Old and New Concepts and Strategies in HIV Vaccinology: A Report from a Workshop held in Rome on 17 June 2016

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Abstract

A workshop entitled: “Revisiting HIV inactivation, elite controllers, immunogenetics and new strategies for developing HIV vaccines” took place during a Eurovaccine Conference held in Rome in June 2016. The purpose of this workshop was to revisit old and new concepts and strategies in HIV vaccinology in the light of novel, and sometimes unexpected, data from recent preventative and therapeutic vaccine approaches that could guide future vaccine research.

Panelists were asked to respond to five questions regarding key points and critical issues and problems in current HIV/AIDS vaccine research. Their responses are summarized.

Keywords: HIV vaccines; Reverse vaccinology; Immunogenicity; Epitopes

Introduction

The HIV pandemic has a detrimental impact that is vast and ongoing, despite therapeutic progress. An estimated 37 million people were living with HIV/AIDS in 2014, most of whom did not know their HIV status and an estimated 1.2 million people died of AIDS-related illnesses during that year [1]. The brunt of this impact is borne by vulnerable and disadvantaged groups, such as those found in Sub-Saharan Africa, which accounts for about 70% of those living with HIV/AIDS and represent an important site of new infections.

Potent biomedical interventions are required to stem the more than 2 million new cases of HIV seen each year, a stark reminder that our public health prevention programs are insufficient, and that the development of an HIV vaccine remains a priority. In fact, given the scale of this harm, the benefits of prophylactic interventions such as vaccination (and other interventions with therapeutic or prophylactic effects) to reduce the disease are potentially enormous. This is especially so if we consider that benefits to the worse off have greater value than benefits to the better off, on egalitarian or other grounds [2].

However, in spite of the substantial efforts and resources that have been devoted to curbing the pandemic during the last 30 years, it has not yet been possible to develop an effective vaccine. Initially, AIDS was perceived as a disease that actually destroys the immune system and this seemed to exclude the possibility of devising an effective method of immunological prevention. As we became more familiar with its physiopathology, it was realized that afflicted subjects did not die immediately as a result of HIV infection. Rather, the opportunistic infections and tumors that caused such havoc and terror during the initial years of the pandemic occurred at the end of the natural course of a disease which, in the absence of therapy, actually spanned years of asymptomatic infection. By way of contrast, we only have to compare it to the natural course of an emergent infection such as Ebola, to clearly see that the natural course of HIV disease is one of a chronic disease and that HIV-infected subjects do not die of acute disease but rather from the consequences of chronic HIV disease. As a result of this perception, for many years HIV disease was characterized by its terminal rather than by its initial phase. This characterization led to many studies being devoted to the specific properties of the virus and of the immune response present at the end rather than at the beginning of the infection. Highly mutated viruses that can only be neutralized by rare antibodies possessing unique structural characteristics that make them broadly neutralizing, became the “holy grail” target of massive HIV vaccine efforts. Unfortunately, these searches failed to deliver a successful HIV vaccine. In recent years, considerable information has become available about the early stage of HIV infection. Nonetheless, investigators are still embarked today in the search for antibodies that appear very slowly following intense internal evolutionary pressures in individual hosts that bear little relationship with the situation during the initial infectious episode.

To date, the only HIV vaccine to show modest efficacy was the RV144 trial, conducted in over 16,000 heterosexual adults in Thailand.
(2003-2009), which proved that a vaccine approach could reduce HIV acquisition and was a major lift for the field [3]. Even though modest efficacy was seen, the RV144 HIV vaccine regimen [a canarypox vector (ALVAC) prime, followed by protein boosts with an alum adjuvant] induced no neutralizing antibody responses, and protection appeared to be correlated with binding antibodies (e.g. IgG antibodies to the V1V2 region of gp120), polyfunctional CD4 T cell responses as well as other non-neutralizing antiviral activity [4]. These findings were a surprise, and a reminder of the importance of advancing candidates to further clinical development. In fact, before this vaccine trial was initiated, numerous renowned and influential HIV researchers published an opinion letter in Science calling into question the rationale for this study of combining two vaccines that had both failed in prior human trials to generate immune responses that they were designed to elicit. This letter stated that spending $119 million when "the overall approval process lacked input from independent immunologists and virologists who could have judged whether the trial was scientifically meritorious" was an ill-advised use of precious resources [5]. Thus, an apparently scientifically unsound approach with no former evidence of potential efficacy proved effective at reducing transmission and this protection was not conferred by neutralizing antibodies, which most vaccinologists still consider an absolute requirement for preventing HIV acquisition.

But there are also other obstacles to HIV/AIDS vaccine development, as Mike King points out: "It is apparent that HIV vaccine development is fraught with many empirical, scientific, philosophical and applied ethical considerations. Factors that delay scientific progress towards vaccine development risk increasing the total harm of the HIV/AIDS epidemic. This can be thought of as a moral form of opportunity cost. Delays can come from a variety of sources, including lack of political, social, ethical, and financial support, as well as inefficient organisation of global HIV research efforts, pursuit of ineffective research programs, and failure to pursue more effective options for vaccine development. Although competing values may explain or justify some impediments, the immense moral value of an effective vaccine remains. Some impediments impose the moral opportunity cost of foregone counterfactual benefits (greater good that could have been achieved through other options). As an example of this, it has been argued that structure-based reverse vaccinology (SBRV) was an ineffective research strategy for HIV vaccine development, and that other approaches should have been used instead [6,7]."

"The more those working in HIV vaccinology are able and willing to consider and discuss their work in all these terms, and not restrict their focus to vaccine science narrowly conceived, the more likely the great good that vaccinology is capable of achieving can be realised." [8]" A number of investigators have proposed outlier approaches and have investigated unconventional HIV/AIDS vaccine candidates, some of which were discussed at the Rome Workshop. Participants confronted their opinions regarding which approaches were most likely to succeed and their viewpoints are summarized below.

**Question 1: What New Approaches should be followed in HIV-1 Vaccinology?**

Barbara Ensoli and Aurelio Cafaro (BE & AC)

It is apparent from the course of the infection that HIV-1 is immunogenic and capable of inducing strong cellular and antibody responses. CD8 responses (CTL) are responsible for blocking virus replication down to the viral setpoint, indicating a partial control, which eventually is overcome, and the infected individual progresses to AIDS if not treated with antiretrovirals. Anti-HIV antibodies do not appear to play a clear protective role throughout the entire course of the infection, although broadly cross-neutralizing anti-Env antibodies do develop in a minority of chronically infected patients a few years after infection, too late to be effective, as indicated by the rapid appearance of escape mutants. Natural immunity may also play a role and contribute to the relative resistance to HIV-1 infection, as shown by a low HIV-1 transmission rate. The crucial question, however, is whether it is possible to protect individuals from infection.

For many years, the prevailing view was that it should be possible to develop preventative vaccines capable of inducing the same cellular and antibody immune responses observed in the course of HIV infection. By pre-arming people with anti-HIV immune responses, it was hoped that one could prevent them from either getting infected (mainly due to neutralizing antibodies against HIV Env) or that it would be possible to control and clear the infection soon after exposure, mainly by anti-HIV CTL. An alternative view was that it may be possible to modify HIV antigens and facilitate the recognition of numerous virus subtypes.

Over the years, these approaches have substantially failed, with the notable exception of the RV144 trial. New immunogens (Env trimers) and delivery systems (adenoviruses, adeno-associated viruses, attenuated CMV vectors, just to mention a few) have been investigated, with the goal to improve specificity, strength and durability of responses, and to elicit new type of responses (MHC-E and class-II restricted CTL responses, as in the case of the CMV vector). In this regard, the protection afforded by MHC-E and class-II restricted CTL responses in the preclinical, attenuated CMV vector model [8,9] or, even more surprisingly, by the induction of suppressive CD8 T cells [10] (see also response to Q2 and Q4) indicate that vaccine efficacy may result from the induction of completely novel and unexpected responses.

We took a different approach, briefly summarized herein. To start, we hypothesized that it might be easier, when studying natural infection processes, to learn which responses are associated with a milder infection (i.e., low progression) that could perhaps be countered by vaccination. Thus, we decided to target the HIV Tat protein, which elicits specific antibodies during natural infection although, when anti-Tat antibodies do develop, progression to disease is significantly delayed [11,12]. The Tat vaccine was first tested in nonhuman primates, demonstrating protection from overt infection after intravenous challenge with the pathogenic SHIV89.6P [13]. Protection was prolonged (2 years), resisted to activation of macaques’ immune system by tetanus toxoid boosting [14], a stimulus known to burst virus replication and controlled virus replication to undetectable plasma levels when macaques became overtly infected upon rechallenge with a 5-fold higher dose of the same SHIV, while all controls progressed to simian AIDS [15]. These results were further confirmed in subsequent preclinical studies, which also revealed the contribution of the Major Histocompatibility Complex (MHC) and of virus challenge dose to the outcome of the experimental infection in cynomolgus macaques [16]. These findings prompted us to evaluate the Tat vaccine in humans, and preventative and therapeutic phase I studies were conducted, which confirmed the safety and immunogenicity of the vaccine in both healthy individuals and infected subjects [17-21]. Moving onwards, we decided to test whether the Tat protein vaccine, administered to infected individuals undergoing effective cART and who were negative for anti-Tat antibodies was able to elicit anti-Tat immunity and protective anti-Tat antibodies. We reasoned that in such individuals, cART may have restored the immune system sufficiently
to lead to a vaccine response. Furthermore, the initial lack of anti-Tat antibodies may have avoided potential interference by a pre-existing immune response, which could have made it easier to observe evidence of efficacy in a therapeutic setting compared to a preventive one, as indeed we found [22-24] and discuss later (see response to Q4).

Massimo Amicosante (MA)

Information relative to host-HIV interaction has greatly increased in the past decade and has given us a better understanding of the intimate interaction between HIV proteins and their targets in host cells as well as of the role that highly variable human genes play in controlling HIV replication. In addition, studies focused on HIV-specific immune responses have demonstrated that the innate immune response plays a major role in delaying HIV disease progression.

Novel vaccine approaches should be able to trigger efficient innate immune responses using both new adjuvants and specific targets. This might lead to the production of antibodies that could help identifying HIV immunogens suitable for either preventive or therapeutic vaccines.

Jean-Marie Andrieu (J-MA)

Since most HIV-1 infections occur across the sexual or rectal mucosal barriers, it is important when designing HIV-1 vaccines to understand how the virus penetrates these vulnerable sites. Recently two new pieces of information have suggested alternative strategies for developing an HIV vaccine. The first piece of information is that as high as 80% of HIV-1 mucosal infections are established by only one infecting virus, called transmitted/founder virus (T/F virus). This T/F virus targets one CD4+ T cell (whereas other types of immune cells, such as dendritic cells and macrophages, are not initially the seat of productive infection). Moreover, the total number of different T/F HIV-1 particles seems to be very limited and each of them bears the same small number of specific amino acid (AA) "signatures" at certain strategic positions on the envelope glycoprotein [25]. Without those AA "signatures", the virus of infected "donor" patients cannot penetrate the mucosa of still uninfected "receivers".

The second piece of information (already hypothesized more than 10 years ago) is that an activated state of the CD4+ T cell is a prerequisite for productive HIV infection although in vivo, replication in quiescent CD4+ T cells is essentially nonproductive and generally abortive [26].

These two pieces of knowledge are also valid for the macaque model, i.e. quiescent CD4+ T cells are not easily infectable, whereas activated CD4+ T cells are the first cells to be productively infected by T/F SIV's that possess specific AA signatures on their Env glycoproteins.

Jean Boyer (JB)

HIV vaccine development is a multidisciplinary task that requires the participation of virologists, immunologists, immunochemists, molecular biologists, geneticists, epidemiologists, statisticians and bioinformatics experts as well as the involvement of the pharmaceutical industry. In addition, it can only succeed if the search for basic immunological knowledge is intimately associated with the search for an effective, vaccine solution to what is the worst pandemic of modern times [27,28].

Felipe Garcia (FG)

Most of the therapeutic vaccines that have previously been tested were previously used as preventive candidates using classical approaches such as whole inactivated virus or recombinant gp120 proteins. The capacity of these early vaccines to increase HIV-specific responses was limited and results were discouraging, as no consistent immunogenicity and no clear impact on viral load could be demonstrated. Subsequently, other approaches using innovative vectors such as DNA, recombinant virus and dendritic cells were investigated in exploratory trials with small numbers of patients. The best results were observed with DC-based vaccines [29,30]. Several other strategies will be explored in the near future such as mRNA-based vaccines, coformulations with nanoparticles and various combined strategies for targeting virus reservoirs.

Glenda Gray (GG)

Our knowledge and experience in HIV vaccine development has increased our capacity to design immunogens that induce competent immune responses. HIV vaccines have become pivotal part of the prevention research agenda and we are optimistic that we can develop both a preventive and a therapeutic HIV vaccine.

Currently, only four vaccine approaches have been evaluated in 6 phase 2b/3 studies. Of course, shortly after the announcement of the RV144 results, a public-private partnership (P5 Partnership) convened to develop the pox-protein regimen for sub-Saharan Africa. HVTN 097 evaluated the RV144 regimen in South Africa, a different setting to Thailand in terms of circulating HIV clade, predominant modes of transmission and average body mass index. Immunologic response in South Africans was comparable to, if not slightly better than, responses induced in Thailand.

The P5 supported the development of the pox-protein regimen for testing in sub-Saharan Africa. In order to better match the "Thai vaccine" to the sub-Saharan population, P5 redesigned the ALVAC vector with a clade C env insert and constructed a new bivalent clade C recombinant gp120 and tested this in HVTN 100 in South Africa, a phase 1 study. Based on promising immunogenicity from this study a large scale efficacy study, HVTN 702 is scheduled to commence at the end of 2016 in South Africa, which will evaluate this regimen in 5400 HIV uninfected individuals aged 18-35 years old.

Thus, it appears that a heterologous prime-boosting approach utilising viral vectors carrying carefully selected antigens that include env for priming and adjuvanted Env based protein(s) for boosting may be a good approach to induce a durable cellular and humoral immune response. To advance from the modest efficacy observed in the Thai trial, additional strategies should be considered, which may include the passive infusion of neutralizing antibodies or the utilisation of more potent vectors like Ad26, MVA or Ad35. Promising viral vectors receiving attention in preclinical development include CMV, while NHP challenge models provide an argument for advancing to clinical development.

Mike R King (MK)

Among HIV vaccination projects, priority should obviously be given to those approaches that seem to provide the most likely means for developing an effective HIV vaccine. Or, minimally, those approaches that have been found to be unsuccessful should be given a very low priority. Such decisions should be made through open, informed, unbiased evaluation of research projects. If new approaches seem to offer a better chance of successful development of a vaccine, they should be the ones that receive funding.

An important consideration is that research aimed at obtaining prescriptive knowledge, i.e., knowledge in the form of a new invention that allows one to do something of practical utility that was previously not feasible, should be prioritized. This is the type of knowledge...
required for obtaining a new vaccine which is nearly always obtained empirically by successfully manipulating and controlling the immune system [31]. Unfortunately, most selection committees tend to prioritize research that is likely to yield novel factual scientific knowledge (i.e., knowledge and discoveries in immunology) that reveals something that existed all along but was unknown to anybody), the reason being that they fund projects proposed by highly productive scientists who have excellent publication records and made important discoveries in basic science. However, curiosity-driven basic research, undertaken without a commitment to the aim of inventing something of such enormous human importance as an effective HIV vaccine, may be unable on its own to lead to the required prescriptive knowledge that is always obtainable empirically [7]. This is not to deny that other good outcomes of research, such as factual, scientific knowledge (whether practically useful or not), are also valuable. However, in a research environment that is finite in its resources, it seems reasonable to prioritise research that is aimed at generating both factual and prescriptive knowledge, which means that the research should not only generate new immunological knowledge but should also strive to obtain the prescriptive knowledge that is only achievable by intervening empirically in the immune system [6].

This would require that current and future proposed research programs should be evaluated in a way that assesses their possible outcomes in scientific, but also ethical terms, taking the issue of AIDS seriously as a major public health matter [32]. This requires the involvement of scientists, ethicists, as well as all the stakeholders who stand to potentially benefit most from the research.

Adan Rios (AR)

With the new understanding of how HIV is transmitted by a single variant in most sexual mucosal transmissions, a strong case can be made that the focus of investigation should be on the initial events in the infection process. It is now obvious that the biology of HIV follows the pattern of an evolutionary process and that the initial transmission of the disease is not a random phenomenon but follows the universal evolutionary principle of strong natural selection. This means that understanding the specific characteristics of the Transmitted/Founder (T/F) virus that initiates the infection is likely to be crucial for developing a preventive HIV vaccine. The antigenic structure of T/F viruses may thus hold the key for identifying which immunogens should be used in a preventive vaccine since it is the neutralization of the viruses that are present initially and can spread easily from cell to cell that should be achieved in order to prevent further viral dissemination.

There is good evidence that the current, improved methods of HIV inactivation offer a unique possibility of developing vaccines that would target specifically the T/F viruses responsible for initiating the infection. Paraphrasing Willie Sutton’s reply to the question: “Why do you rob banks?” “Because that’s where the money is!” It seems equally relevant to direct a preventive HIV vaccine to where it matters most. The possibility of using safe, chemically inactivated HIV creates a biological scenario that allows for a potential new beginning in the search for a preventive HIV vaccine, either by rational design, notwithstanding its failure thus far or by the use of empirical strategies that have a past track record of success [33-35].

Given that over the last 30 years, there have been only six clinical trials of potential HIV vaccine candidates, all based on rational vaccine design, with only one trial showing a less than modest success due to the inherent difficulties in the approach, it could be argued that there are compelling scientific and moral reasons to explore the potential development of the HIV inactivation approach. This argument was made painfully valid during the last 2016 Durban International AIDS Conference, where it became clear that once again timetables and milestone projections to cure HIV are far from being achieved and are in fact becoming a cruel mirage (Table 1).

Eric Sandstrom (ES)

In the search for ‘new’ approaches, it is often forgotten that when the ‘new’ gets well documented, it soon is no longer new and may fall by the wayside to be replaced by other ‘new’ approaches, as a result the initial new approach will not get the benefit of a definitive evaluation. This is the case with the DNA prime MVA boost regimen that we have pursued for a number of years [36-38]. In spite of similar or higher immune responses reminiscent of the correlates of protection found in RV144 (such as impressive CD8 induction and a durable immune response that can be boosted by a single late MVA administration), it has proven impossible to find resources for another efficacy trial. If indeed laboratory assays suggest that there may be protection from infection, then an efficacy trial is needed to corroborate these assays and provide the material for further investigation of possible correlates of protection. If, on the other hand, there is no protection, then an in depth reevaluation of all the criteria for selecting which vaccines should move forward for efficacy trials is mandatory. Not to proceed to an efficacy trial of these vaccine candidates remains a major missed opportunity.

Marc Van Regemortel (MVR)

It is essential that HIV-1 vaccine developers critically examine the validity of the research paradigms and conceptual frameworks they use when trying to develop a preventive HIV-1 vaccine. For more than 10 years the paradigm of structure-based reverse vaccinology (SBRV) proposed by Dennis Burton [39] was pursued by large teams of investigators, although it was not based on sound immunological theory [7]. SBRV assumed that it should be possible to generate an HIV-1 vaccine by first determining the crystallographic structure of a complex between an HIV-1 Env epitope and a neutralizing (n) monoclonal antibody (Mab) and then reconstructing the epitope by reverse molecular engineering outside the context of the native Env.

This strategy was based on the assumption that the epitope designed

Table 1: Factors affecting the UNAIDS “ending AIDS” goal [62].

1. Worldwide funding for ARV therapy is becoming increasingly and woefully inadequate.
2. South Africa has 6.6 million persons infected, of which 3.4 million are on ARV. With a decline in gross domestic product and currency devaluation, expenses are increasing making more difficult to sustain the current expenses.
3. Worldwide new yearly infections have remained at nearly 2 million for 5 years.
4. Eastern European and several Asian countries where new infections jumped 57% between 2000 and 2015 only have 18% of the infected persons currently receiving ARV.
5. There is a looming crisis of insufficient supply of ARV as profit margins of generic companies’ compromise production capacity.
6. Only 30% of HIV infected persons in the United States fully suppress the virus and the number is far lower in many countries, making the UNAIDS’s goal of the “90-90-90” by 2020 clearly unrealistic.
7. Infected children and adolescents constitute an increasing burden because of limited access to care and poor retention in therapy.

Infect children and adolescents constitute an increasing burden because of limited access to care and poor retention in therapy.
to fit the nMab should have acquired the immunogenic capacity to elicit a polyclonal Ab response with the same neutralizing capacity as the Mab used as template. The proponents of SRBV called this approach “rational vaccine design” although they were only improving the capacity of an epitope to bind one particular Mab (i.e., its antigenicity) and were actually not designing a hypothetical immunogenic epitope able to elicit Abs endowed with the same neutralizing capacity as the Mab. Antigenicity was simply confounded with immunogenicity [40].

When an antigen or epitope is introduced in a host immune system (IS), it becomes known as an immunogen, although it is of course the IS that produces the antibodies, the epitope being only a triggering agent that initiates a chain of reactions in the IS. This is only successful if the given host IS also possesses B cell receptors that can recognize the immunogen, as well as various types of T cells and other regulatory mechanisms. Reverse vaccinologists, however, assume that these required features are always present in the individual IS to whom they administer the engineered epitope, although this is obviously not the case [41,42]. Since they only consider the specificity of the epitope-Ab interaction and not the relevant biological characteristics of the IS that allows it to produce neutralizing Abs, reverse vaccinologists never succeeded in obtaining an effective HIV vaccine. They also ignored the fact that the epitope engineered by SRBV is only one of the many epitopes that the polyspecific nMab is able to recognize, which means that their engineered epitope is not necessarily the one that elicited the nmAb and should not be expected to be able to elicit protective antibodies [7].

The SRBV approach used in hundreds of studies thus failed to yield a preventive HIV-1 vaccine [6,43,44] because it did not take into account that Abs are polyspecific and that the antigenicity and immunogenicity of a viral protein are different properties that are not necessarily located in the same regions of the protein [31].

In a similar way, the success of the currently popular paradigm in HIV-1 vaccine research which assumes that it may be possible, by sequential immunization with various Env immunogens [45], to drive human immune responses towards the production of highly mutated anti-HIV nAbs will depend on whether the stochastic nature of successive mutations in Ab genes can indeed be controlled to achieve the required degree of Ab affinity maturation in large numbers of human vaccinees.

Question 2: Should HIV-1 Inactivation be reconsidered?

BE & AC

Recent technical progresses make chemically inactivated HIV a valuable vaccine candidate [35]. However, just to pre-expose an individual to a nonviable form of the virus may not be sufficient to induce the protective responses that occur when he/she gets exposed to the viable counterpart. Many variables make it difficult to predict the outcome of using the different delivery systems and adjuvants that need to be tested. It must also be kept in mind that a preventive vaccine against HIV/AIDS is intended for the general population, whereas a therapeutic vaccine will be directed to a restricted number of people with altered immune systems.

MA

The use of whole virus particles as vaccine presents a number of advantages compared to purified viral components or synthetic HIV peptides. Both the modalities of HIV-inactivation and the type of HIV used (wild type strains or modified strains carrying different viral proteins) may influence viral antigen preservation and determine whether an efficient and protective immune response is obtained. This is particularly critical for preventive vaccines where an efficient protective antibody response is likely to be crucial for blocking virus.

J-MA

During the last 5 years, Wei Lu and I developed a strategy, based on inactivated virus, that was tested in the Chinese (Guangxi) macaque model to determine whether it was possible to suppress the activation of SIV-positive CD4+ T lymphocytes in vivo in order to prevent SIV replication and protect the animals from virus challenge [10,29]. We developed a new oral vaccine made of inactivated SIVmac239 adjuvanted by Lactobacillus plantarum, a commensal bacterium of the digestive tract known to be associated with immune unresponsiveness/ immunological tolerance. We also used Lactobacillus rhamnosus (a commercially available Lactobacillus prepared under GMP conditions) as well as the bacillus of Calmette Guerin (BCG). In contrast to all known vaccines, these oral vaccines elicited neither SIV-specific antibodies nor cytotoxic T-lymphocytes. Instead, they induced a previously unrecognized population of non-cytolytic MHCIIB/F-restricted CD8+T-regulatory cells that specifically suppressed the activation of SIV positive CD4+T lymphocytes. The suppression of activation also interfered with SIV reverse transcription in CD4+ T cells, thereby preventing the initial burst of virus replication, which in turn protected the macaques from infection. Of the 24 macaques orally vaccinated in this way and challenged intra-rectally 3 to 14 months later with a high dose of SIVmac239 or with the heterologous strain SIVB670, 23 remained solidly protected for up to five years whereas all 24 control macaques became infected [10]. This approach will be investigated in humans in the near future.

A second strategy based on inactivated virus which is potentially applicable for constructing a prophylactic vaccine (to our knowledge this has not yet been tested) would be to immunize macaques, and later humans, with a vaccine based on a mixture of several killed/inactivated T/F viruses [35]. Presently available technologies (i.e., sequencing, cloning, gene modification, cell culture and different methods of virus inactivation/killing that do not modify the Env glycoprotein) should allow the preparation of T/F HIV-1-based vaccines. Such vaccines may be the most promising candidates for inducing antibodies able to neutralize the T/F viruses that have entered the mucosa but not yet their target CD4+ T cells.

FG

It has not yet been demonstrated that vaccination with whole inactivated HIV can be successful although some encouraging new data have been obtained. This might be particularly suitable for DC-based vaccines that have shown promising results. We have recently started a new clinical trial with an intranodal DC-based vaccine pulsed with whole inactivated virus.

GG

The most important consideration for HIV inactivation or a killed virus approach would be immunogenicity, and whether this approach would yield an immunogenic vaccine. Novel approaches to inactivate the HIV vaccine should continue and be evaluated in a pre-clinical challenge model to demonstrate proof of concept efficacy. Inactivated HIV vaccine approaches have, to date been limited by scientific, technical and sociological issues [46]. Use of physical and chemical elements to inactivate HIV have impacted on the structural integrity of the HIV virus [47] and other technologies such as radiation technology
or the use of "light" therapy may leap-frog this approach, making for a cheap and effective vaccine, should issues of safety and immunogenicity be resolved.

A live-attenuated, or genetically attenuated HIV vaccine approach, may solve the issue of immunogenicity, but safety issues abound, limiting its evaluation in humans. Future work aimed at ensuring the stability of a genetically attenuated HIV vaccine should be funded, as this approach may lead to high vaccine efficacy that is not be attainable using current methods.

MK

There is good reason to believe that HIV-1 inactivation may offer a viable approach to HIV vaccine development. This strategy was initially rejected on grounds of safety and the initial scepticism still persists today in spite of the development of greatly improved inactivation techniques that preserve the structure and antigenic properties of the Env glycoprotein [33-35,46,48]. Research funding agencies as well as ethics committees and institutional review boards should thus reconsider their earlier systematic rejection of projects utilizing chemically inactivated HIV. In addition to considering any possibility of harm to trial participants, review boards have an obligation to allow promising research (even if previously considered too risky), provided it is conducted in a scientifically and ethically robust way and offers the possibility of promoting the development of an HIV vaccine.

Question 3: Can Therapeutic Vaccines help the Development of Preventative Vaccines? Should Therapeutic Testing Precede Testing in Healthy People?

BE & AC

This is certainly the case since it is exactly what has been done in the case of the Tat vaccine. If a vaccine is safe, immunogenic and effective in a person with an immune system that is incompletely restored, it is plausible that it may work in a healthy individual. In addition, if vaccine efficacy is first evaluated in therapeutic trials, this could significantly speed up the development of preventative vaccines, since 1) a smaller number of volunteers may be needed to assess efficacy, 2) trials may be of shorter duration which would allow faster comparisons of multiple vaccine candidates, 3) logistic and infrastructure requirements may be reduced which would make testing more feasible and less costly in developing countries. The adoption of an adaptive design of the trial may further speed up the vaccine development process [49].

MA

Information obtained during trials of therapeutic vaccines together with other immunotherapeutic treatment of HIV might lead to the identification of novel approaches for blocking virus entry and replication. In view of the different stages of HIV infection, going from persons with a fairly healthy condition to people with advanced disease, it may for safety reasons only be acceptable to evaluate certain products in non-infected HIV subjects. Efficacy trials could, however, be rationally designed in HIV-infected persons.

FG

The therapeutic vaccine pipeline is complementary to the development of preventative vaccines. Since immune responses that prevent infection are different from those that help to control the viral load, the development of both types of vaccines could run in parallel, and findings obtained with the one vaccine may be relevant for the other type of vaccine.

GG

Therapeutic vaccines can assist the development of preventive vaccines. Understanding the mechanisms which control viral replication may lead to a vaccine approach that may assist in the attenuation of disease post HIV infection by lowering viral load set-point, viral control and immune preservation. Concomitant evaluation in both HIV infected and uninfected participant will advance the clinical development of these candidates.

MK

Testing therapeutic vaccines may offer some advantages compared to the testing of preventive vaccines in healthy people. Trial participants who are already HIV-positive may view the potential benefits of a vaccine more favourably than do HIV-negative individuals who expect only the benefits of prophylaxis and this may facilitate the recruitment of participants in therapeutic trials. A draw-back is that such trials usually require an interruption of antiretroviral therapy which may be perceived as entailing a certain risk. This could be mitigated by recruiting individuals for whom ART is less effective because of the development of drug resistance. On the other hand, participants in preventive vaccine trials risk being exposed to HIV infection, which is a requirement for being able to evaluate the effectiveness of prophylaxis in comparison to the control group [50]. Such a risk is obviously not present in therapeutic vaccine trials with HIV-positive participants.

The perception of risk taking by potential trial participants is influenced by numerous factors, trial design being one of them [51,52]. Regardless of what type of vaccine is being tested, researchers should of course strive to build trust among communities in which they work. Open, understandable, and consistent communication about the risks and potential benefits of trials, including community and broader societal benefits, is important, and not only when seeking informed consent [53,54].

It should also be borne in mind that it can be rational for individuals who participate in a trial to accept certain risks and that this should not be seen by ethics committees as being unreasonable [55-57]. The enormous social benefits of preventing HIV infection can be a value that participants and ethics committees must promote by allowing reasonable decisions to be made concerning both trial participation and granting permission of undertaking certain types of research [56].

AR

Although it cannot be denied that the study of a therapeutic vaccine might shed some light on immunological responses to HIV, it is not clear whether such information would be relevant to immune responses occurring in healthy uninfected subjects and would inform the design of a preventive vaccine. It may therefore be counterproductive to use the results of a therapeutic vaccine trial as a gateway for developing a preventive HIV vaccine for healthy subjects.

In view of the efficacy of current therapies for HIV infection, additional advantages afforded by a therapeutic vaccine for patients undergoing interrupted ARV may perhaps be limited although this will of course only become clear once the trials have been conducted.

ES

Generally therapeutic trials should not be required for developing a preventive vaccine. There are many difficulties in interpreting the results of therapeutic vaccines since the immune system may already be too altered in order to respond optimally when the burden of virus is
too great or the virus has already reached many hidden reservoirs. Thus a potentially effective vaccine might erroneously be ruled out because of the failure a therapeutic vaccine trial. However, if preclinical work has indicated that an established infection can be curbed, moving to a preventive vaccine may be a rational option.

**Question 4: Do you think that Therapeutic Vaccines could lead to a Functional Cure?**

**BE & AC**

Felipe Garcia was the first to show a significant, although transient, control of viremia by the use of a DC-based personalized therapeutic vaccine [30]. This was the first step towards a functional cure, i.e., a condition of long-term remission without need of therapy. We took a different approach by intensifying cART with the Tat vaccine [22-24]. Apart from the amelioration of several immune parameters (most noticeable were the restoration of CD4 T cell and B cell numbers beyond the cART-induced level, and the restoration of central memory CD4 and CD8 T cells accompanied by a concomitant decrease of effector cells) we also observed an increased decay of proviral DNA.

DNA decay started late (3 years) after vaccination, but continued to decline 8 years after vaccination, as indicated by the increasing number of volunteers (30 out of 92, i.e., 33%) with undetectable (<2 copies/μg) proviral DNA; the proportion that was even higher for vaccinees who had received 30 μg Tat three times (12 out of 26, i.e., 46%), the most effective of the four regimens compared in the trial. To our knowledge this is unprecedented and very promising. It should be stressed that cART was never interrupted and may have contributed to viremia control and DNA decay. This is intriguing, because it would suggest that exit from latency may not be necessary to reduce the virus reservoir and that residual ongoing replication is key to replenish it. Of course, these results need confirmation and it should be emphasized that we only examined the blood, whereas virus reservoirs might be present elsewhere in the body.

Of importance, the immunogenicity and safety of this B-clade Tat vaccine was confirmed in a subsequent phase II study (ISS T-003, ClinTrials.gov NCT01513135) conducted in cART-treated South African adult volunteers, that is in a population with a different genetic background and mainly infected with a C clade virus [24]. Strikingly, B clade Tat induced anti-Tat antibodies with a kinetics and titers that cross-recognized Tat from different clades (A, B, C, D) and cross-neutralized the Tat-mediated entry of both oligomeric B- and C-clade envelope in dendritic cells. This is a novel assay we set up upon the discovery that Tat binds oligomeric Env and enters dendritic cells through a Tat-mediated pathway involving Tat binding to integrins [58]. Anti-Tat antibody titers correlated positively with neutralization. Tat vaccination increased CD4+ T-cell numbers, particularly when baseline levels were still low after years of therapy, and this had a positive correlation with HIV neutralization. Interestingly, in some patients non-compliant to cART (n=24), vaccination contained viral load rebound and maintained CD4+ T-cell counts above study entry levels as compared to placebo, suggesting that Tat vaccine intensification of cART may indeed counterbalance and hopefully abrogate, the consequences for reduced adherence to treatment, including selection and transmission of cART resistant strains.

Another issue that we believe is very important is the ample cooperation, which we deemed as necessary and actually build up with the South African public authorities, to make the vaccine development programme a successful one. The agreement, signed in 2007 by the Directorate General for Development Co-operation (DGCS) of the Ministry of Foreign Affairs of Italy and the National Department of Health (NDOH) of SA, included, in fact, several components, all considered important to provide the South African government with instruments for undertaking preventive and therapeutic vaccine programs. In particular, the bilateral program foresaw three components: i) to support the development and/or the strengthening of a network of clinical sites and laboratories located in the area of intervention capable of providing quality health care, particularly in the HIV/AIDS sector; ii) to support the development of a GMP line of production to manufacture vaccines in South Africa; and iii) the conduct of a Phase II therapeutic clinical trial with the Tat vaccine developed at ISS. All the three objectives were met, and both the United Nations Industrial Development Organization (UNIDO) and a Panel of Experts convened by the National Department of Health (NDOH) evaluated as highly relevant the program outcomes, advocating completion of vaccine clinical development and registration. To this end, a Private Public Partnership (PPP) is being established in SA.

Thus, we think it is ethically important not only to evaluate vaccine candidates in the countries that need them the most, but also to undertake all those initiatives that render the countries independent with respect to vaccine production and conduction of vaccination campaigns.

**MA**

The complete clearing of HIV from infected subjects has been a “chimera” pursued for the past 30 years. The existence of small numbers of elite controllers and long term non-progressors among HIV–infected individuals gave rise to the hope that it may be possible to uncover which biological features or biomarkers in these subjects allowed them to evade or overcome the development of AIDS. It seems that these individuals were able to improve the immune response sufficiently to control the infection without drug treatment, thereby avoiding the problems linked to prolonged antiretroviral treatment. The limited success of therapeutic vaccine trials obtained in recent years has given rise to the hope that it might become feasible to replace ART treatment with therapeutic vaccines.

**J-MA**

When attempting to develop therapeutic vaccines, vaccinologists face the problem that the presence of large number of HIV mutants in every patient is unlikely to allow the immune system to produce the variety of neutralizing antibodies required for completely blocking virus multiplication. This seemed to exclude the possibility that a classical immune response based on neutralizing antibodies could be achieved that would entirely stop viral replication in infected patients. On the other hand, if it proved possible to induce an immune response towards the unmutated HIV epitopes present in T/ F viruses, it is conceivable that it might be possible to prevent HIV transmission from one patient to another or to interfere with the spread of the infection in individual subjects.

Among the promising current strategies tested in clinical trials, the Tat vaccine has not yet been shown to be able to completely stop viral replication in the absence of antiviral therapy, although there is evidence that it could increase the number of CD4+ T-cells and could slowly decrease the number of HIV DNA reservoir cells [22-24]. An alternative approach aims at reinforcing the capacity of dendritic cells to activate CTLs specific for Gag epitopes. We were partially successful with this strategy in 2002-2004 [29,59] and other groups are currently pursuing this approach that could perhaps be improved [30].
Another, alternative strategy, similar to the one we used with Chinese macaques, would be to suppress virus-specific CD4+ T cell activation with the objective of suppressing viral replication [10,60]. At the moment, a therapeutic vaccine composed of inactivated HIV-1 associated with Lactobacillus rhamnosus has been prepared and will be tested in 2017 with a group of 20 patients undergoing antiviral therapy. Three months after oral vaccination, the antiviral treatment will be interrupted and the ability of the vaccine to suppress viral replication will be determined.

FG

It seems unlikely that a therapeutic vaccine could be completely successful on its own, and a functional cure may require the combination of several different approaches. Numerous pathogenic issues need to be considered such as the possible inability of the immune environment (because of inflammation and Tregs) to generate an effective immunity, the expansion of pre-existing clones targeting escape variants, ineffective DC antigen presentation and the existence of B cell follicle sanctuaries that would allow persistent, productive virus infection.

GG

Therapeutic vaccines could lead to a functional cure by impacting on viral replication with an immune response that assists in the containment or eradication of latent viral reservoirs. HIV vaccination in combination with other agents that seek to stimulate the resting/latent virus may assist in functional cure. Immune based therapy may be a promising strategy in this respect.

ES

The results of Lu et al. [60] indicate that viremia in SIV-infected macaques that are depleted of HLA E restricted CD8 cells, are controlled when the CD8 cells return. Furthermore, Hansen et al. [9] have demonstrated that a complete eradication is possible after induction of HLA E restricted CD8 cells.

Vaccines that induce HLA E restricted CD8 cells, therefore, hold great promise both for a functional cure and as part of “shock and kill” protocols aimed at eradication.

Question 5: If you had the Authority to do it, What Vaccine Concepts would you Support for Testing?

BE & AC

Despite all our efforts to rationalize vaccine design, it must be accepted that only efficacy trials can demonstrate whether a vaccine is effective. The results of Jean-Marie Andrieu show that a tolerogenic vaccine may possibly be as protective as a sensitizing one, at least in the macaque model, indicating that it is extremely difficult to decide upfront what vaccine to go for. Rather than selecting a priori vaccine candidate on the basis of fashionable design, technical appeal, preliminary in vitro results or preclinical testing, it may be best to use a therapeutic setting in order to facilitate and speed up the clinical testing and comparison of multiple candidates. As Maurice Hilleman used to say, "The only correlate of protection is protection".

MA

Although multiple-epitope based vaccines have not been found to be effective against HIV, it might nevertheless be possible to modulate and manipulate different arms of cellular immune responses (both specific and innate as in the case of NK cells) together with antibody responses, in order to achieve some degree of protection against HIV.

This could be coupled with other interventions that may be able to lead to either the complete maturation of the immune response against HIV or could contribute to other critical features that could make it possible to control virus replication over time.

FG

I think that Tat-based vaccines, personalized vaccines, mRNA and certain coformulations with nanoparticles should be explored. However, such prototypes need to take into account the numerous issues that were discussed in this workshop.

GG

I would support any concept that has been found to be safe, addresses a novel approach with new immunogens or platforms with a different humoral or cellular immune response or correlates of protection elicited, than seen previously. Nonhuman primate challenge data supporting protection with putative correlates of protection that can be evaluated in the human would be advantageous for advancement into clinical efficacy.

MK

Based on the points discussed previously, support should be given to concepts that hold the greatest promise for the development of an effective HIV vaccine, while also abiding by considerations dictated by the need for ethical conduct of research. Such an assessment is largely, although not purely, a scientific matter, and requires that the assumptions and hypotheses underlying any proposed research strategy should be critically examined by scientists free of any potential conflict of interest. The continued pursuit of the unsuccessful structure-based reverse vaccinology approach that was based on unsound immunological hypotheses [7] illustrates the need for critically appraising fashionable strategies even when they are followed by large teams of highly respected scientists.

AR

For the reasons outlined above (questions 1 and 2), novel methods of inactivation and novel approaches for rationally designing an HIV vaccine, focused on the initial stage of HIV infection, should be investigated. Other strategies such as those developed by Barbara Ensoli and Jean-Marie Andrieu are also worth of further support and development since these concepts are backed by preliminary data that require continued investigation.

ES

Given my bias stated under section 1, I would rapidly proceed to an efficacy trial of the DNA prime MVA boost concept as the most rapid way to move the traditional HIV vaccine field forward. In parallel, a vigorous effort should be made to evaluate the new finding that HLA E restricted CD8 T cells are present during HIV infection. HLA E restricted CD8 cells can home in on the most important targets for HIV infection, namely T helper cells in germinal follicles to which HLA E restricted CD8 T cells are present during HIV infection. The results of Lu et al. [60] indicate that viremia in SIV-infected Indian macaques in US colonies which could have severely restricted the expansion of pre-existing clones targeting escape variants, (because of inflammation and Tregs) to generate an effective immunity, to either the complete maturation of the immune response against HIV or could contribute to other critical features that could make it possible to control virus replication over time.

Concluding Remarks

In the vaccine field in general and in the HIV/AIDS vaccine in particular, it seems that the rational design is gaining an increasing consensus and...
it is proposed as the only way to get a vaccine. The substantial failure of most efficacy trials and the moderate protection unexpectedly afforded by the ALVAC-HIV/AIDSVAX vaccine in Thailand has forced the scientific community, which had relied mostly on a rational design and preclinical efficacy results in a nonhuman primate model that mimics but does not reproduce entirely what happens in the human, to reconsider various issues of HIV vaccine development.

Here a few investigators in the field convened and discussed freely what they think is the way to go. The results presented in the workshop indicate that empirical approaches, although driven by the current knowledge of virus host interaction, may be as valuable, if not more, in the run for inventing an effective HIV/AIDS vaccine.

All the participants agreed that the natural history of infection is key to vaccine design, from the choice of the target (Tat, T/F viruses, etc.) to the identification of relevant immune responses to control/eradicate the virus and the discovery of relevant effector and regulatory responses in elite controllers. Recent acquisitions have clearly shown that the selection of viral antigens (biologically relevant viruses or proteins) and of the delivery system (dendritic cells, DNA, MVA, Adenoviruses, CMV vectors, bacterial vectors) are also critical although they could generate unexpected immune responses, underscoring the limitations of our current knowledge. This is illustrated, for example, by the still incompletely understood negative influence of pre-existing immunity to Adenovirus serotype 5 as well as by the newly discovered protective role played by both cytotoxic and regulatory MHC E restricted CD8 T cells in nonhuman primates. It is also apparent that the regimen utilized affects the outcome: the moderate success observed in the RV144 trial was obtained with a prime-booster regimen of two vaccine candidates that had both failed when given singly. The preclinical testing in nonhuman primates appears very controversial, and a reappraisal of what can actually be obtained and relied on in these models is needed. Conceivably, a similar reasoning applies to the novel and improved humanized mouse models, although they were not discussed here, possibly because they are still seldom used.

It also appears that vaccine development is a multidisciplinary task, requiring strict collaborations of investigators with expertise in different fields as well as the involvement of the pharmaceutical industry, always keeping in mind that the vaccine to develop has to be a feasible one, that is, easy to produce, store and deliver to the population.

The speed at which the HIV/AIDS vaccine field is moving forward is sometimes perceived negatively, since promising candidates (for example the DNA prime-MVA boost approach) may be neglected because a promising new vaccine approach has come into the arena. Since the capability of evaluating vaccine efficacy is always limited, a selection must necessarily be made. However, recent data suggest that virus reactivation and reservoir replenishment in the chronic phase occur with modalities similar to those HIV exploits to establish the primary infection. This has led to the proposal that a therapeutic setting might be used to evaluate preventative vaccine candidates, since this would make it possible to compare them at an affordable cost [61,62]. An adaptive design of the trial could further speed up the development process.

Evidence from the therapeutic trials presented at the workshop suggest that a functional cure may be achievable using different strategies by 1) instructing the host to control virus replication without cART, as demonstrated by Felipe Garcia with his DC-based vaccine, 2) suppressing the immune response against HIV as achieved by J-M Andrieu, and 3) attacking the hidden reservoirs of the virus that continuously generate new copies of viral proteins and virions, as shown by the CART intensification observed with the Tat vaccine.

Of all the discussed candidate vaccines, a modified version of the preventative vaccine tested in the Thai trial and a therapeutic Tat vaccine are advancing to phase III trials in South Africa. An intranodal DC-based therapeutic vaccine pulsed with whole inactivated virus will soon enter clinical testing, and a preventative vaccine based on a mixture of T/F viruses that have undergone a chemical inactivation that preserves the Env structure will also be evaluated in nonhuman primates in the near future. We all hope that these novel strategies will lead to an effective vaccine.

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