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International Clinical Trials of HIV Vaccines: II. Phase I Trial of an HIV-1 Synthetic Peptide Vaccine Evaluating an Accelerated Immunization Schedule in Yunnan, China

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SUMMARY **A Phase 1, double-blind, placebo controlled trial was conducted in Longchuan County, China, to evaluate the safety and immunogenicity of a prototype HIV-1 synthetic peptide vaccine in a target population at risk for HIV infection, and to establish the infrastructure for future large-scale HIV vaccine efficacy trials. Subjects were randomly assigned to receive 100 μg or 500 μg of vaccine or alum placebo, and were given three injections at an accelerated 0, 1, and 2 month schedule. The vaccine was well tolerated with no significant local or systemic reactions observed in any subjects. Fifty-five percent (100 μg dose) and 64% (500 μg dose) of subjects who received the vaccine produced binding antibody to the immunogen as determined by ELISA. However, HIV-1 (MN) neutralizing antibody was detected in only 23% (3/13) of subjects with detectable HIV-1 specific binding antibody. It was concluded that this prototype HIV-1 synthetic peptide vaccine was well tolerated, safe and immunogenic, and that a 0, 1, 2 month schedule was not as effective in stimulating HIV-1 specific neutralizing antibodies compared with previous trials utilizing a 0, 1, 6 month schedule. Finally, this trial demonstrated that well-designed HIV vaccine trials can be performed at this clinical trials site in Yunnan, China, and that this site should be considered for conducting larger safety, immunogenicity and efficacy trials of candidate HIV vaccines.**

The development of a safe and globally effective HIV vaccine will require the parallel development of candidate vaccines capable of providing long-term protective immunity against multiple worldwide subtypes of HIV and establishing international capabilities for large-scale efficacy trials of the most promising vaccine candidates.[1] It is anticipated that extensive field evaluation of any HIV vaccine candidate will involve populations in developing regions of the world where the HIV epidemic is most severe.[2] In this regard, public health agencies including the World Health Organization[3] and the National Institutes of Health[4] have begun identifying potential international sites for field evaluation of HIV vaccines.

In the USA, most clinical experience in the conduct of HIV vaccine trials has been acquired through the repeated performance of small Phase I and Phase II clinical trials with candidate vaccines at specific centers forming the National Institutes of Allergy and Infectious Diseases (NIAID) AIDS Vaccine Clinical Trials Network.[5] Many developing countries have already recognized that Phase I/II clinical trials of candidate HIV vaccines in their own populations are likely to provide the most relevant information and experience for

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consideration of future large-scale efficacy trials.[6] In preparation for conducting international efficacy trials using a multicomponent HIV vaccine, United Biomedical, Inc. (UBI®) in cooperation with a network of international clinical investigators from Asia, Africa, South America and the United States, has developed a clinical research program to provide the essential experience in the conduct of HIV vaccine trials in the proposed target populations. This program includes the development of methods for recruitment, counselling, informed consent, and clinical practice consistent with the accepted codes of Good Clinical Practice, and to develop the laboratory and clinical infrastructure necessary to perform HIV vaccine clinical trials in less developed regions. These studies build on the existing local, social, political, and clinical infrastructure to develop a locally adapted process applicable to the region and its population.

The prototype HIV-1 vaccine used in the study reported here is a monovalent HIV-1 synthetic peptide vaccine based on the clade B, MN sequence which overlaps a portion of the HIV-1 principal neutralizing determinant (PND) of the V3 loop.[7-9] In preclinical studies, this immunogen demonstrated the capacity to stimulate long-term high titer neutralizing antibodies directed against HIV,[8,9] and mixtures of similar immunogens representing different subtypes of HIV were capable of boosting humoral and cellular immune responses of a recombinant poxvirus vaccine expressing HIV gp160 to sufficient levels to confer protection against subsequent virus challenge.[10] This prototype HIV-1 MN synthetic peptide vaccine has previously been evaluated in the United States, and found to be immunogenic and safe, with no significant adverse events attributable to the vaccine being reported.[11,12] Based on these preliminary findings, this prototype HIV-1 vaccine became the first vaccine endorsed by the WHO Global Programme on AIDS Steering Committee on Vaccine Development for evaluation in developing countries.

The purpose of this present study in China was to extend the evaluation of the prototype HIV-1 synthetic peptide vaccine using an accelerated immunization schedule, and to develop the social, political, and clinical trials infrastructure at the Longchuan site for future HIV vaccine field trials in IDU populations at higher risk for HIV infection in Yunnan, China.

METHODS

Clinical trial site and study population

The study site comprised the town and county of Longchuan, Dehong prefecture, in Yunnan province, China. Longchuan is a rural county of 150,000 residents located adjacent to the border with Myanmar, in what is commonly known as the "Golden Triangle". It is estimated that there are approximately 4,000 drug users in the county, and as a result of this high number, local authorities implemented a comprehensive HIV-1 surveillance program as part of a national surveillance program. This was achieved through the establishment of 29 outpost surveillance centers for counselling and HIV testing of higher risk individuals throughout the province.

Between 1989 and 1992, 88,965 subjects in Yunnan province were screened for HIV-1 infection identifying 774 confirmed HIV-1 infections. Of these, 743 (95%) of all infections were in Dehong prefecture, with the principal risk factor being injecting drug use (82%), followed by prostitution (13.3%). In 1992, of 277 randomly screened injecting drug users (IDU) in Longchuan county, 119 were confirmed HIV-1 positive (43.5%), an increase of 8.2% over a similar survey in 1990.[13] HIV-1 virus isolations from Yunnan, China have included multiple intraclade B isolates (*eg.* Divergent North American and Thai B) and clade C subtypes which reinforces the importance of developing HIV vaccines capable of protecting against variable subtypes of HIV.[14-16]

Study approvals

Approval for the Phase I clinical trial of the UBI candidate HIV-1 vaccine was obtained from the Ministry of Public Health of the People's Republic of China, following review of preclinical data, and preliminary safety and immunogenicity data obtained from Phase 1 studies performed in the USA. Approvals for the clinical trial were also obtained from the governments of the Yunnan province, Dehong prefecture and Longchuan county.

Subjects

The volunteer subjects for this study were drawn from villages located within 20 km of Longchuan town. Subjects were HIV-negative, healthy adults engaging in higher risk behavior for HIV infection as determined by interview questions, who fully understood the purpose and details of the study, and gave their written informed consent. Eligibility for the trial was dependent on results

of physical examination and a health and lifestyle review. Table 1 describes the inclusion and exclusion criteria for the clinical trial. On initial screening, 30 subjects were found eligible for enrollment into the study. This group comprised 26 male and 4 female subjects, with all male subjects known to be IDU and with all four female subjects being sexual partners of four male subjects. The ethnic distribution of the first 30 subjects initially enrolled were as follows: 15 Chinese; 13 Jingpo; and 2 Dai. Due to the withdrawal of 8 Jingpo subjects following trial commencement for personal reasons, an additional 8 Chinese male volunteer subjects were subsequently recruited from a regional voluntary drug rehabilitation center. A total of twenty-nine (29) subjects completed the full three immunization regimen: 12 subjects received the 100 μg dose; 12 subjects received the 500 μg dose; and 5 received placebo. One male IDU subject died on day 25 of the study in a lifestyle related event not attributable to vaccine or vaccination as determined by the Longchuan Department of Public Security, Health and Anti-Epidemic Station.

All subjects were HIV-1 seronegative using a diagnostic kit (UBI HIV-1 EIA) licensed by the U.S. Food and Drug Administration (FDA) and all were Mantoux (PPD) test negative. Three subjects tested positive for antibody to hepatitis C virus, but remained eligible as this does not form one of the exclusion criteria due to the very high prevalence of HCV infection in IDU in this region.[17]

Counselling

All subjects received counseling prior to their enrollment, and throughout the course of the study. Counselling was required to assist the subjects with developing a complete understanding of the study procedures and the possible consequences. Specific counselling was necessary before subjects agreed to HIV antibody testing. Information and education was provided to subjects on risk behavior changes and ways to make and sustain these changes throughout the study. Counselling was made available to subjects at any time during the study.

Synthetic peptide candidate HIV-1 vaccine

The prototype vaccine was synthesized and formulated at United

Table 1 Subject inclusion and exclusion criteria for UBI® HIV-1 MN peptide prototype vaccine clinical trial, China

A. INCLUSION CRITERIA

1. **Age**: 18 and 50
2. **Sex**: Male or female
   A negative pregnancy test at time of entry and assurance that adequate birth control measures for duration of the study were required for all female subjects
3. **Normal history and physical examination**
4. **Negative ELISA for HIV-1**
5. **Availability for 16 weeks of follow-up**
6. **Either a history of injection drug use in the previous 12 months, or sexual behavior of higher risk**

B. SUPPLEMENTAL INCLUSION CRITERIA

1. **Sexually transmitted disease in the previous six months**
2. **Receipt of blood or blood products in the previous six months**
3. **Positive circulating hepatitis B surface antigen**
4. **Positive for hepatitis C virus antibody**

C. EXCLUSION CRITERIA

1. **Active tuberculosis**
2. **History of immunodeficiency, chronic illness, autoimmune disease or use of immunosuppressive medications**
3. **Evidence of psychiatric, or medical problems during the previous six months which the investigator believed would adversely affect the subject's ability to participate in the trial, or other responsibilities which would prevent completion of participation in the study**
4. **Prior receipt of an HIV vaccine**
5. **Pregnant or lactating women**

Biomedical, Inc., and consisted of eight homologous peptides corresponding to the gp120 principal neutralizing domain of HIV-1 MN (amino acid residues 295-325) linked to a heptalysyl core to form a radially branched structure by modification of the Merrifield solid-phase procedure for peptide synthesis.[8,9]

The peptide immunogen was purified to greater than 95% by high pressure liquid chromatography, and formulated using water for injection into a phosphate buffered saline (PBS) solution employing aluminum hydroxide gel as adjuvant at a final concentration of 0.2%. Thimerosal was used as an anti-microbial preservative at an effective concentration of 0.01%. The placebo control consisted of 0.2% aluminum hydroxide gel in PBS with thimerosal (0.01%) included as preservative. All vaccine and placebo doses were supplied in coded, single dose vials, with all local investigators and subjects remaining blinded to vaccine dose and placebo. The treatment code was retained at UBI, Hauppauge, NY, USA and was released to the study site investigators upon completion of the study.

Vaccination and follow-up

All subjects were randomized to one of three study groups, to receive three doses of either 100 μg or 500 μg of study vaccine, or placebo, on a 0, 1, 2 month schedule which was administered by intramuscular injection in a 0.5ml volume. All subjects were vaccinated either at the Longchuan Health and Anti-Epidemic Station in Longchuan town, or at their village for subjects at more remote locations. All subjects were monitored throughout the study for local and systemic adverse reactions, and for HIV infection using the UBI HIV-1 EIA diagnostic test. This ELISA test permits the discrimination between an immune response to naturally-acquired HIV-1 infection from that induced through vaccination,[18] by targeting HIV-1 specific epitopes not included in the prototype vaccine. The ability to distinguish natural infection from seroconversion induced by a candidate HIV vaccine based on viral envelope proteins or peptides, is considered an important prerequisite to the evaluation of candidate HIV vaccines in developing countries.[19]

Subjects were observed for one hour post-vaccination, and vital signs and any reactions were assessed by the clinical staff at the end of this observation period. At 6 hour and 12 hour time points, the subjects recorded their temperatures and any symptoms they experienced in a personal diary provided. Final protocol-follow-up occurred two months after the administration of the final vaccine dose (at 20 weeks) but subjects continued to be monitored as part of the routine community health program in the region.

Immunogenicity determinations

Evaluation of HIV-1 peptide binding and virus neutralizing antibody was determined by the following algorithm. All samples were initially screened by ELISA for binding antibody to the homologous HIV-1 MN PND peptide octamer.[8,9] ELISA responses are presented as the highest serum dilution giving an ELISA absorbance of at least 0.2 plus background. A positive ELISA result was defined by an ELISA absorbance value of 0.2 plus background at a 1:31 dilution. ELISA positive sera were screened in a standard neutralization assay using the HIV-1 MN laboratory strain, grown in MT-2 cells, and using plaque reduction as an indicator of reactivity.[20] Neutralization titers are reported as the reciprocal of the serum dilution which yields a 50% reduction of plaque counts compared with control sera.

RESULTS

Demographic data and clinical safety evaluations

Table 2 summarizes the demographics of the study population. It was necessary to enroll thirty-eight (38) subjects to assure that twenty nine (29) subjects completed the entire immunization sequence. This was due to the voluntary withdrawal of eight Jingpo subjects prior to the second vaccination following the drug-related death of a male Jingpo subject (see Methods). The 29 subjects consisted of 25 males and 4 females, with a mean age of 27.2 years. The ethnic demography of the group was: 23 Chinese, 4 Jingpo, and 2 Dai. The demographic characteristics of the trial were not significantly different among the vaccine groups.

The vaccine was found to be safe, and was well tolerated by the subjects (Table 3). Following vaccination, the majority of subjects exhibited either no symptoms or mild symptoms, with no significant differences among vaccine groups. No severe pain or tenderness was reported at the injection site following the first, second, or third injection. These findings were comparable to those observed in studies previously conducted in the United States.[11,12]

Binding antibody determinations

Post vaccination serum was

Table 2. Demographic characteristics of subjects for Longchuan HIV-1 vaccine clinical trial

	Group 1	Group 2	Group 3	Total
Vaccine	Placebo	100 μg	500 μg	
Number[a]	5	12[b]	12[c]	29
Mean age (yrs.)	24.8	26.3	29.2	27.2
Male	5	11	9	25
Female	0	1	3	4

[a] Indicates the number of subjects who received the full course of immunizations at 0,1,2 months; Note that 9 other subjects received an immunization at Day 0; 8 withdrew for personal reasons before the second immunization; 1 subject died of a suspected IDU related death independent of the vaccine or vaccination on day 25 of the study
[b] One subject withdrew after completing the three immunization regimen for personal reasons
[c] One subject withdrew post third immunization due to a family emergency

Table 3. Local and systemic clinical reactions

	Placebo	100 μg	500 μg
Total number of subjects	8	15	15
I. Visible reactions at injection site			
Post 1st vaccination	0	2	0
Post 2nd vaccination	0	2	1
Post 3rd vaccination	0	1	1
II. Pain and tenderness at injection site			
Post 1st vaccination	1	2	0
Post 2nd vaccination	0	2	1
Post 3rd vaccination	0	1	1
III. Systemic symptoms			
Post 1st vaccination	0	0	1[a]
Post 2nd vaccination	0	0	0
Post 3rd vaccination	0	0	0

[a] Muscle soreness, dizziness, and nausea.

available from 27 of 29 subjects who received all three vaccinations. One subject withdrew from the trial following the complete series of immunizations for personal reasons, and was not available to provide post-vaccination sera for weeks 12, 16, and 20. Another subject withdrew and returned to his home province due to a family emergency and was not available to provide post-vaccination sera on weeks 16 and 20. The HIV-1 PND-specific antibody responses as measured by ELISA are presented in Fig. 1. No HIV-1 antibody responses were detected in any of the subjects who received all three doses of placebo (Group 1). Serum antibody responses were observed in both the 100 μg and 500 μg groups beginning at week 12. By week 16 (eight weeks following the third immunization), 6/11 subjects who received the 100 μg dose were positive by HIV-1 PND specific ELISA (GMT=1:93, range=1:33-1:214) and 7/11 subjects who received the 500 μg dose (GMT=1:74; range=1:37-1:283).

Neutralizing antibody determinations

Serum which scored positive by HIV-1 PND specific ELISA was evaluated for HIV specific neutralizing antibody (Table 4). Three of 13 sera demonstrated detectable HIV specific neutralizing antibody versus the HIV-MN strain, which contains amino acids of the V3 loop of the envelope glycoprotein gp120 homologous to the prototype vaccine. Neutralization titers ranged between 1:20 and 1:100. By comparison, 90% of subjects receiving this immunogen on a 0, 1, 6 month schedule produced neutralizing antibodies with titers ranging from 1:19 to 1:355 in a Phase 1 trial conducted by the NIAID AIDS Vaccine Clinical Trials Network,[12] and 95% of volunteers immunized with the 0, 1, 6 month schedule elicited neutralizing antibodies with titers ranging from 1:14 to 1:1,294 in a trial conducted with the Thai Red Cross Pro-gram in Bangkok, Thailand.[21]

DISCUSSION

This study in Longchuan, China, demonstrated the feasibility of conducting HIV vaccine clinical trials in a developing region, by building on

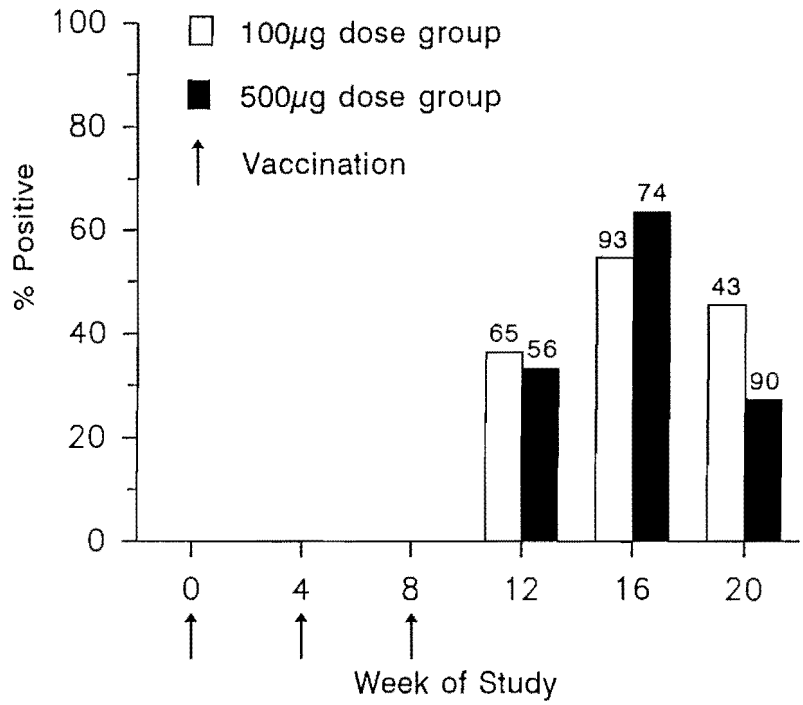

Fig. 1. Binding antibody to HIV-1 V3 branched peptide by EIA. EIA assays conducted as described in Methods. Figure shows the percent samples positive over the number tested for both the 100 μg and 500 μg dose groups. Sera from placebo recipients were negative on the HIV-1 V3 branched peptide EIA at all time points. Values above the bars indicate geometric mean titers (GMT). Range of titers for week 16 samples are shown in Table 4.

Table 4. HIV-1 binding and neutralizing antibody determinations

Dose	Subjects	Anti–V3 ELISA[a]	Neut. Ab-MN[b]
100 μg	11	6/11 (1:33-1:214)	2/6 (1:20-1:100)
500 μg	11	7/11 (1:37-1:283)	1/7 (1:72)

[a] Values indicate number positive/number tested at week 16 of the study (8 week post the third immunization); Range of titers indicated in parentheses.
[b] Values indicate number positive/number tested. Note that only ELISA positive sera were evaluated for neutralizing antibodies. Range of titers indicated in parentheses.

existing social, political and epidemiological/public health services. The region had already established an open HIV surveillance program based on a network of field stations in collaboration with the central and regional governments. In recognition of the severity of the HIV epidemic in the populations of this region, these agencies have implemented local education and counselling programs. However, despite these efforts in AIDS education, HIV continues its insidious spread in this region, reinforcing the need for the development of a safe and effective vaccine. During the five months of preparation from time of approval to implementation of this study, additional education was provided to the local population in order to establish a satisfactory level of understanding of the clinical trial process of HIV vaccine development.

The prototype vaccine evaluated in this study had previously been evaluated in the United States in subject groups at lower and higher risk for HIV infection under an FDA Investigational New Drug (IND) application prior to its evaluation in China. It has also been evaluated in Thailand,[21] Australia[22] and is currently under test in Brazil. In the completed U.S. and Thai studies, the vaccine has been demonstrated to be safe, well tolerated with minimal reactions, and without any systemic adverse events attributable to it. It has been shown to induce both HIV-specific neutralizing antibody and T-helper memory responses.[11,12,21]

The 0, 1, and 2 months vaccination schedule used in this study differed from the 0, 1, and 6 month used in other studies with this UBI® prototype HIV vaccine and commonly used with other subunit HIV-1 vaccine candidates.[23-25] The use of the shorter schedule was intended to provide an opportunity to determine if biologically relevant antibody responses could be induced at an earlier time point than has been observed, since the long vaccination schedules over many months may not be appropriate in the setting of field trials in a developing region with high incidences of HIV infection. The choice of 0, 1, and 2 months was derived from previous successful studies with accelerated schedules with recombinant hepatitis B surface antigen vaccine,[26] and provided an opportunity for a direct comparison with an HIV gp160 vaccine previously evaluated on both a standard and accelerated schedule.[27]

The present study has demonstrated that while HIV-1 specific binding antibody can be induced by this accelerated schedule in approximately 60% of subjects, only 23% of subjects eliciting binding antibody were capable of inducing HIV-1 specific neutralizing antibody. These observations indicate that the accelerated schedule is less effective in stimulating neutralizing antibody than the 0, 1, 6 month schedule utilized in previous trials.[11,12,21] Similarly, studies utilizing an HIV gp160 comparing a 0, 1, 6 month schedule to an accelerated monthly immunization schedule suggested that lengthening of the resting period between the second and third immunizations led to more potent and durable HIV specific immune responses.[25,27]

Finally, the death of a volunteer by a lifestyle related event not attributable to vaccine or vaccination, in this initial HIV vaccine trial in China, provided an opportunity to address the public health infrastructure associated with vaccine trials in a developing country. One male subject belonging to the Jingpo ethnic minority and known to be an active intravenous drug user was found dead, and the provisional diagnosis of sudden unexplained death was noted by the local physician. This diagnosis was a frequent diagnosis of young male drug users within this community, and was confirmed by further inspection as determined by the Longchuan Department of Public Security, Health and Anti-Epidemic Station. Following the drug-related death of the male Jingpo subject, eight additional Jingpo subjects voluntarily withdrew from the trial prior to the second vaccination for personal and religious reasons. After consultation with local representatives conducting the clinical trial, a decision was made to recruit additional subjects to complete the enrollment of the trial.

Incidents unrelated to vaccine or vaccination such as the one described can have a significant impact on the ability to successfully conduct clinical trials, if not handled appropriately and professionally. The Longchuan Department of Public Security, Health and Anti-Epidemic Station, in consultation with clinical associates conducting this Phase 1 trial, handled this incident in a highly effective manner enabling the additional recruitment to occur and completion of the clinical trial as planned.

Although continued assessment of safety and immunogenicity of this prototype vaccine was an important goal of this study, the key accomplishment was the establishment of the infrastructure within China for the meaningful evaluation of next generation candidate HIV vaccines. We have been able to demonstrate that where there is a poli-

tical will to collaborate in a development program targeted at generating a safe and globally effective HIV vaccine, the experience gained is likely to significantly shorten the time frame for development and eventual delivery of the vaccine. In this regard, additional Phase I studies have now been undertaken in multiple sites both in the U.S. and abroad, in preparation for efficacy trials of a final HIV vaccine candidate. While the difficulties in con-ducting studies in less developed regions of the world must not be underestimated, it is important that these challenges are not overstated such that they paralyse the process of continuing HIV vaccine development.[28] When an HIV vaccine has demonstrated in Phase I/II safety and immunogenicity trials the capacity to stimulate both humoral and cellular immune responses in a significant majority of volunteers, including neutralizing antibodies against divergent HIV subtypes, HIV-specific T-helper memory responses, and HIV-specific cytotoxic T cell responses, it should be considered for large-scale efficacy trials. When these product development milestones are achieved and the international clinical sites where variable HIV subtypes are currently circulating have effectively demonstrated the capacity to conduct HIV vaccine trials, a large-scale multinational clinical trial should be undertaken to evaluate the efficacy of the HIV vaccine. The study conducted in Longchuan takes another important step towards the goal of a safe and effective HIV vaccine, and has established the process and feasibility for conducting additional HIV vaccine clinical trials in China.

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