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International Clinical Trials of HIV Vaccines: I. Phase I Trial of an HIV-1 Synthetic Peptide Vaccine in Bangkok, Thailand

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SUMMARY **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, to evaluate the safety and immunogenicity of the vaccine in a population of healthy adults at low risk for HIV infection, and to establish essential infrastructure for future HIV vaccine trials in Thailand. Thirty volunteers (25 males; 5 females) were recruited and randomized into 3 groups, receiving 3 intramuscular injections of either 100 μg vaccine (N=12) or 500 μg vaccine (N=12) or alum placebo (N=6) on weeks 0, 4 and 25. The vaccine was well tolerated without any serious adverse effects. HIV-1 specific ELISA responses were detected in 20/24 subjects who received the vaccine, with V3 binding antibody titers ranging from 1:69 to 1:5,041. HIV-1 (MN) specific neutralizing antibody was detected in 19/20 of subjects with detectable HIV-1 specific binding antibody. Neutralization titers ranged from 1:14 to 1:1,294, which were less than titers observed in HIV-infected subjects. The results of this study indicate that the vaccine was well tolerated, and that the vaccine stimulated anti-HIV humoral immune responses in Thai subjects. The successful undertaking of this first HIV vaccine trial conducted in Thailand provided important preparatory information surrounding volunteer recruitment and motivations, and paves the way for future trials of HIV vaccines in Thailand.**

Currently, the World Health Organization estimates that 18.5 million persons worldwide are infected with HIV, with more than 40 million persons likely to become infected by the year 2000.[1] Following a similar pattern to the spread of HIV in Africa during the late 1970's and early 1980's, Asia is now confronting a massive epidemic of HIV, making the development and delivery of effective HIV vaccines an urgent public health priority. In this context, Thailand has been designated by the World Health Organization as an important site for HIV vaccine trials, due in large part to the high incidence rates of HIV infection coupled with a progressive public health infrastructure and a history of successfully undertaking prophylactic vaccine trials.[2]

The HIV epidemic in Thailand began in the 1980's with the introduction of the virus through homosexual men and intravenous drug users, and later through commercial sex workers.[3] The prevalence of HIV in Thailand has increased significantly during recent years, and multiple subtypes of HIV are currently circulating in the population, including several variants of the predominant clades E and B.[4] The recent introduction of clade C viruses to the "Golden Triangle" area of Asia further supports the need for the development of HIV vaccines capable of protecting against variable subtypes of HIV.[5]

The development of a safe and effective vaccine for prevention of HIV and AIDS requires that not only the

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scientific challenges of designing an appropriate immunogen capable of providing long-term protective immunity against HIV be addressed, but that the requisite clinical trial infrastructure for HIV vaccine trials be established in different geographic locales in order to prepare for large-scale international trials to establish the level of vaccine efficacy.[6]

As a first step toward the development of a multicomponent synthetic HIV vaccine, a prototype monovalent synthetic peptide vaccine based on the HIV-1 clade B, MN sequence which overlaps a portion of the HIV-1 principal neutralizing determinant (PND) of the V3 loop,[7,8] has been utilized to initiate the clinical infrastructure evaluation of multiple international sites[9] while simultaneously obtaining important safety and immunogenicity determinations of the vaccine's performance in diverse population groups. Preclinical studies with this immunogen demonstrated its capacity to stimulate significant levels of HIV specific neutralizing antibodies[7,8] and Phase I trials conducted in the United States established that the vaccine was well tolerated and capable of stimulating HIV-specific humoral and cell-mediated immune responses in the majority of vaccinated subjects.[10]

The purpose of the present study in Bangkok, Thailand was to continue the evaluation of the prototype HIV vaccine, in a group of highly educated and motivated volunteers at low risk of HIV infection and to compare the safety and immunogenicity profile of the vaccine using the same 0, 1, 6 month schedule previously undertaken in the United States trials. In addition, since this was the first HIV vaccine trial to be carried out in Thailand, an equally significant goal of the study was to evaluate the process for conducting HIV vaccine trials in the Thai population and to facilitate the preparations and infrastructure for accelerating the evaluation of future HIV vaccine trials in Thailand.

METHODS

Subjects

Thirty volunteer subjects were recruited for the Phase I clinical trial. The volunteers were HIV-negative healthy adults between the age of 21-48, who were determined to be at low risk for HIV infection based on medical history and interview questions. Utilizing an age inclusion criteria of 20-50 presented no difficulty in recruiting sufficient numbers of volunteers for the trial. Inclusion and exclusion criteria were similar to previous Phase I trials of HIV vaccines.[11-13] Briefly, inclusion criteria for the trial was based on a normal physical examination, and subjects were qualified based on a complete blood count and differential in the normal range, including hemoglobin, hematocrit, white blood cell count, total lymphocyte count, platelet count, urine dipstick with esterase and nitrite, ALT, creatinine, and negative ELISA for HIV Subjects with active tuberculosis, active malaria, history of immunodeficiency, chronic illness, autoimmune disease or use of immunosuppressive medications were excluded from consideration. Additional exclusion criteria included evidence of psychiatric, medical or substance abuse during the past six months; receipt of a live attenuated vaccine in the past 60 days; use of illicit or experimental agents during the past 30 days; history of anaphylaxis or other serious adverse reactions to vaccines; high risk sexual behavior as determined by interview and questionnaire; history of injecting drug use during the past 12 months; history of recurrent genital ulcer disease, history or evidence of sexually transmitted disease; current or past history of pelvic inflammatory disease or proctitis; positive ELISA for HIV. Pregnant or lactating women were also excluded from participation in the trial.

Subjects were provided detailed background regarding the purpose and design of the study, and gave their written informed consent to participate in the study. The trial was approved by the Human Ethical Committee of Chulalongkorn University Hospital and the National AIDS Committee of Thailand.

Synthetic peptide candidate HIV-1 vaccine

The prototype vaccine was produced by United Biomedical, Inc., Hauppauge, New York, and consisted of eight homologous peptides corresponding to the gp120 PND of HIV-1 MN (amino acid residues 295-325) linked to a heptalysyl core to form a radially branched structure.[7,8] The vaccine was purified and formulated in 0.2% aluminum hydroxide gel in phosphate buffered saline with 0.01% thimerosal included as preservative as previously described.[10]

Vaccination and follow-up

The trial was conducted as a double-blind, placebo controlled study. Subjects were randomized into three groups: 12 each in 100 μg and 500 μg vaccine groups, and 6 in the placebo group. The placebo contained an equivalent amount of alum adjuvant and preservative as the vaccine. The randomization code was maintained at United Biomedical, Inc., and the code was not broken to either the principal investigator or the subjects until the completion of the trial. Vaccinations were administered by

intramuscular injection in the deltoid area at weeks 0, 4, and 25. All subjects were enrolled on the same day, and received their immunizations on the same days (June 6, July 4, and November 28, 1994).

Subjects were observed by clinical staff for one hour post vaccination, and were supplied with a thermometer to record body temperature twice daily for 3 days after each vaccination, and recorded any adverse events for 14 days following each vaccination. On days 1-3 following the first vaccination, and for the first three days following each subsequent vaccination, subjects were contacted by investigators to assess any symptoms reported by the subject. If significant symptoms were reported by the subject, he/she was then evaluated by the principal investigator. Subjects returned for physical examinations and blood drawings at weeks 4, 8, 16, 25, 27, 29, and 33. At each visit, 50 ml of blood was obtained and serum was stored frozen in aliquots for immunogenicity determinations. Final protocol follow-up occurred at week 33.

Counselling

All subjects received counselling throughout the course of the trial. Two registered nurses and a social scientist assisted with the counselling of the subjects. Each volunteer received at a minimum a 15 minute confidential counselling session during each visit. In addition, group counselling discussions were conducted at each visit.

Immunogenicity determinations

Sera were screened by ELISA for binding antibody to the homologous HIV-1 MN peptide octamer as previously described.[10] Sera were screened at 1:31 dilution for the assay, and a positive ELISA was defined by an absorbance value of 0.2 above background. ELISA positive sera were screened in a neutralization assay using the HIV-1 MN laboratory strain, grown in MT-2 cells, using plaque reduction as a read-out.[14] Neutralization titers are reported as the reciprocal of the serum dilution which yields a 50% reduction of plaque counts compared with control sera.

RESULTS

Recruitment and demographics of subjects

Since this trial represented the first clinical trial of an HIV vaccine in Thailand, significant education and communication efforts were undertaken in preparation of the recruitment process. This included several public presentations, media interviews and debates. Following approval of the study by the National AIDS Committee, the recruitment efforts began in earnest at the Thai Red Cross Anonymous Clinic, resulting in 50 potential volunteers registered within a two-week period. An additional 25 volunteers were rejected during the recruitment sessions due to high-risk behaviors, expected poor compliance, or HIV positive status. Following a thorough interview and informed consent process, the 50 potential volunteers were screened to derive the final 30 subjects who participated in the trial. There were no significant differences in demographics among the placebo, low dose and high dose vaccine groups in the trial.

Twenty five (83.3%) of the subjects were male and 5 (16.7%) were female, with ages ranging from 21-48 (mean= 32.5). Twenty five of the subjects were regular blood donors. Levels of education of the subjects ranged from grade 4 to masters degree, with 80% of the subjects continuing education past grade 12. Altruism was a significant reason given by the subjects for willingness to participate in the trial.

Clinical safety evaluations

The vaccine was well tolerated without any serious adverse events. Mild pain following the injection was the most common event, which was occasionally accompanied by mild swelling and redness (Table 1). Fever of 37.5°C was observed in two subjects following the second and third injections. Generalized urticaria occurred in one subject, however, this was not viewed as significant due to the subject's history of intermittent urticaria for several years. There were no clinically signficant changes in blood chemistries, hematological and biochemical assessments noted throughout the course of the trial. Generally, side-effects were mild, self-limited within a few days and had no relation to the vaccine or the vaccine dose.

Due to the extensive media and publicity associated with this clinical trial, psychosocial evaluations of the subjects were conducted by social scientists associated with the clinical trial. No psychosocial adverse events were associated with being a volunteer in the study. Several of the volunteers indicated that participation in the trial was an extremely positive experience for them individually. Collectively, the volunteers participated in giving AIDS education to school children, visiting AIDS patients and AIDS hospice, and giving public seminars on radio and television. AIDS prevention efforts by the volunteers was both a positive psychosocial outcome for the individual volunteers, but also played an important role in promoting the national AIDS prevention agenda in Thailand.

Table 1. Local and systemic clinical reactions

Side effects	Placebo (N = 6)			Vaccine 100 μg (N = 12)			Vaccine 500 μg (N = 12)		
	#1[a]	#2	#3	#1	#2	#3	#1	# 2	#3
[b]1. Pain-mild	2	0	1	3	3	2	1	5	3
-moderate	0	0	1	0	1	0	0	0	0
2. Swelling	0	0	0	1	0	1	0	3	0
3. Redness	0	0	0	0	0	0	1	3	0
4. Itching, local	0	0	0	0	0	0	0	1	0
5. Itching, generalized	0	0	0	0	0	0	1[c]	1[c]	0
6. Fever (>37.5°C)	0	0	0	0	1	1	0	1	1
7. Malaise	0	0	0	0	0	0	1	1	1
8. Myalgia	0	0	0	0	0	0	1	1	0
9. Dizziness	0	0	0	0	1	0	0	1	0
10. Headache & nausea	1	0	0	0	0	0	0	0	0

a #'s indicate 1st, 2nd, and 3rd vaccinations
b Indicates injection site reactions
c The same single patient has had history of intermittent urticaria for several years, not aggravated by vaccination

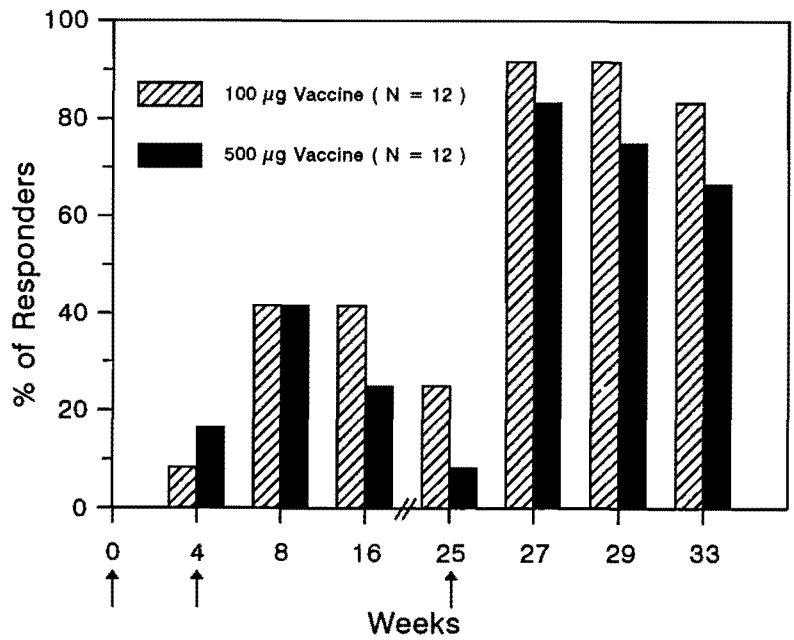

Fig. 1 Binding antibody assay to HIV-1 branched peptide by EIA. See Methods for description of EIA. Figure shows the percent of responders for both the 100 μg and 500 μg groups at various time points after immunization. Sera from placebo recipients were negative by the HIV-1 branched peptide EIA at all time points.

Table 2. ELISA titers to HIV-1 peptide vaccine

	HIV-1 MN V3 Titers[a]						
SUBJECT[b]	WK4	WK8	WK16	WK25	WK27	WK29	WK33
100-1	<31	<31	<31	<31	367	449	123
100-2	<31	<31	<31	<31	389	855	157
100-3	<31	199	327	57	356	494	141
100-4	89	875	1185	283	1044	1813	266
100-5	142	2491	1927	1010	8973	5041	638
100-6	<31	<31	<31	<31	NT[c]	259	77
100-7	<31	66	37	<31	431	280	135
100-8	<31	173	96	<31	914	996	958
100-9	<31	<31	<31	<31	<31	<31	<31
100-10	<31	295	321	171	1280	1265	858
100-11	<31	<31	<31	<31	130	82	66
100-12	<31	<31	<31	<31	273	235	167
GMT[d]	**112**	**337**	**308**	**230**	**621**	**591**	**214**
500-1	<31	<31	<31	<31	46	69	<31
500-2	<31	276	168	<31	383	420	97
500-3	<31	<31	<31	<31	<31	<31	<31
500-4	<31	<31	<31	<31	325	237	106
500-6	<31	160	39	<31	381	346	231
500-6	323	357	352	190	385	310	306
500-7	87	304	90	<31	1724	2055	1063
500-8	<31	<31	<31	<31	<31	<31	<31
500-9	<31	<31	<31	<31	<31	<31	<31
500-10	<31	288	199	55	667	653	536
500-11	<31	<31	<31	<31	167	124	95
500-12	<31	<31	<31	<31	1427	790	1150
GMT[d]	**168**	**268**	**133**	**102**	**394**	**358**	**286**

[a] Methodology for HIV-1 MN V3 titer assay is described in Methods section.
[b] Subject #'s beginning with 100 received 100 μg of vaccine at 0, 4 and 25 weeks.
Subject #'s beginning with 500 received 500 μg of vaccine at 0, 4 and 25 weeks.
[c] Not tested.
[d] Geometric mean titer for those individuals demonstrating a positive antibody responses.

Binding antibody determinations

Fig. 1 describes the kinetics of antibody responses for anti-PND (HIV-MN) antibodies stimulated in subjects receiving the prototype vaccine. Table 2 outlines the individual responses, including geometric mean titers. For reference, HIV-infected seropositive individuals generate anti-PND titers in the range of 1:600 to 1:4,000 (Potts, B.P., personal communication). None of the 6 volunteers in the placebo group elicited any HIV specific antibodies. By week 29 (4 weeks after the third immunization), 20/24 subjects seroconverted, with titers ranging from 1:69 to 1:5,061. No significant differences were observed between the 100 μg and 500 μg groups, either in percent responders or levels of response.

Neutralizing antibody determinations

Serum which scored positive on the HIV-1 PND ELISA was further evaluated for HIV specific neutralizing antibodies (Table 3). HIV-1 (MN) specific neutralizing antibody was detected in 19/20 of subjects with detectable HIV-1 specific binding antibody at week 29. Neutralization titers ranged from 1:14 to 1:1,294. For reference, HIV-infected seropositive subjects generate neutralizing antibodies to HIV-MN with titers in excess of 1:10,000.[14]

Table 3. Vaccine induced neutralizing antibody responses: individual subjects

	Neutralization vs. HIV-1 MN[a]					
SUBJECT[b]	WK4	WK8	WK16	WK27	WK29	WK33
100-1	NT[c]	NT	NT	87	117	35
100-2	NT	NT	NT	132	270	120
100-3	NT	<10	<10	47	130	94
100-4	<10	<10	<10	129	285	270
100-5	<10	<10	<10	1066	522	115
100-6	NT	NT	NT	NT	14	10
100-7	<10	<10	<10	66	55	44
100-8	NT	<10	<10	142	157	42
100-9	NT	NT	NT	NT	NT	NT
100-10	NT	<10	<10	54	112	35
100-11	NT	NT	NT	58	33	18
100-12	NT	NT	NT	119	86	34
GMT[d]				**110**	**108**	**50**
500-1	NT	NT	NT	<10	<10	<10
500-2	NT	<10	<10	856	419	144
500-3	NT	NT	NT	NT	NT	NT
500-4	NT	NT	NT	55	107	55
500-6	NT	<10	<10	90	107	40
500-6	<10	<10	<10	17	16	17
500-7	<10	<10	<10	396	1294	665
500-8	NT	NT	NT	NT	NT	NT
500-9	NT	NT	NT	NT	NT	NT
500-10	NT	<10	24	100	152	72
500-11	NT	NT	NT	28	43	33
500-12	NT	NT	NT	397	551	124
GMT[d]			**24**	**116**	**156**	**75**

[a] Methodology for neutalization assays is described in Methods section.
[b] Subject #'s beginning with 100 received 100 μg of vaccine at 0, 4 and 25 weeks. Subject #'s beginning with 500 received 500 μg of vaccine at 0, 4 and 25 weeks.
[c] Not tested.
[d] Geometric mean titer of the neuralization titers for those individuals demonstrating a positive response.

DISCUSSION

This Phase I trial of a prototype (HIV-1) synthetic peptide demonstrated the safety and immunogenicity of the vaccine in a population of healthy Thai adults at low risk for HIV infection. In addition, the trial served to validate the infrastructure for conducting future AIDS vaccine trials in Thailand. Moreover, the thirty volunteers who participated in the study helped to stimulate additional public awareness on AIDS prevention efforts in Thailand, through extensive media coverage associated with the study.

The clinical trial confirmed the safety and immunogenicity of the candidate vaccine determined from earlier Phase I trials in the United States.[10] Approximately 80% of vaccinated subjects generated binding antibodies to the HIV-PND, at titers within the a fourfold range observed for HIV seropositive subjects, indicating the capacity of branched peptide immunogens to stimulate levels of binding antibody approaching those elicited by natural infection. In addition, 95% of subjects who elicited binding antibodies also produced HIV-specific neutralizing antibodies. In contrast to levels of binding antibody, levels of neutra-

lizing antibodies induced by the prototype vaccine were less than 10% of those observed in HIV-infected subjects, suggesting that additional epitopes besides the PND were responsible for higher levels of neutralizing antibodies in HIV-infected subjects.

Gorse *et al.*[10] demonstrated that 100% of subjects with the HLA B7 allele produced HIV-specific binding antibody following vaccination with this prototype immunogen, compared with 43% of subjects who did not possess this allele. Although this trend was not statistically significant after adjustment for multiple alleles, these data raised concerns that diverse population groups may not respond comparably to a synthetic peptide immunogen. Similar Phase I clinical trials of the prototype vaccine are currently being conducted in the People's Republic of China, Brazil, and Australia, and will offer an opportunity for retrospective analysis of HLA profiles among divergent populations with respect to responsiveness to the synthetic peptide immunogen. However, the present results from the Thai trial indicate that HIV-specific binding and neutralizing antibody responses are quite similar to populations of volunteers from the United States, suggesting that the candidate vaccine is capable of being recognized immunologically by genetically diverse populations.

Recent studies have demonstrated that chimpanzees immunized with vaccines specific to clade B subtypes of HIV and challenged with the clade E subtype are not protected (M. Girard, personal communication). The recent introduction of other subtypes of HIV to Asia demonstrate that an effective AIDS vaccine will need to protect against multiple subtypes of HIV.[6,15-17] The prototype synthetic peptide vaccine evaluated in the current clinical trial was designed for pilot studies aimed at optimizing HIV specific immune responses, based on a single clade B isolate (HIV-1 MN). A second generation candidate vaccine, consisting of 15 peptides covering a broad spectrum of HIV isolates is currently being evaluated in Phase I trials in the United States, with the aim of extending anti-HIV immune responses across the variable clades. Since synthetic peptide vaccines elicit highly specific immune responses directed against a target sequence, it might also be possible to combine peptide immunization with other strategies for HIV vaccines such as DNA immunization to maximize both humoral and cell mediated immune responses against HIV. The goal of these pilot trials is to develop a vaccine with the capability of conferring protective immunity against the globally diverse strains of HIV.

The successful undertaking of this initial Phase I clinical trial in Thailand has important ramifications for accelerating AIDS vaccine trials in Thailand and in other international sites. The vaccine trial also served to complement ongoing AIDS prevention campaigns in Thailand.[18] The process for launching the Phase I trial involved approvals from local and national scientific and ethical committees, and review by the World Health Organization Global Programme on AIDS Steering Committee for Vaccine Development. The demonstration that motivated volunteers can be effectively recruited and followed throughout the course of this vaccine trial has enabled other AIDS vaccine trials to recently be initiated in Thailand (J. Esparza, personal communication), and continues to advance Thailand as a leading site for initiation of future AIDS vaccine efficacy trials. Similar trials should be undertaken in other regions of the world where the incidence of HIV infection coupled with an effective public health infrastructure will enable promising candidate AIDS vaccines to be evaluated worldwide to determine their efficacy in protecting against the highly variable circulating subtypes of HIV.

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