

HIV-1 Prophylactic Vaccine Trials in Thailand

Punnee Pitisuttithum*

Clinical Infectious Diseases Research Unit, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand

Abstract: The HIV epidemic has resulted in medical, social and economic consequences. There is general agreement that a safe, effective and affordable preventive HIV vaccine is urgently needed to control the epidemic. To date, over 60 phase I/II trials of about 30 candidate vaccines have been conducted worldwide. In 1991, Thailand was selected by WHO, UNAIDS as one of the countries for potential HIV vaccine evaluation sites, and 10 projects with HIV phase I, II and III trials have been conducted since 1994. Strong national commitment, collaboration both at national and international levels together with infrastructure strengthening and capacity building, are very important for success. The vaccine designs pursued included synthetic peptides, recombinant protein and recombinant viral vectors followed by or with boosting doses of recombinant proteins. All phase I/II trials indicated that the candidate vaccines were safe and produced binding and a certain level of neutralizing antibodies. The recombinant vector vaccines produced both humoral and cell-mediated responses. The AIDS-VAX phase III trial conducted in 1999 was the first efficacy trial of HIV vaccine in Thailand that brought valuable information for further HIV vaccine development. Recently, a phase III trial of ALVAC-HIV priming with AIDS-VAX[®] B/E boosting was launched in 2003, and the findings of this trial will be shared with the international community. With committed parties in medical science, government, industry and the community, we hope that we can achieve success in developing a safe and effective HIV vaccine in the near future.

Keywords: HIV, candidate vaccine, clinical trial, binding antibody, neutralizing antibody.

INTRODUCTION

The first cases of Acquired Immune Deficiency Syndrome (AIDS) were identified in the United States of America, in 1981, and AIDS became epidemic since then. Currently, the UNAIDS/WHO global report estimated that 40 million people were living with AIDS at the end of 2003. There were 5 million newly infected people, 3 million deaths due to HIV/AIDS, and about 14,000 people becoming infected per day in 2003. More than 95% of them are in low and middle-income countries (<http://www.unaids.org> on 18/3/04).

There were 4.6–8.2 million people living with HIV/AIDS in South & South east Asia at the end of 2003. In Thailand, the first cases of HIV/AIDS were identified in 1984 and the HIV epidemic has exploded since 1987. It was estimated that about 670,000 were living with HIV/AIDS in 2002, and about 30,000 new infections occur in Thailand each year (UNAIDS/WHO Epidemiological Fact Sheet, 2003, <http://www.unaids.org> on 18/3/04).

Since the Human Immunodeficiency Virus (HIV) epidemic has resulted in very significant medical, social and economic consequences, the need for prevention is clear. Public health strategy for preventing and controlling HIV infection depends on changing human behaviour to reduce IDU (Intravenous Drug Use), numbers of unprotected sexual encounters and sexual partners. Changes in human sexual behavior are possible, but may require intense intervention that may not be practical or affordable in all risk

settings. Therefore, a safe, effective, accessible and affordable vaccine becomes the single most important long-term research goal for the prevention of HIV/AIDS infection [19, 11]

Nowadays, HIV/AIDS vaccine research is considered a global priority in addition to education for behavioral change, care and treatment. To date, over 60 phase I/II trials of 30 candidate vaccines have been conducted worldwide (HIV Vaccine Development Status Report, 2000, <http://www.niaid.nih.gov> on 7/3/04). Although most of the early preclinical investigations and early phase I trials have been conducted in industrialized countries, repeat phase I/II trials are now being conducted in developing countries [13].

Thailand, with its history of participating in the development of other vaccines, such as dengue, is a model of industrialized-developing country collaboration [HIV Vaccine Development Status Report.2000, <http://www.niaid.nih.gov> on 7/3/04]. Thailand is also one of the four countries (Brazil, Rwanda, Uganda, and Thailand) selected in 1991 by the World Health Organization (WHO) as potential HIV vaccine evaluation sites [13] and Phase I/II clinical trials for HIV-1 vaccines have been conducted here since 1994.

NATIONAL PLAN FOR HIV VACCINE RESEARCH AND DEVELOPMENT

Realizing the serious impact of the HIV epidemic, the Royal Thai Government is strongly committed to active participation in the global effort to develop and evaluate HIV vaccine [41] and a comprehensive National Plan for Prevention and Alleviation of HIV/AIDS was formulated. The Thai National Plan for HIV/AIDS Vaccine Development

*Address correspondence to this author at the Clinical Infectious Diseases Research Unit, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand; Tel: (662) 6435599, (662) 6435584; Fax: (662) 6435598; Email: tmppt@mahidol.ac.th

is an operational extension of the National Plan for Prevention and Alleviation of HIV/AIDS.

The first "National Plan for HIV/AIDS Vaccine Development and Evaluation" was approved by the Ministry of Public Health (MOPH) in 1992, and the plan was endorsed by the World Health Organization/Global Programme on AIDS (WHO/GPA) Steering Committee on Vaccine Development, in 1993. The aim of the first plan was to develop a comprehensive, well-coordinated, long-term strategy for evaluation of safety, immunogenicity and efficacy of preventive, therapeutic, and perinatal HIV/AIDS vaccines in Thailand.¹

The first plan served to create consensus among the Thai scientific community in relation to HIV/AIDS vaccine research, build the capacity of Thai scientists and stimulate the conduct of related research. It also helped in creating an enabling environment, which allowed the initiation of the clinical trials of HIV candidate vaccines in Thailand, as well as promoting collaboration and technology transfer of the national and international organizations to evaluate HIV/AIDS vaccines in Thailand in accordance with international standards.

Considering the great achievements of the first plan, 1993-1999, and the rapidly evolving area of HIV/AIDS vaccine research, the MOPH requested the collaboration of the Joint United Nations Program on AIDS (UNAIDS), to revise and update the first plan. In 1999, the new plan "National Plan for HIV/AIDS Vaccine Development (NPAVD, 1999)" was approved by the National AIDS Committee (NAC) and endorsed by UNAIDS.

The objectives of the new plan were:

- 1) To implement a comprehensive strategy aimed at promoting the development, evaluation and future availability of safe, effective and affordable HIV/AIDS vaccine(s) for the people of Thailand and neighbouring countries.
- 2) To promote infrastructure strengthening, training and transfer of knowledge and expertise in technical, managerial and operational areas, to support the long-term involvement of Thailand in HIV vaccine research;
- 3) To foster and coordinate collaboration on HIV/AIDS vaccine research within different situations in Thailand and with international institutions; and,
- 4) To make a contribution to the global effort to develop HIV vaccine(s), with special consideration of other countries in the region.

APPROVAL PROCESS OF HIV/AIDS VACCINE IN THAILAN [www.aidsthai.org on 2/7/04]

The principal investigator, who was the local investigator, and other investigators of the institution prepared a well-developed scientific protocol to assure that Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) were used, and the results of the trial would produce

interpretable and applicable data. The good clinical protocol prepared by the principal investigator and investigators was approved by Institutional Review Boards (IRBs). To carry out the scientific review, proposals and protocols pertaining to the conduct of HIV/AIDS vaccine-related research in Thailand must be submitted to the AIDS Vaccine Coordinating Unit (AVCU) with previous approvals by the respective IRBs.

The AVCU transferred the proposal to the Technical Subcommittee on AIDS Vaccine (TSAV) for evaluation of the scientific merits of the proposal, including the adequacy of the study design and the level of readiness of investigators and institutions in charge of conducting the research. The TSAV also considered how the proposed research contributed to the training, technology improvement and capability strengthening of national institutions.

Before submission for ethical review by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, the TSAV would seek the advice of external national and/or international experts and of the advisory committee of UNAIDS, if necessary. Both the TSAV and the Ethical Review Committee would express their opinions by recommending approval, recommending modification of the proposal, or not recommending the proposal. In the first two instances, the proposal is returned to the principal investigator with comments and recommendations justifying the decisions. The principal investigator can resubmit a modified proposal for a second review.

The ethical review of the clinical protocol is made by the Ethical Review Committee following the recommendation of the TSAV for the scientific merit of the candidate vaccine. The Ethical Review Committee would follow internationally accepted guidelines, such as "International Ethical Guidelines for Biomedical Research involving Human Subjects" prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with WHO and "International Guidelines for Epidemiological Studies". The Ethical Review Committee might seek the advice of the international experts if needed.

As a final step, the proposals and protocols are submitted to the Director General of the Department of Disease Control (DDC) for administrative approval. Acting on the positive recommendations of the TSAV and the Ethical Review Committee, the Director General of the Department of Disease Control would give the final administrative approval for the initiation of the research proposal.

In protocols that include the import of candidate vaccines, the Director General of the Department of Disease Control would seek the advice or agreement of the Secretary General of the Office of Food and Drug Administration (OFDA), who is responsible for the decision. The administrative approval by the Director General of the DDC is transmitted to the principal investigator through the AVCU, with a copy to the TSAV, the Ethical Review Committee, and the OFDA, for appropriate follow-up.

The letter of approval contains qualifying statements in relation to the implementation of the proposed research proposal. The AVCU & TSAV are responsible for ensuring that the research is carried out according to the approved

¹ Thailand National Plan for HIV/AIDS Vaccine Development and Evaluation, 1999, Ministry of Public Health.

proposal and the recommendations from the review committees.

CLINICAL TRIALS IN THAILAND

In 1991, the WHO designated Thailand as one of the important sites for HIV vaccine trials, due in large part to the high incidence rate of HIV infection, coupled with a progressive public health infrastructure and a history of successfully undertaking prophylactic vaccine trials [35]. The major contributor for HIV-vaccine research on capacity building and infrastructure strengthening in Thailand so far has been coming from the Military HIV Research Programme, USA.

Since 1993, a series of projects on clinical trials of HIV vaccines have been carried out in Thailand. To date, there were 10 projects on phase I/II and phase III trials, as shown in Table (1). At least seven different gp120 and gp160 envelope candidates have been evaluated in phase I/II trials and the vaccine designs pursued included synthetic peptides, recombinant proteins, and recombinant viral vectors, followed by, or with, boosting doses of recombinant protein. In the trials, candidate vaccines were evaluated as to whether they were safe and well-tolerated in doses capable of inducing HIV-1 specific immune responses. These immune responses include binding antibodies (Bab), as well as functional antibodies, which have the ability to neutralize virus infectivity, to mediate cell cytotoxicity, to block binding of gp120 to the CD4 molecule, and to initiate fusion of HIV-1 infected cells [13].

Phase I, Safety and Immunogenicity Trial of HIV-1_{MN} Synthetic Peptide Prototype Vaccine [35]

A randomized, double-blind, placebo-controlled Phase-I trial of a prototype human immunodeficiency virus type-1 (HIV-1) synthetic peptide vaccine was conducted in Bangkok, Thailand, from June 1994 to January 1995. It was conducted to evaluate the safety and immunogenicity of the vaccine in a population of healthy adults at low risk of HIV infection, to compare the safety and immunogenicity of the vaccine using the same 0, 1, 6 month schedule previously undertaken in United States trials and to establish essential infrastructure for future HIV vaccine trials in Thailand. Thirty volunteer HIV-negative healthy adults between the ages of 21-48 years, who were determined to be at low risk for HIV infection based on medical history and interview questions, were recruited at the Thai Red Cross Anonymous Clinic for this Phase-I clinical trial [35].

The subjects were randomized into three groups: 12 each in the 100 µg and 500 µg vaccine groups, and 6 in the placebo group. The prototype vaccine used was oligomeric V3-Multiple Antigen Presenting System (V3-MAPS) synthetic peptide candidate vaccine produced by United Biomedical, Inc. (UBI), and the placebo contained an equivalent amount of alum adjuvant and preservative as a vaccine. Immunogenicity parameters measured included binding antibody to the homologous the HIV-1_{MN} peptide, and neutralizing antibody responses to the HIV-1_{MN} laboratory strain.

The vaccine was well-tolerated, without any serious adverse event. It was found that 20 out of 24 subjects who received prototype vaccine seroconverted, with binding titers

Table 1. HIV-1 Prophylactic Vaccine Trials in Thailand

Vaccine	Year	Phase	No. of Participants	Dose interval	Type of Immunity
Peptide V3-MAPS, subtype B	1994	I/II	30 Low risk adults	0, 1, 6 months	Humoral
Subunit (Recombinant Protein)					
gp120/alum, subtype B	1995	I/II	33 Recovering IDU	0, 1, 6, 12 months	Humoral
gp120/alum, MF59, subtype B	1995	I/II	54 Low risk adults	0, 1, 6 months	Humoral
gp120/MF59, subtype E antigen alone or combine with B antigen	1997	I/II	380 Low risk adults	0, 1, 6 months	Humoral
gp120/alum, combined subtype B/E (AIDSVAX B/E)	1998	I/II	92 Recovering IDU/ Low risk adults	0, 1, 6, 12 months	Humoral
rgp120/alum combined subtype B/E (AIDSVAX B/E)	1999	III	2500 High risk IDUs	0, 1, 6, 12, 18, 24, 30 months	Humoral
Recombinant vectors					
ALVAC-HIV Oligo gp160 or gp120 B/E Boost	2000	I/II	130 Low risk adults	0, 1, 3, 6 months	Cell-mediated + Humoral
ALVAC-HIV rgp120 B/E Boost	2000	I/II	125 Low risk adults	0, 1, 3, 6 months	Cell-mediated + Humoral
ALVAC-HIV rgp120 B/E Boost	2003	III	16,000 Low risk adults	0, 4, 12, 24 weeks	Cell-mediated + Humoral
MRKAd5 HIV-1 gag Vaccine	2003	I	82 Healthy adults	0, 4, 26 weeks	Cell-mediated + Humoral

ranging from 1:69 to 1:5,061 by week 29 (4 weeks after the third immunization). Furthermore, HIV-1_{MN} specific neutralizing antibody titers in the range 1:14 to 1:1,294 was detected in 19 out of 20 subjects with detectable HIV-1 specific binding antibody at week 29.

This Phase-I trial of prototype (HIV-1) synthetic peptide demonstrated and confirmed the safety and immunogenicity of the candidate vaccine determined from earlier Phase-I trials in the United States. However induction of very low levels of neutralizing antibodies, i.e. less than 10% of those observed in HIV-infected subjects, suggested that additional epitopes besides the principal neutralizing determinants (PND) were responsible for higher levels of neutralizing antibodies. The results of HIV specific binding and neutralizing antibody responses from the Thai trial, however were quite similar to those conducted in a population from the United States, indicating that the candidate vaccine was capable of being recognized immunogenically by genetically diverse populations [35]. Moreover, this trial demonstrated that HIV vaccine testing could be done in Thailand.

Phase I/II, Evaluation of Safety and Immunogenicity of rgp120/ subtype B in Alum Adjuvant [28]

A Phase I/II study of AIDS_{VAX}[®]_{MN} was initiated in 1995 by using a randomized, double-blind, placebo-controlled design among 33 recovering injecting drug users (IDUs). The study was conducted from February 1995 to June 1996. Volunteers who were HIV-1 antibody negative, had received methadone maintenance for at least 2 months, were between the ages of 20-50 years and passed the study-specific comprehension test, were recruited from one drug treatment clinic operated by Bangkok Metropolitan Administration (BMA). The objectives of the study were to evaluate the feasibility of conducting vaccine trials in this population and to determine the safety and immunogenicity of this candidate vaccine [28].

The vaccine used was a single, highly-purified envelope protein gp120 from the MN strain of HIV-1 adsorbed onto alum, AIDS_{VAX}[®]_{MN}, produced by recombinant DNA technology in Chinese hamster ovary cells. AIDS_{VAX}[®]_{MN}, based on the envelope glycoprotein (gp120) of HIV-1, had been previously shown to induce protective immunity in chimpanzees [3,4]. It was also demonstrated to be safe and immunogenic in Americans at low or moderate risk of HIV-1 infection [5]. The placebo, being alum alone, was identical to the vaccine except that it was free of gp120. Four doses of vaccine (300 µg of MN-rgp120) were given to 22 volunteers and 11 volunteers received placebo at 0, 1, 6, and 12 months.

Measures for safety monitoring, risk counselling and routine HIV-1 testing were conducted with all volunteers at each vaccination visit. Mild reactogenicity was noted, which was similar in both vaccine and placebo recipients. Three ELISA-based assays to measure antibodies that bind to gp120 antigen (MN) (anti-gp120), bind to a peptide consisting of MN V3 loop (anti-V3) and block gp120 binding to the CD4 binding site in the surface of lymphocytes (CD4 blocking), were carried out. Two neutralization-based assays were used to measure antibodies that neutralize T cell-tropic virus (MN) and primary macrophage-tropic viruses [27, 28, 41] (Mucosal

Immunology Laboratory Assays available at www.scharp.org/public/redbook/assays.htm on 5/3/04).

It was found that 91% (20/22) of patients had detectable binding antibodies to rgp120_{MN} at one month after the first dose of vaccine. This rate increased to 100% with an additional injection and remained so until the end of the study (month 18). But anti-V3 and CD4 blocking antibodies did not develop in all recipients until after the third or fourth doses, and the proportion testing positive for anti-V3 declined to 88% at 6 months after the last dose. In contrast, 100% of recipients developed neutralizing antibodies with geometric mean titer (GMT) (log₁₀) of 2.8 after dose-3 (at 6 months). After the 12-month boost, all recipients maintained detectable antibody, with a peak GMT of 3.1, and the antibody level was still detectable with a GMT of 2.1 at 6 months after the last dose, without boosting [28].

Moreover, the neutralizing antibody titer continued to rise after each boost, while the binding antibodies (anti-gp120 and anti-V3) showed little change after the second, third, and fourth immunization [28]. Using the 90% neutralization cutoff, no titer exceeded 1:400 after the second immunization at 1 month. But 6 of 18 volunteers had titers in excess of 1:1000, and 14 of 17 vaccinees had titers over 1:1000, with 5 of these having titers over 1:2000 at the 6-month and 12-month immunization, respectively.

To determine the ability of volunteer serum to neutralize the primary B subtype isolates, post-6-month-injection serum samples and post-12-month-injection serum samples of volunteers were selected and tested for their ability to neutralize the macrophage-tropic, subtype B viruses (301660 and JRCSF isolates) in a peripheral blood mononuclear cell (PBMC) assay. The gp120 sequence of JRCSF is 80% homologous to MN and the sequence of 301660 shares the GP_{GRAF} V3 loop crown sequence with MN [10, 32]. It was found that 4 of 7 vaccinee sera tested positive against 301660 (1:20) and 5 of 7 tested positive against JRCSF (two at 1:10, two at 1:20 and one at 1:70) after 12-month boost. Two vaccinees became infected during the trial with subtype E virus after the second dose and the third dose of immunization, respectively.

This study demonstrated that AIDS_{VAX}[®]_{MN} is safe and highly immunogenic, inducing high titers of binding antibodies (anti-gp120_{MN}) in all recipients after the 6- and 12-month booster doses. It also showed that vaccine-induced antibodies were able to neutralize macrophage tropic (primary) subtype B virus – the same subtype as MN, but these neutralizing antibodies (NAb) declined about 10-fold during the 6 months after the last immunization. According to some previous reports, neutralizing titers against primary isolates were typically 10-to 100-fold lower than titers to T-cell adapted viruses [29]. Some have contended that a vaccine that does not neutralize primary isolates has little chance of inducing protection and did not recommend the advancement of monovalent gp120 vaccines to phase III testing [28].

However the protection that AIDS_{VAX}[®]_{MN} induced was not predicted with the PBMC neutralization assay in an earlier experiment with chimpanzees using PBMC-grown challenge virus to challenge AIDS_{VAX}[®]_{MN}-immunized animals [4]. In that study, it was found that the chimps were protected despite negative PBMC neutralization results, so

the value of the PBMC assay in predicting potential protection from this assay was not known. Another interest was whether the negative result in this assay system, which appears to be quite insensitive, indicates a virus that falls outside the potential protection of a given candidate vaccine [28].

Nevertheless a decision on proceeding with an efficacy trial of this vaccine was made after balancing the potential negative health risk to the volunteers and the potential of this vaccine to induce protection from HIV-1 infection. Since the epidemiological study of Bangkok IDUs in 1995-1996 indicated that currently predominant infecting virus among newly infections were HIV-1 subtype-E² [42], there was concern about using monovalent AIDSVAX[®]_{MN} as the vaccine for a phase III trial in Bangkok. Considering this point, VaxGen produced a bivalent vaccine (AIDSVAX[®] B/E) consisting of antigens from a subtype B virus (MN) and a subtype E virus (A244) [1] and Phase I/II studies of this vaccine were launched in the U.S and Thailand, in 1998.

Phase I/II Trial of HIV_{SF2} gp120/MF59 Vaccine in Seronegative Volunteers [31]

In August 1995, a multicentre, randomized, double-blind, placebo-controlled Phase I/II trial of HIV_{SF2} gp120/MF59 vaccine was conducted to determine the safety and immunogenicity of the recombinant, B clade, HIV envelope protein vaccine [31]. This Chiron vaccine HIV_{SF2} gp120/MF59 had been safely administered and had demonstrated immunogenicity in trials among volunteers in studies conducted in the United States [18, 20, 22]. Since there is significant geographic genetic variation among HIV-1 viral isolates, the evaluation of candidate vaccine for safety or immunogenicity needed to be carried out in different locations and populations.

This Phase I/II trial of HIV_{SF2} gp120/MF59 was conducted at two sites in Thailand, Bangkok and Chiangmai, from August 1995 to November 1996. A total of 52 healthy, HIV-negative volunteers aged 20-50 years, with no high risk behaviour for HIV exposure from the community, were enrolled. All volunteers were counselled regarding HIV risk behaviours prior to enrolment and at each visit after enrolment. Twenty-six subjects enrolled at each site were grouped into two groups: group A received vaccine (50 µg) or placebo at 0, 1, and 4 months, and group B at 0, 1, and 6 months [31].

The vaccine used contained the recombinant subunit HIV_{SF2} gp120 antigen that was derived from the SF2 strain of HIV-1, a B genotype virus. This HIV recombinant protein gp120/SF2 vaccine was produced by Chiron Vaccines in genetically-engineered, Chinese hamster ovary (CHO) cells. The adjuvant, MF59, consisted of an emulsion of polysorbate 80 and sorbitantriolate [20]. The placebo was vehicle material, containing MF59 [31].

Clinical symptoms were recorded and laboratory tests on haematology, blood chemistry, liver and renal function

measurements, CD4 and CD8 cell counts, serum for antibody assays and cells for lymphoproliferation studies were done at regular scheduled visits. An enzyme-linked immunosorbent assay (ELISA) was performed using sera to test for binding antibodies (anti-rgp120/SF2) at six time points: (1) immediately prior to each immunization; (2) one month post-second immunization; and (3) one and 4 months post-third immunization [31]. Neutralizing antibody was measured by using H9 target cells, as previously described with minor modifications [25, 26]. Lymphoproliferative assays (LPA) were performed using cryopreserved PBMC obtained from all subjects at two time points: pre-immunization and 1 month post-third immunization.

There was no significant difference in reactogenicity between vaccine and placebo nor between vaccine schedules (0, 1, and 4 months vs 0, 1, and 6 months) in the occurrence of moderate and severe reactions. Neither the frequency nor the severity of the reactions appeared to increase with succeeding injections. Binding antibody to rgp120 was detected in 37 out of 40 vaccinees after the 2nd immunization. These antibodies persisted in forty-five percent of subjects in the 4-month schedule, and 50% of those in the 6-month schedule at the third injection. Moreover, binding antibody was present in all vaccinees 1 month after the third dose and persisted in 39 of 40 subjects for at least 4 months. Binding antibody titers (geometric mean titers) to rgp120 of subjects in the 6-month regimen were higher than those in the 4-month regimen, reaching significance ($p=0.003$) 4 months after the third injection [31].

There was no significant difference in response of neutralizing antibody between the two immunization schedules. Neutralizing antibody to homologous virus (HIV-1_{SF2}) was present in 97.5% (39/40) of subjects and those antibodies to heterologous virus (HIV-1_{MN}) were present in 55% (22/40) of subjects 1 month after the third immunization. But titers of neutralizing antibodies were lower to the heterologous, than the homologous, virus [31].

Paired LPA responses to the immunogen could be assessed in 43 subjects (35 vaccinees and 8 placebo recipients) and that no positive baseline LPA responses to any form of the immunogen in any of the volunteers were observed. One month after the third immunization, positive stimulating indices (SI) to the native rgp120/SF2 were measured in 54% of 35 vaccinees and those to REDrgp120/SF2 and Env 2,3 were 77 and 80%, respectively. One of eight placebo recipients responded to REDrgp120/SF2 and Env 2,3 at one month post-third immunization and three of 35 vaccinees responded to the control antigen, CHOC, at one month post-third immunization. The difference in the frequency of responders to the different immunization regimens was not statistically significant ($p>0.05$) to any form of immunogen [31].

This clinical vaccine trial demonstrated safety and immunogenicity, and was well tolerated in Thai adults of the candidate vaccine previously demonstrated to be safe in Western developed countries. All vaccinees seroconverted to the immunogen after three doses and more than 90% after the second dose. Based on the results of binding and neutralizing antibody responses, this antigen was planned for testing in combination with a gp120 antigen derived from a

² Kitayaporn D, Vanichseni S, Mastro TD, Choopanya K, Raktham S, Sujarita S, Des Jarlais DC, Wasi C, Subbarao S, Mock P, Heyward WL, Esparza J. (1998). Abstract 13127, XIIth World AIDS Conference, Geneva, Switzerland.

primary isolate, to assess potential broadening of antibody specificity [30].

Phase I/II Trial of Combinations of Recombinant Subtype B (HIV-1_{SF2} gp120) and E (HIV-1_{CM235} gp120) in Healthy Thai Adults [36]

HIV exhibits a remarkable degree of genetic variability and genetic diversity among virus clones within an individual, at about 2-3%. HIV subtypes, also known as clades or genotypes, are designated by letters A through K [34]. These subtypes are approximately equidistantly related, exhibiting 25-35% amino acid sequence difference within their Env proteins, and up to 20% difference within subtypes [17]. In some geographic regions, (e.g., sub-Saharan Africa), multiple subtypes (e.g., A, C, D, G, and O) of HIV 1 are in circulation, whereas in other regions (e.g., United States, Western Europe, Thailand) the representation of genetic subtypes is more restricted, with the majority of infections limited to a single subtype, B or E [2].

By the mid-1990s, it was demonstrated that individuals could be dually infected with viruses from two different subtypes. A prospective study of IDUs in Thailand documented two cases of sequential superinfection with two different subtypes, B and CRF01_AE [17, 37]. Genotype and serotype analysis also showed that strains were largely subtype B in IDU of Bangkok, and subtype E in the larger national heterosexual epidemic [30]. To match the trial vaccine with circulating HIV subtypes of the virus that are common among studied subjects, the safety and immunogenicity of combinations of 2 recombinant HIV-1 gp120 vaccines derived from SF2 (subtype B) and CM235 (CRF01_AE, Thai E) were evaluated among 370 Thai adults, in 1997.

A randomized, double-blind, placebo-controlled, multicentre trial was conducted by TAVEG at 4 collaborating centres (3 in Bangkok and one in Chiangmai). A total of 370 volunteers in the phase II trial were healthy HIV-seronegative adults (aged 20-50 years) from the community who did not have high-risk behaviour for HIV exposure. After counselling regarding HIV risk behaviour and testing for HIV, the volunteers were randomly assigned to 1 to 10 groups receiving various dose combinations of vaccine and placebo [36].

Two vaccines (CM235 gp120 and SF2 gp120) were produced in genetically- engineered CHO cells at Chiron Vaccines. SF2 is a T-cell-line-adapted (TCLA) subtype B, and CM235 is a PBM-derived primary HIV-1 isolate of subtype E. The two vaccines were used with the adjuvant MF59. The 4 collaborating centres evaluated CM235 gp120/MF59 vaccine at 25, 50, and 100 µg alone or combined with SF2 gp120 vaccine at doses of 25 or 50 µg. Doses of 25, 50, and 100µg were chosen for CM235/MF59 based on previous experiments with monovalent SF2/MF59. The placebo contained MF59 adjuvant without immunogen. 92 volunteers at each centre were randomly assigned to receive one of the vaccine combinations or placebo and immunized at months 0, 1, and 6 [36].

Clinical evaluation, haematologic studies, serum chemistry measurements, liver and renal function tests, serum for antibody assays, were obtained during the course

of study. In addition, binding antibody assays, neutralizing antibody assays, and cells for lymphoproliferation studies, were also carried out. Anti-gp120 binding antibody titers were determined by ELISA for each volunteer at 4 time points: baseline and at 1 month after the first, second, and third immunizations. Binding and neutralizing antibody assays were performed as described elsewhere [26, 31, 36]. Lymphoproliferation assays were performed as in a previous study [31].

The immunizations were well tolerated with no serious adverse events related to the vaccines. The most frequently reported local reaction was pain, occurring in 70% (50 µg E antigen/25 µg B antigen and 25 µg E antigen/50 µg B antigen groups) to 89% (100 µg E antigen/50 µg B antigen group) of subjects. Adverse events possibly related to vaccination were found only in the groups receiving both antigens, and included headache [6 subjects (3%)], injection site reaction [11 subjects (6%)] and myalgia [6subjects (3%)]. Eleven subjects experienced serious adverse events, the majority of which were due to motorcycle accidents. None of these were considered to be related to the study vaccines and there were no significant differences in the distributions of reported reactogenicity among the groups.

Vaccination induced binding antibodies to CM235 gp120 or SF2 gp120 or both in all recipients. Binding antibody was observed as early as after the first immunization, increasing to maximum titers after the third immunization. No dose response was observed for CM235gp120, whereas there was a clear increase in SF2 gp120 binding antibody titer with increasing SF2 gp120 dose. Anti-CM235 gp120 BAb titers in the recipients of bivalent (E plus B gp120) and monovalent (E only) groups were similar [36], but immunization with CM235 gp120 (E) alone resulted in BAb that cross-reacted with SF2 gp120 (B) antigen, although the response was augmented >5-fold when SF2 gp120 was included in the immunization regimen.

Before studying for neutralizing antibody to the heterologous T-cell line adapted (TCLA) subtype E strain NP03 (X4) and the homologous TCLA subtype B strain SF2 (X4) from pre- and post-serum samples of 287 vaccine recipients and 80 placebo recipients, screening for neutralizing antibodies (NAb) to NP03 and SF2 was done. It was found that 84% of vaccine recipients neutralized NP03 and 82% neutralized SF2. Two out of 80 (2.5%) placebo recipients had ≥ 50% neutralization of NP03, whereas 6% (5/80) had NAb against SF2. There were no statistically significant differences between dose groups among the recipients of various combinations of gp120 CM235and SF2.

Although the recipients of gp120 CM235 alone did have NAb responses to SF2 (subtype B), these were lower than the responses seen in recipients of bivalent combinations. NAb responses to SF2 were found in 35, 59, and 66% of recipients of the 25, 50 and 100 µg doses of gp120 CM235, respectively, compared with an SF2 response of 82% overall. NAb responses to subtype E NP03 in recipients of the bivalent combinations were equal to or higher than responses to subtype E gp120 alone.

No dose response was observed between the dose of CM235 gp120 and neutralization of NP03. This lack of a dose response between the CM235 immunogen and NAb

against NP03 is similar to that observed in the whole study group. The lack of apparent relationship between the dose of CM235 gp120 and the induction of NAbs detected by MP03 virus may be explained by V3 loop differences of NP03 from the CRF01_AE consensus. The lymphoproliferative responses were equivalent in magnitude and frequency in the assays at both time points, demonstrating that the PBMCs were well cryopreserved and retained their functional ability to respond to both mitogen and recall antigen. It was also found that the percentage of responders to reduced carboxymethylated gp 120 (rcm gp120) SF2 in each of the 3 Thai E dose groups was > 95% after 3 immunizations, whereas that of CM235 was the highest (97%) for the combinations with the lowest dose of Thai E antigen (25 µg) and lower with the higher dose of Thai E antigen (75% for 50 µg and 78% for 100 µg of CM235) [36].

The current trial used a coformulation of the previous SF2 gp120 vaccine with a gp120 derived from the subtype E, R5-tropic primary isolate CM235. Similar to the safety profile of other gp120 and gp160 subunit vaccines [8, 22], gp120 CM235 alone or combined with SF2 gp120 was well tolerated. The reported reactogenicity was similar to that of SF2 gp120/MF59 alone in Thai adults [31] and there were no serious vaccine-related adverse events and no immunizations had to be withheld because of intolerability. The magnitude of the CM235 gp120-induced binding antibody and neutralizing antibody were independent of the presence of SF2 gp120 antigen, suggesting that there was no antigen interference between CM235 and SF2 gp120 [36].

All these data demonstrated that the bivalent combination of gp120 CM235 and SF2 was safe, well tolerated and immunogenic in low-risk, HIV-seronegative Thai adults. Based on these results, further human studies of the combined vaccines, after priming with canarypox-vectored HIV vaccine containing subtype B and E antigens, should be pursued in Thailand, to provide further information on potential antibody specificity, both breadth and magnitude, which would be useful in the context of the planned prime-boost phase 3 trial.

Phase I/II, Evaluation of Safety and Immunogenicity of AIDSVAX B/E Vaccine (VaxGen) in Bangkok³

Before going further to a Phase III trial, in the Phase I/II trial of AIDSVAX[®] B/E, a bivalent subunit vaccine prepared by combining recombinant gp120 from a subtype B virus (HIV-1_{MN}), with gp120 from a subtype E virus (HIV-1_{A244}) in alum adjuvant was conducted. Based on preclinical studies in animals, it was found that simultaneous injection of MN-rgp120 and A244-rgp120 induced antigen-specific immune responses and suggested that HIV-1 envelope glycoproteins could be combined without evidence of antigenic interference [2].

The primary purpose of this Phase I/II study was to evaluate the safety and immunogenicity of three different doses of AIDSVAX[®] B/E vaccine in humans. This study was also designed to provide information regarding the dose dependence, magnitude, specificity, and duration of the antibody responses to each of the two antigens in the bivalent AIDSVAX[®] B/E vaccine. Results of this phase I/II study supported the initiation of a phase III efficacy trial of

AIDSVAX[®] B/E, begun in March 1999 among 2500 IDUs in Bangkok, Thailand.

This study was an open-label trial of the safety and immunogenicity of different dosages of AIDSVAX[®] B/E vaccine, conducted from January 1998 to August 1999. Treatment assignment followed a dose escalation scheme. The subjects were assigned into groups of ten based on the order of enrolment, with the first ten receiving 100 µg, followed subsequently by ten subjects each receiving 300 µg, and 600 µg. Both IDUs and non-IDUs were recruited from five narcotic treatment clinics operated by the Bangkok Metropolitan Administration (BMA), where services including methadone maintenance were delivered to approximately 8,000 drug users per year. The vaccine consisted of 300 µg/ml of subtype B, MN-rgp120/HIV-1 antigen (derived from lab-adapted strain) and 300 µg/ml of subtype E, A244-rgp120/HIV-1 antigen (derived from primary macrophage-tropic virus) per 1 ml of vaccine formulated with alum adjuvant (0.6 mg/ml alum adjuvant). Either one injection of 0.3 or 1.0 ml (100 µg and 300 µg, respectively) or two 1.0 ml intramuscular injections (600 µg of each antigen) in the deltoid region were given at 0, 1, 6, and 12 months.

As in previous studies, volunteers were counselled to reduce their HIV infection risk from unprotected sexual intercourse, while the IDU volunteers were counselled at each visit to use sterile injection equipment and not to share injection equipment. All volunteers were repeatedly informed not to assume any protection from the vaccine.

The immunogenicity parameters measured included MN-rgp120 and A244-rgp120 binding antibody, V2 domain and V3 domain reactive antibodies, and MN-rgp120 and A244-rgp120 spec CD4 blocking antibodies, antibodies able to neutralize the MN strain of HIV-1, and a flow cytometry assay that measured the ability of antibodies to bind to the surfaces of cells infected with the A244 and 92TH009 subtype E strains of HIV-1. The detail of the gp120, V3 domain, CD4 blocking assays, and MN neutralizing assay in MT4 cells have been described previously [2, 23, 33].

Of 92 volunteers enrolled in the study, 31 were in the 100 µg dose group, 31 in the 300 µg group, and 30 in the 600 µg group. Sixty-five percent were healthy individuals with no history of using parenteral drugs. Thirty-five percent reported injection drug use within 6 months prior to enrollment, and all had negative urine opiate tests at enrollment. Three volunteers were discontinued after first immunization because of pregnancy, acute tuberculosis, and loss to follow-up, respectively. One subject chose to discontinue after three immunizations and the fifth one discontinued the study due to death (lung abscess), but received all four immunizations³.

80.4% of the volunteers reported at least one reactogenicity event. The most common symptoms reported were pain and tenderness at the injection site (75%), followed by malaise (25%), myalgia (21.7%), fever (9.8%)

³ Pitisuttithum P, Berman PW, Phonrat B, Suntharasamai P, Raktham S, Srisuwanvilai L, Hirunras K, Kitayaporn D, Kaewkangwal J, Migasena S, Sheppard HW, Li E, Chernow M, Peterson ML, Shibata R, Heyward WL, Francis DP. Phase I/II Study Of A Candidate Vaccine Designed Against The B And E Subtypes Of HIV-1. *Journal of Acquired Immunity Deficiency Syndrome* (2004). In press.

and local erythema (8.7%). All local and systemic reactogenicity symptoms were mild to moderate in nature and volunteers in the 100 µg group reported fever reactogenicity events compared to the 300 µg and 600 µg groups.

90.2% of volunteers reported at least one adverse event during the course of the study, and among them, 19.6% were considered possibly or probably related to the vaccine. None of the reported serious adverse events was attributed to the vaccine. Two HIV-1 infections were reported and thought to be acquired by high-risk behaviours in spite of risk reduction counselling. Molecular sequence analysis indicated that both infections were caused by subtype E viruses. One infected volunteer indicated a history of sharing injection equipment with a HIV-positive friend prior to the third immunization (month 6). The other infection was thought to be acquired by sexual transmission.

The magnitude of the antibody responses to MN-rgp120 and A244-rgp120 ranged from 3.7-4.2 (\log_{10} titers) after the second immunization (at month 1.5) and modestly increased in range from 4.1-4.4 (logs) after the fourth immunization (month 12.5). At 6 months after the last immunization, the mean titer remained above 3.5 logs. It was found that the titers in the 100 µg dose group were significantly lower than the 300 µg and 600 µg dose groups at month 12.5 ($p < 0.001$). After three injections, it was also found that significantly higher ($p=0.0015$) antibody responses to A244-rgp120 were elicited by the bivalent AIDS-VAX[®]B/E vaccine than the AIDS-VAX[®]_{MN} vaccine.

Since the antibody responses to the gp120s from the different subtypes were highly cross reactive, synthetic peptides with sequences unique to each antigen were used to document that both components of the vaccine were immunogenic. It was found that after the third immunization (month 6.5), seroconversion rates in the 100 µg, 300 µg, and 600 µg dose groups to the MN-V2 domain peptide were 40, 80, and 97%, respectively, and the rates to the A244-V2 domain peptides were 97, 100, and 100%, respectively. Conversely, seroconversion rates to the MN-V3 peptide (100% for all three dose groups) were somewhat higher than to the A244-V2 peptide (67, 97, and 100%, respectively).

It was also found that 100% of sera from volunteers immunized with AIDS-VAX[®] B/E developed antibodies able to inhibit the binding of sCD4 to MN-rgp120 and A244-rgp120 after the third injection (month 6.5). The magnitude of the sCD4 blocking response was significantly higher in the 600 µg dose group (64% for MN; 79% for A244) compared with the 300 µg dose group (47% for MN; 64% for A244) after the third injection (month 6.5). Although there was no significant difference between the magnitude of the MN CD4 blocking antibody response when the AIDS-VAX[®] B/E sera was compared with the AIDS-VAX[®]_{MN} sera, the latter were significantly less effective ($p=0.001$) in blocking the binding of A244-rgp120 to CD4.

The presence of virus neutralizing antibodies is thought to be a key correlate of protective immunity, and several studies have shown that *in vitro* neutralizing antibodies correlate with protection from HIV infection in chimpanzees [3, 4, 6, 12, 16] and protection from SHIV infection in macaques [38]. In the study, neutralizing activity could be

detected after two immunizations, but the data revealed that three injections of vaccine were required to achieve high levels (2.8-3.2 \log_{10} titers) of neutralizing activity. Interestingly, the virus neutralizing response did not exhibit dose dependence, as observed in the V2, V3, and CD4 blocking assays. The HIV-1_{MN} neutralizing response did not differ significantly ($P=0.06-0.9$) from the 300 µg or 600 µg dose groups after the second and fourth immunizations.

Again, a flow-cytometry-based assay was used to assess whether the AIDS-VAX[®] B/E vaccine elicited antibodies able to bind to envelope glycoprotein oligomers on the surface of cells infected with primary isolates of HIV-1. It was found that 67% of volunteers developed antibodies reactive with cell surface CM244 gp120 by 2 weeks after the second immunization (month 1.5), and 100% of subjects achieved seroconversion after the third and fourth immunizations (months 6.5 and 12.5). Similarly, 100% of subjects showed seroconversion to 92TH009 gp120 binding at months 6.5 and 12.5.

Based on the results, the study showed that the bivalent vaccine appeared safe and exhibited a safety profile closely resembling previously tested monovalent vaccines. In addition, the HIV envelope glycoproteins from different genetic subtypes can be combined into a bivalent vaccine to expand the breadth of antibody binding to functionally significant epitopes, and immunization with monomeric rgp120 elicits antibodies able to bind to oligomeric gp120 on cells infected with primary isolates of HIV. It can also be concluded that a 300µg dose of each antigen is superior to a 100µg dose, and nearly equivalent to a 600µg dose for eliciting functionally significant antibodies.

This study demonstrated that rgp120s from genetically distinct subtypes can be combined into a bivalent vaccine and that this vaccine expands the breadth of the antibody response to functionally significant epitopes. These results are significant because they represent a potential strategy to overcome the problem of virus variation in HIV vaccine development. The phase I/II trial described in this study provided important data, enabling the advancement of this vaccine to a phase III efficacy trial among 2500 IDUs in Bangkok. This Phase III trial, begun in 1999, would be the first in a developing country.

A Phase III Efficacy Trial of Bivalent B/E rgp120 HIV Vaccine (AIDS-VAX[®] B/E) in Bangkok, Thailand^{4,5,6}

From the previous studies, the vaccine, AIDS-VAXTM, appeared to be safe, highly immunogenic and judging from chimpanzee experiments, protective. What remained to be determined was whether this vaccine was protective in humans, how broadly, across different strains, how this protection will act and how long. To gain insight into these questions, two efficacy trials were conducted; one in North America and one in Thailand[14]. There were some challenges in the preparation and conduct of the Phase III efficacy trial. Epidemiologic feasibility studies on target population and circulating virus, availability of suitable vaccine for the target population and political commitment, including availability of scientists of the host country, were needed⁴.

In March 1999, a Phase III HIV vaccine trial of AIDS-VAX[®] B/E was initiated among IDUs attending drug

treatment clinics operated by the BMA. The primary objective of the trial was to determine whether immunization with AIDSVAX[®] B/E protects injecting drug users from HIV-1 infection, and the secondary objectives were to confirm the safety of AIDSVAX[®] B/E vaccine in high-risk populations, to determine whether prior immunization with AIDSVAX[®] B/E vaccine prevents persistent viremia and reduces the viral load of HIV-infected subjects.

A total of 2545 out of 4943 IDUs were screened and recruited from 17 BMA drug treatment clinics to conduct a randomized, double-blinded and placebo-controlled trial. Recruitment and screening began on March 1999 and enrollment of all IDUs was completed in August 2000. Recruitment methods included TV and radio spots, telephone hotline, posters, fliers, a friend-help-friend referral program and extended clinic hours. Prior to enrollment, eligible participants were required to pass a knowledge test consisting of 20 true-false questions about the study design, vaccine, informed consent etc.

The vaccine used was 300 µg MN rgp120 combined with 300 µg A244 rgp120 in alum adjuvant and the placebo was alum adjuvant alone⁵. Following informed consent, HIV-seronegative IDUs who met the eligibility criteria were randomized to receive AIDSVAX[®] B/E (300 µg of each antigen) or placebo at months 0, 1, and 6, with booster doses at months 12, 18, 24, and 30. The ratio of vaccine to placebo recipients was 1:1. All participants were followed for 3 years to detect a minimum 30% efficacy⁶.

There will be primary and secondary endpoints to evaluate the efficacy of AIDSVAXTM. The primary endpoint will be evidence of infection, measured by enzyme-linked immunosorbent assay (ELISA) and immunoblot. The secondary end points will be the extent and duration of viremia, measured by quantitative RNA polymerase chain reaction (PCR) [14].

In addition to analysis of all 2546 randomized initially HIV-uninfected subjects, two subsets of the cohort were also considered in this study. These were the ITT cohort, defined as randomized subjects who were confirmed to be HIV-uninfected on the date of entry, and the weighted immunized (WI) cohort, defined as the subset of participants in the ITT cohort who received months 0, 1, and 6 immunizations and were not diagnosed with HIV infection within 8 months of the month 0 immunization. Vaccine efficacy was defined as the percent reduction in the hazard rate of HIV infection diagnosis during the 36 months after entry follow-up period 6.

The most common reactogenicity event reported was pain and tenderness in both vaccine (48.8%) and placebo (46%) recipients. Serious adverse effects were found in 16.6% (211/1272) of placebo recipients and 15.9% (203/1274) of vaccine recipients. None of these effects were attributable to the vaccine. Immunization compliance was >97% and follow-up rate was 90%.

⁴ Punnee Pitisuttithum, Presentation on Practical Policies and Challenges in HIV Vaccine Research and Development. 2002. Barcelona Conference, Spain.

⁵ Pitisuttithum P. Presentation on Efficacy on AIDSVAX[®] B/E Vaccine in Injecting Drug Users. CROI Meeting, San Francisco, California, USA.

⁶ Pitisuttithum P, The Bangkok Vaccine Evaluation Group. Thailand's success in moving to the first phase III HIV vaccine efficacy trial in a developing country. Program and abstracts of AIDS Vaccine 2001; September 5-8, 2001; Philadelphia, Pennsylvania. Abstract S11. Available at: <http://www.aidsvaccine2001.org/Pages-/Abstract/S11.htm> on 16/4/04

Of 2546 randomized subjects, 2527 were in the ITT cohort and 2360 subjects were in the WI cohort. In the ITT cohort, 106 of 1267 (8.4%) vaccine and 105 of 1260 (8.3%) placebo participants became infected with HIV-1 during the trial. In the WI cohort, 86 of 1193 (7.2%) vaccine and 79 of 1167 (6.8%) placebo participants were infected during the trial. The annualized HIV-1 incidence rate was 3.4 per 100 person-years, 95% confidence interval (CI) 2.8–4.1, for both arms. For the ITT cohort, the unadjusted estimated overall vaccine efficacy to prevent HIV infection was 0.1%, 95% CI -30.8% to 23.8% and $p = 0.99$ and that for the WI cohort was -7.5%, 95% CI -45.9% to 20.8%, $p = 0.64$.

The phase III trial in Thailand was well-conducted, in accordance with international good clinical practice. The vaccine candidate appeared to be well-tolerated, with no serious adverse events related to the vaccine, and the vaccine could not cause HIV infection. But the vaccine did not show efficacy for either primary or secondary endpoints (VaxGen announces results of its phase III HIV trial in Thailand: vaccine fails to meet endpoints, <http://www.vaxgen.com> on 20/4/04). There was also no difference in disease progression as measured by viral load or CD4 T cell counts.

The results seemed to confirm the findings of the companion trial conducted by Vaxgen in North America. In that trial, more than 5000 volunteers consisted of men who had sex with men and women who were at high risk of infection because they had a partner who was infected, had multiple partners or traded sex for drugs or money. They received a similar vaccine, AIDSVAX B/B, which targeted strain subtypes B: MN and GNE8, common in North America. At the end of the 3-year study, 5.8% of those who received placebo and 5.7% of those who were vaccinated became infected, a difference that was not statistically significant. (Michael McCarthy. AIDS vaccine fails in Thailand, <http://www.vaxgen.com> on 20/4/04).

Phase I/II Trial of Aventis Pasteur Live Recombinant ALVAC-HIV (vCP1521) Priming with either Oligomeric gp160 or Chiron Vaccine HIV Thai E (CM235) gp120 + SF2 gp120 Boosting in Thai HIV-seronegative Adults⁷

In an effort to induce both cell-mediated and humoral responses, attention has turned to evaluating a combination vaccine approach in which two types of vaccines were used and most commonly referred to as “prime-boost”. This involved an immunization (priming) with a recombinant viral vector followed by or combined with boosting doses of recombinant protein (HIV Vaccine Development Status Report, May 2000, <http://www.niaid.nih.gov/daids/vaccine> on 18/3/04).

In February 2000, the first phase I/II trial of live-recombinant ALVAC-HIV (vCP1521), priming with either gp160 or gp120 boosting, was launched among Thai HIV-seronegative adults. The objective of the study was to evaluate the safety and tolerability of two prime-boost HIV vaccine combinations in HIV-negative, healthy Thai adults.

⁷ Suriyanon V, Gurunathan S, Thongcharoen P, Khamboonruang C, Miland S, Kim J, Brown AE. Safety and Tolerability of live Recombinant- ALVAC/HIV (vCP1521) Priming with Either gp160 or gp120 Boosting in Thai seronegative.

⁶ International Congress on AIDS in ASIA and the PACIFIC; October 5-10, 2001; Melbourne, Australia.

ALVAC-HIV (vCP1521) was a recombinant canarypox vector vaccine developed by Aventis Pasteur, and it contained a clade E rather than clade B envelope gene; this envelope was from the primary isolate 92TH023. The study was divided into two phases: Phase I was open-labeled for acute safety and Phase II was double-blind. Participants in each phase were divided into two groups (groups 1 and 2) and there were 5 participants in each phase I group.

Subjects in group-1 received live-recombinant ALVAC-HIV (vCP1521) vaccine and those in group-2 received 50 µg of gp160 TH023/LAI-DID at months 0, 1, 3, and 6. There were 45 participants in each group (groups 3 and 4) in phase II. Subjects in both groups 3 and 4 were given ALVAC-HIV (vCP1512) at months 0, 1, 3, and 6. For group 3, at month 3 and 6, ALVAC-HIV (vCP1521) vaccine was administered with 50 µg of gp160 TH023/LAI-DID, and for group 4, with (100/50 µg) of Thai E (CM235) gp120 + SF2 gp120. Neutralizing antibody assay, cytotoxic T-lymphocytes (CTL) responses and enzyme-linked immunospot (ELISPOT) assays were conducted to evaluate antigen-specific responses in HIV-1-vaccinated individuals.

The frequency of local reaction was not significantly different between groups 3 and 4, and was comparable to those reported in another study [9]. Sera from vaccinees in 2 trials of canarypox ALVAC-HIV E prime, followed by either bivalent gp120 B/E or oligomeric gp160 E boost, were tested for HIV-1 neutralizing antibodies 2-4 weeks after completion of vaccination. It was found that only volunteers in the groups who received subunit boosts had neutralizing antibodies. Ninety percent of vaccinees responded to E viruses in the boosted group and the greatest cross-reactivity to both B/E was found in group 4. However, the mean 50% neutralizing titers of subtype E viruses were approximately 10-fold lower in vaccinees compared to the titers of subtype-E-positive patients.

CTL assays were conducted at baseline, and at 1.5, 3.5, 6.5, 7, 9, and 12 months. Freshly-isolated peripheral blood mononuclear cells were stimulated *in vitro* with clade E and B antigens and used as effectors in a standard chromium release assay with autologous EBV-transformed B cells infected with recombinant vaccinia virus expressing clade E *env* and clade B *gag/pro* antigens as targets. From the study, it was found that a total of 37 subjects had CTL activity, of whom 11 demonstrated repeated CTL responses. Most of the initial CTL responses were seen following the third injection, but continued to be seen as late as 3 months after the last injection. 89% of CTL responses were CD-8 mediated, with 4 subjects demonstrating bulk CTL, and 6 subjects demonstrating CD-8-mediated CTL only. Most CTL activity (68%) was to HIV-*env* antigen and cross-clade CTL HIV-*gag* activity was observed in PBMC from 27% (3/11) of participants.

ELISPOT assay was used to examine interferon gamma (IFN- γ) releases from T-cells of individuals enrolled in the trial. Freshly isolated PBMC were stimulated *in vitro* with HIV clade E or B antigens and used as effectors in a standard chromium release assay, or PBMC were incubated with a pool of overlapping HIV clade B and E *env* or *gag* peptides, and the release of interferon gamma (IFN- γ) was measured in an ELISPOT assay. A cutoff of >20 responding IFN- γ secreting cells/10⁶ PBMC and at least a "two-fold" increase

over background was considered a positive response. The study showed that 5% of Thai volunteers demonstrated a CD8-mediated ELISPOT response. Most IFN- γ were CD4-mediated and CD8-mediated IFN- γ responses were due to *env* and *gag*. Further studies to validate whether ELISPOT is a suitable test for CTL were necessary.

Phase I/II Trial of Live Recombinant ALVAC-HIV (vCP1521) Priming with AIDSVAX[®] B/E Boosting in HIV-negative Participants⁸

A Phase I/II trial of ALVAC-HIV (vCP1521) priming with AIDSVAX[®] B/E boosting was conducted in the year 2000 to assess the safety and immunogenicity of ALVAC-HIV (vCP1521) priming with two doses of AIDSVAX[®] B/E boost in HIV-negative Thai adults. The prime vaccine used was a recombinant canarypox vector vaccine, ALVAC-HIV (vCP1521) that had been genetically engineered to express subtype E HIV-1 gp120 (92TH023) linked to the transmembrane anchoring portion of gp41 (strain LAI), and HIV-1 *gag*, and protease (LAI strain). The booster vaccine was AIDSVAX[®] B/E, a bivalent HIV-1 vaccine of recombinant gp120 from MN (subtype B) and A244 (subtype E).

The study was a randomized, double-blinded, placebo-controlled trial and 123 volunteers were enrolled into the study. Participants were divided into two groups based on dose of booster vaccine, i.e., 200 µg and 600 µg, and were randomized into vaccine or placebo at a ratio of 3:1. For group one, 45 subjects were given ALVAC-HIV (vCP1512) at months 0, 1, 3, and 6. At months 3 and 6, ALVAC-HIV (vCP1521) vaccine was administered with 200 µg of bivalent AIDSVAX[®] B/E gp120 (100 µg for each B and E gp120 antigen). Fifteen other subjects received placebo injections. In group-2, 45 subjects were given ALVAC-HIV at months 0, 1, 3, and 6. At months 3 and 6, ALVAC-HIV was administered with 600 µg of AIDSVAX[®] B/E. Fifteen other subjects received placebo injections. Participants were followed for 6 months after completion of immunization. Blood was collected per schedule for testing for cellular antibodies (CTL and LPA) and humoral antibodies (neutralizing and binding antibodies).

The most common reactogenicity was pain in both groups of vaccine and placebo recipients. Most of the observed reactogenicity was mild to moderate. CTL is defined as positive if the HIV-1 antigen expressing targets relative to control have a specific lysis $\geq 10\%$. CD8 + CTL occurs when lytic activity decreases by 50% after removal of CD8 cells, while removal of CD4 cells maintains specific lysis of at least 5%. An analogous rule is applied for CD4 + CTL (Mucosal Immunology Laboratory Assays available at www.scharp.org/public/redbook/assays.htm on 10/3/04). It was found that 23% (20/88) of all vaccine recipients and no placebo recipients showed CTL responses after the last immunization⁸.

Lymphoproliferation assays were performed as in previous studies and positivity was defined as

⁸ Nitayaphan S, Pitisuttithum P, Karnasuta C, Eamsila C, Souza MD, Morgan P, Polonis V, Benenson M, Vancott T, Kim SR, Kim J, Thapinta D, Garner R, Bussaratid V, Singharaj P, Habib RE, Guranathan S, Heyward W, Birx D, McNeil J, Brown AE. Safety and Immunogenicity of an HIV Subtype B and E Prime-Boost Vaccine Combination in HIV-negative Thai Adults. *Journal of Infectious Diseases* (2004). In press.

lymphoproliferative stimulating index (LSI) ≥ 5 and paired LPA responses to the immunogen were assessed at pre-immunization and one month following completion of immunization. Among recipients of the 200 μg dose of AIDSVAX[®] B/E boost, 67% of vaccine recipients had lymphocyte proliferation to gp120 CM244 and 60% had lymphocyte proliferation to gp120 MN. Among recipients of the 600 μg dose of AIDSVAX[®] B/E boost, lymphocyte proliferation to gp120 CM244 was found in 58% of vaccine recipients and to gp120 MN in 60% recipients.

Binding antibody was measured at one month after last immunization and positivity was defined as antibody reactivity at serum dilution $\geq 1:50$. Among the recipients of the 200 μg dose of AIDSVAX[®] B/E boost, 85% of recipients had binding antibody response to gp120 A244 and 95% had antibody response to gp120 MN. The binding antibody response among recipients of the 600 μg dose of AIDSVAX[®] B/E boost to gp120 A244 and gp120 MN were 96 and 100% of vaccine recipients, respectively. Among recipients of the 600 μg dose of AIDSVAX[®] B/E boost, 71 and 98% of vaccine recipient volunteers respectively, had neutralizing antibody to subtype B and E TCLA HIV strain, whereas 47 and 100% of the recipients of 200 μg dose of AIDSVAX[®] B/E boost had neutralizing antibody to subtype B and E.

Prime-boost vaccination with ALVAC-HIV (vCP1521) and AIDSVAX[®] B/E appeared to be safe and well-tolerated in Thai adults. This vaccine combination induced both cellular and humoral HIV-specific immune responses. It was an appropriate candidate for advancement to phase III evaluation, and this vaccine combination using higher dose of AIDSVAX[®] B/E is being advanced to phase III testing in Thailand, in October 2003.

Phase III Trial of Live Recombinant ALVAC-HIV (vCP1521) Priming with AIDSVAX[®] B/E Boosting in HIV-uninfected Thai Adults⁹.

This was the second phase III trial in Thailand, with an innovative and promising immunization concept and general population approach. The priming vaccine was live-recombinant ALVAC-HIV (vCP1521) and AIDSVAX[®] B/E from VaxGen was used for boosting. The primary objective of the trial was to determine whether immunization with an integrated combination of ALVAC-HIV (vCP1521) boosted by AIDSVAX gp120 B/E could prevent HIV infection in healthy Thai volunteers.

The study was a community-based, randomized (1:1), multicentre, double-blinded, placebo-controlled clinical trial, which started in October, 2003. With a drop-out rate of about 5% per 6-month period, 16,000 volunteers were required to enrol within the 1-year enrolment period. Endpoints of the study were HIV infection by serologic and nucleic acid testing and CD4 quantitation for volunteers who had HIV infection during the trial, and genetic characterization of infective viruses for comparison with vaccine antigens.

The volunteers recruited were Thai nationals, 20-30 years, old from Chonburi and Rayong provinces who were HIV-negative, had no systemic disease or immunodeficiency syndrome, passed the test of understanding and were available for 3.5 years' participation. Volunteers will be randomized into vaccine and placebo groups, with 8,000 subjects in each group. Among the vaccine group, ALVAC-HIV (vCP1521) will be given to the volunteers at weeks 0, 4, 12, and 24. At weeks 12 and 24, ALVAC-HIV (vCP1521) will be administered with AIDSVAX B/E. Follow-up duration will be 3 years, and counselling, assessment of risk behaviour, and test for HIV infection, will be carried out every 6 months. The trial started in September 2003, and approximately 3,000 participants are currently enrolled.

Since it is a community-based trial, community participation and engagement is important. To obtain community engagement, informal and formal consultative meetings with NGOs, participation of NGOs in consent review and counselling training and information dissemination to the public and the community are being carried out. For management of inter-current infection, counselling about disease and transmission, referral to MOPH facilities for HIV care and medical treatment under national guidelines are also provided.

Although this trial is the second phase III trial in Thailand, it is the world's first efficacy trial of a HIV prime-boost vaccine combination. The predominance and relative narrow diversity of HIV subtype E in Thailand provide an optimal setting to test this concept, utilizing vaccines derived from Thai primary isolates. An update of this phase III trial will be shared with the international community.

A Worldwide Phase I Dose-Escalating Study of the Safety, Tolerability and Immunogenicity of a Three-Dose Regimen of the MRKAd5 HIV-1 gag Vaccine in Healthy Adults¹⁰.

Human adenovirus types 4, 5, and 7 offer several potential advantages as vectors for live recombinant vaccines. They can be administered orally, in the form of gelatin-coated tablets from which the virus is released in the intestine, or intranasally, and they can induce both systemic and mucosal immunity. Recombinant adenoviruses expressing the *env* or *gag* antigens from HIV-1 or SIV have been tested in animals and shown to induce long-lasting protective immunity in chimpanzees [7, 18, 25]. More recently, defective adenovirus type 5 (Ad5) vectors have been developed that bear a deletion of the E1 gene, which can be complemented using a packaging cell line that supplies the E1 gene product in trans [17].

An Ad5 recombinant expressing HIV-1 gag was found successfully to induce cellular immune responses in rhesus macaques and to attenuate infection and mitigate disease progression after challenge with a pathogenic SHIV [39]. A phase I clinical study combining a DNA vaccine for priming, and an Ad5 recombinant vaccine for boosting, is underway [17].

⁹ Pitisuttithum P, Rerks-Ngarm S, Wiriyaakijja W, Wattana S, Worathanarat T, Bebyasuvann T, Benenson M, Brown AE. Challenges and Approach to Conducting HIV Vaccine Trial in the Community. AIDS Vaccine 2004 International Conference.

¹⁰ http://www.ihv.org/clinical_trials/HVTN050.html 12/07/04

A worldwide phase I dose-escalating study of the safety, tolerability and immunogenicity of a three-dose regimen of the MRKAd5 HIV-1 gag vaccine in healthy adults was launched in Thailand, in November 2003. 12 volunteers were recruited and vaccines will be given at 0, 4, and 26 weeks. The findings from this study will elucidate whether the vaccine is safe and immunogenic in humans especially who have background antibody to adeno virus.

Another vaccine trial involving fowl-pox-based vaccine is being proposed now by HIV Netherlands Australian Thai Collaboration. The plan is to move to a phase I trial soon.

CONCLUSIONS

To achieve the goal of providing a safe and effective HIV vaccine for those in need, industry, investors/funders, clinical and research partners and collaboration of both public and private institutions are needed [15]. With the strong national commitment to HIV vaccine development and with multi-partner collaboration, Thailand has successfully conducted more than 7 phase I and II HIV vaccine trials of peptides, subunit vaccines and recombinant vector vaccines. The results of these trials showed that these vaccines were safe and immunogenic. Successful implementation of the trial is assured by the feasibility of conducting clinical trials, including a large-scale one, the ability to recruit and follow the volunteers and community support. Recruitment from the community in phase I/II can be successfully achieved by booth presentations, focus group presentations, press releases by radio and television, through universities and monks' colleges, from friend, to friend and through community leaders. Demographic and motivational variables were most important in predicting retention. Altruistic or mixed altruistic with non-altruistic motives were associated with greater retention. Furthermore, close relationships with providers and volunteers, providing intermittent meetings, frequent reminders and thank you parties can also result in 100% retention.

The first efficacy trial of HIV vaccine in a developing country using AIDS VAX[®] B/E was started in 1999, and ended in 2003. Although these phase III trial results showed no efficacy of the vaccine, it did not mean that the trial 'failed'. The trial showed that the IDU population can be educated, like other healthy individuals. Recruitment and follow-up of 2500 IDUs was possible in spite of 16 visits during the 3-year follow-up. Proper education, counselling and sincerity in trying to solve volunteers' problems are very important in building trust between volunteers and staff. Thorough education, sincerity and care can lead to high levels of understanding, high retention rates and compliance. The other key to this success depends on the talents and dedication of clinic staff. In summary, the trial could be conducted according to the highest international clinical standards in a developing country.

All the abovementioned studies were designed to test the concept of humoral immunity alone. As knowledge advances, there is growing evidence that an efficacious HIV-1 vaccine should be able to stimulate both cell-mediated and humoral immunity. So, another phase III trial of prime-boost recombinant vector vaccine was launched in September 2003. The challenge is community engagement, since

16,000 participants are going to be enrolled from Eastern Seaboard province communities.

Several candidate vaccines based on different concepts, are at different stages in the HIV vaccine development pipeline [13]. To date, eleven subtypes of HIV-1 have been identified and one of the major challenges in HIV vaccine development is to develop one or multiple vaccines effective against all major HIV subtypes. More trials will be needed to develop effective vaccines, particularly against the most prevalent subtypes, which are having a devastating impact on populations in Asia and Africa.

The vaccine research effort is of compelling public health importance and continued HIV vaccine research remains an urgent global need. We hope that vaccine trials in Thailand can provide a safe, effective and affordable HIV vaccine that will be of advantage to all people in the world, especially people in developing countries, who are the most severely stricken by the AIDS pandemic.

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LIST OF ABBREVIATIONS

AIDS	=	Acquired Immune Deficiency Syndrome
AIDS VAX [®] B/E	=	A bivalent vaccine consisting of antigens from a subtype B virus (MN) and a subtype E virus (A244)
AIDS VAX [®] MN	=	Purified envelope protein gp120 from the MN strain of HIV-1 adsorbed onto alum
ART	=	Antiretroviral therapy
AVCU	=	AIDS Vaccine Coordinating Unit
Bab	=	Binding antibodies
BMA	=	Bangkok Metropolitan Administration
CHO	=	Chinese hamster ovary
CIOMS	=	Council for International Organizations of Medical Sciences
CTL	=	Cytotoxic T-cell Lymphocytes
DDC	=	Department of Disease Control
DSMB	=	Data and safety monitoring board
ELISA	=	Enzyme linked immunosorbant assay
ELIS POT	=	Enzyme-linked immunospot
GCP	=	Good Clinical Practice
GLP	=	Good Laboratory Practice
GMT	=	Geometric mean titer
HIV	=	Human Immunodeficiency Virus
IFN-g	=	Interferon gamma
IRBs	=	Institutional Review Boards
IDUs	=	Intravenous Drug Users

IVDU = Intravenous drug use
 LPA = Lymphoproliferative assays
 LSI = Lymphoproliferative stimulating index
 MOPH = Ministry of Public Health (Thailand)
 NAb = Neutralizing antibody
 NAC = National AIDS Committee
 NGO = Non-government organization
 NPAVD = National Plan for HIV/AIDS Vaccine Development
 OFDA = Office of Food and Drug Administration
 PCR = RNA polymerase chain reaction
 PBMC = Peripheral Blood Mononuclear Cell
 PND = Principal neutralizing determinants
 SI = Stimulating Index
 TCLA = T-Cell line adapted
 TSAV = Technical Subcommittee on AIDS Vaccine
 UBI = United Biomedical, Inc.
 UNAIDS = Joint United Nations Program on AIDS
 V3-MPS = V3- Multiple Antigen Presenting System
 WHO = World Health Organization
 WI = Weighted immunized

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