIV multigene [Nef, Rev, Tat (B) and Gag, RT, Env (A, B, C)]	HIV Nef, Rev, Tat (B)	D	D	D	D	D	D	Completed (HIV+)
	HIV Gag, RT Env (A, B, C)	D	D	D	ND	D	Ongoing	Ongoing (HIV–, HIV+)

* Multi HIV A, B, C, or FGH clade antigens and epitopes = full length *rev, tat, nef, gag* (p17, p24) antigens and over 20 T cell epitopes from Pol, Protease, Env antigens; ** D = Done; *** ND = Not done; **** HIV–: in non-infected individuals; ***** HIV+: in HIV-infected individuals.
[a] The SIV Nef is used for monkey efficacy studies.
[b] First generation vector.

tant to highlight that AVIP focuses on HIV-1 clade B, since the phase I trials will be performed in the EU. However, it should be pointed out that, at least for regulatory genes, interclade issues may not be as critical as for Env, since recent data indicate cross-recognition of the Tat vaccine (clade B), which is presently in phase I trials in Italy, by sera from individuals infected with clade A, B, C, or D circulating viruses (from Italy, Uganda, South Africa) [15]. Moreover, the clade-B ΔV2 Env used in this project exposes cryptic neutralizing epitopes that are conserved among different clades, and preliminary data indicate cross-recognition of clades B and C by sera of animals immunized with clade B Env [27,31,32,46]. On the other hand, clade-A and clade-C immunogens are already being developed by the AVIP Consortium, as part of parallel national, bilateral, and EU vaccine programs, related to AVIP.

Two of the four different vaccine candidates that are being evaluated within AVIP are based on the combination of the same structural gene product (ΔV2 Env) with two different regulatory proteins:

- Tat ± ΔV2 Env.
- Nef ± ΔV2 Env.

These formulations will allow assessment of the contribution of each regulatory protein to the overall immunogenicity in animal models and humans, as well as to evaluate efficacy in preclinical studies.

The other two vaccines are complex combinatory vaccine formulations involving the use of blocks encompassing multiple structural and nonstructural antigens.

The first candidate is a pure DNA vaccine, which is based on a single vector coding for selected antigens, namely, the

- multi HIV antigens/epitopes [full-length *rev, tat, nef, gag* (p17, p24) antigens, and over 20 T cell epitopes from Pol, Protease and Env antigens]. Preclinical studies will indicate whether this strategy will require boost with viral vectors expressing the identical Multi HIV antigens, with the Multi HIV proteins or with single proteins/genes.

With regard to the second candidate, the antigens are administered as individual DNA constructs or proteins according to a prime-boost protocol, in which GM-CSF is used as adjuvant. The specific composition being:

- HIV multigene (nef, rev, tat, gag, rt, env).

The modular format of this specific vaccine formulation will permit a clear evaluation of the contribution of each component to the overall immunogenicity and efficacy of the vaccine formulation in preclinical studies and immunogenicity in humans.

The efficacy of the different antigens present in the AVIP vaccine candidates has been shown in animal models and these antigens have been or are being tested in humans (Table 1). Comparative analysis in both preclinical and phase I trials of these vaccines will be key for the selection of vaccine candidates for phase II/III trials in DC. To this end, clinical sites have already been set up in Estonia, Finland, Germany, Italy, Sweden, and the United Kingdom. Partners involved in the clinical development have wide experience with regulatory issues, GMP production, study design and site preparation, ethical issues, and community advisory board (CAB) involvement. In addition, the support of the already established European Vaccine against AIDS (EVA) Program Centralized Facility for AIDS Reagents (NIBSC, UK) with repository and distribution functions is of key value to this project.

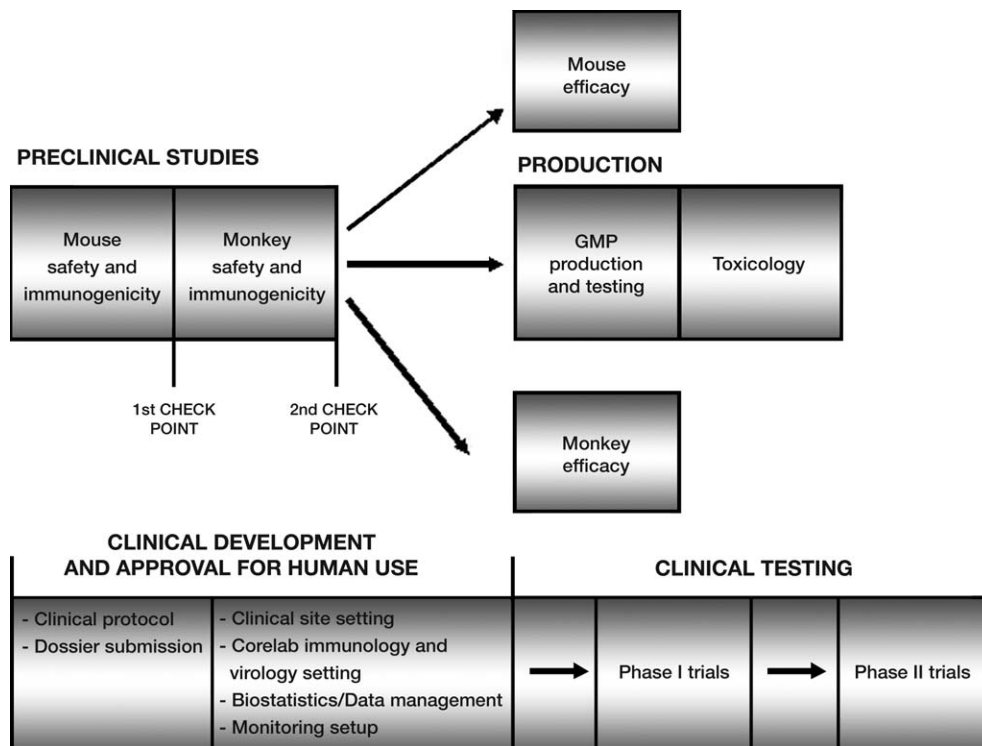

Fig. 1. Vaccine development pipeline.

2.1.2. Capacity building and technology transfer to DC

Cooperation with DC is one of the main activities of AVIP. In particular, the current collaboration with South Africa, aimed at the preparation of sites for future phase II/III vaccine trials, constitutes the basis for building up a partnership with other DC (i.e. Swaziland, Uganda, Tanzania) that already collaborate with participants of the AVIP Consortium. Both laboratory and clinical capacities will be implemented in DC through appropriate training in the specifics of the work needed for the project. Clinical capacity development will include joint writing of the future vaccine clinical protocols for phase II/III trials. The training and standardization of techniques and procedures, in both the clinical sites and core laboratories for performing phase I clinical trials in the EU with DC, will ensure the reproducibility of the results at the various sites, and maintenance of high levels of internal and external quality controls.

To this end, as part of the innovation of this proposal, training is seen as a two-way process in which European investigators will also visit and learn from the African sites. As part of the training program, African investigators will visit the sites in Europe where phase I trials will be conducted to make them fully acquainted with the clinical, immunological, ethical and analytical aspects of the phase I trials in the EU. The participation of African researchers will therefore promote continuity in the development of the vaccine. The establishment or expansion of CAB in the EU and DC will be of critical importance to ensure the ethical recruitment and briefing of volunteers, quality of life assessment, risk assessment and communication programs. Vice versa, the participation of the European researchers in the development of future phase II and III sites will also serve to ensure compatibility and a smooth transition between phase I trials in Europe and phase II trials in Africa. The AVIP International School will be the main instrument for the training activity.

2.1.3. Community involvement

The project will also take advantage of (and will support) the existence of CAB in Africa and joint CAB with the EU. These Boards, while similar to some extent to such committees in the US and Europe, also have unique aspects that the European researchers will be exposed to. For example, traditional leaders still have substantial authority in rural Africa and, hence, the consent to participate in clinical studies is not only the responsibility of the individual but also of the community. The "community consent" should be therefore taken into account, as articulated through traditional authority structures.

Finally and more generally, another value of this project is the well established collaboration with South Africa, which was initiated several years ago through other funding resources. After the first year of the program, another application is planned to include additional DC where cooperation with participants of the Consortium is already ongoing through EU Member States and EU-funded programs (i.e. Swaziland, Uganda, Tanzania). This will allow a broader partnership and synergy with DC.

2.2. Vaccine testing and selection

The AVIP program will be implemented through the following activities (Fig. 1):

- *safety and immunogenicity in mice and/or rabbits* will be performed with all candidates and compared with single components to ensure selection of the best vaccine formulation to avoid antigen interference, which can easily occur between structural and regulatory genes/products (first decision-point);
- *safety and immunogenicity in monkeys*. Vaccine combinations that fulfill safety and immunogenicity requirements in small animal models will progress to monkey safety and immunogenicity evaluation studies. This will be a second decision-point for both human trials and preclinical efficacy studies (mice, monkeys);
- *mouse and monkey efficacy studies*. Mouse efficacy studies will be performed for formulations that will have passed the second decision-point. Two different models (HIV pseudotypes and herpesvirus infection models) will be used. Monkey efficacy studies will be performed for the two vaccine candidates for which appropriate SHIV are available (Tat ± ΔV2 Env and Nef ± ΔV2 Env). These monkey studies will start in parallel with mice efficacy studies allowing comparison and validation of the novel murine models. The continuation of the efficacy studies in parallel with clinical trials will allow comparison and validation of the animal models used;
- *clinical trials in non-infected and HIV-infected individuals*. To assess the safety and immunogenicity of the vaccines, clinical trials will be conducted in parallel in both seronegative and seropositive individuals. This will be essential to determine the impact of vaccination, since it cannot be anticipated which vaccine candidate may be optimal for seronegative versus seropositive individuals. Immuno-monitoring and prolonged follow-up of the vaccinees will enable head-to-head comparisons of the vaccine candidates, as well as an efficient selection of the best candidates for advanced clinical testing in DC. This is of great importance for field vaccination campaigns in DC, where uninfected and infected individuals can be reached without knowing the infection status. Capacity building and implementation of virological, immunological and epidemiological studies in South African sites will be pursued. This will permit to clarify the requirement of either clade-specific antigens or the effectiveness of a vaccination approach inducing cross-reactive immune responses for future vaccine formulations.

All activities described above will be conducted upon harmonization of the different centers that are involved in the AVIP activities.

3. Conclusions

Results obtained through the AVIP program are expected to contribute toward the improvement of life expectancy, quality of life, health and safety on a worldwide scale by providing vaccine candidates for the fight against HIV/AIDS, and may lead to a significant improvement of living conditions in both developing and developed countries. In DC, a successful vaccine strategy may contribute to ameliorate human life expectancy and to greatly decrease the number of children orphaned by AIDS, therefore reducing the tremendous socioeconomic loss due to AIDS. Furthermore, the development of vaccines against HIV/AIDS may reduce the risk of emergence and transmission of drug-resistant viruses from noncompliant patients.

The major objectives of the AVIP program will be achieved through the establishment of a unique partnership between complementary academic, government, regulatory, national health and industrial laboratories, which are at the "cutting edge" of HIV/AIDS vaccine research, and have structured vital cooperation with centers of excellence in Africa. This unique constellation of expertise and know-how generates an added value, which is essential for the fulfillment of the main AVIP goals. Finally, AVIP may represent one of the first structured cooperations between the EU and US international organizations, to achieve the first joint platform, which can contribute to vaccine development within the Global HIV Vaccine Enterprise [47].

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