Published in final edited form as:

Vaccine. 2016 November 4; 34(46): 5571–5578. doi:10.1016/j.vaccine.2016.09.041.

A Phase 1, Randomized, Controlled Dose-Escalation Study of EP-1300 Polyepitope DNA Vaccine Against *Plasmodium Falciparum* Malaria Administered via Electroporation

Paul Spearman, MD^{a,*}, Mark Mulligan, MD^b, Evan J. Anderson, MD^{a,b}, Andi L. Shane, MD MPH MSc^{a,c}, Kathy Stephens, RN^a, Theda Gibson^a, Brooke Hartwell, RN^a, Drew Hannaman^d, Nora L. Watson^e, and Karnail Singh, PhD^a

^aDepartment of Pediatrics and Children's Healthcare of Atlanta, Atlanta, GA, USA

^bDepartment of Medicine, Emory University School of Medicine, Atlanta, GA, USA

^cRollins School of Public Health, Emory University School of Medicine, Atlanta, GA, USA

^dIchor Medical Systems, Inc., San Diego, CA, USA

^eEMMES Corporation, Rockville, MD, USA

Abstract

Plasmodium falciparum malaria is one of the leading infectious causes of childhood mortality in Africa. EP-1300 is a polyepitope plasmid DNA vaccine expressing 38 cytotoxic T cell epitopes and 16 helper T cell epitopes derived from *P. falciparum* antigens expressed predominantly in the liver phase of the parasite's life cycle. We performed a phase 1 randomized, placebo-controlled, dose escalation clinical trial of the EP-1300 DNA vaccine administered via electroporation using the TriGrid Delivery System device (Ichor Medical Systems). Although the delivery of the EP-1300 DNA vaccine via electroporation was safe, tolerability was less than that usually observed with standard needle and syringe intramuscular administration. This was primarily due to acute local discomfort at the administration site during electroporation. Despite the use of electroporation, the vaccine was poorly immunogenic. The reasons for the poor immunogenicity of this polyepitope DNA vaccine remain uncertain.

*Corresponding Author: Division of Pediatric Infectious Diseases, 2015 Uppergate, Atlanta, GA 30323; paul.spearman@emory.edu; Fax Number: (404) 727-9223; Telephone Number: (404) 727-5642.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions

PS, MM, DH, designed the study. PS, ALS, KS (RN), TG, BH performed the study. PS, KS (PhD), TG performed the immunologic assays. NLW contributed to the design and conduct of statistical analysis for the study. All authors contributed to the analysis and interpretation of the data. All authors were involved in the production of the manuscript and approved the submission for publication.

Clinical Trials Registration: ClinicalTrials.gov NCT01169077

Conflict of interest statement

DH is an employee of Ichor Medical Systems. All other authors declare no conflicts of interest.

Keywords

Plasmodium falciparum; Malaria; Vaccine; Phase 1; Electroporation; Polyepitope DNA Vaccine

1. Introduction

Malaria is a mosquito-borne disease caused by protozoan parasites of the genus *Plasmodium*. Malaria is a major global health concern, associated with approximately 198 million infections and 584,000 deaths in 2013 [1]. The majority of deaths from malaria occur among children under five years of age in Africa, especially in those areas with poor access to healthcare services. Severe disease occurs most frequently with *P. falciparum*, due to its ability to infect a higher percentage of erythrocytes. Development of a safe and effective malaria vaccine is a major public health goal.

Malaria vaccines targeting a number of distinct steps in the *Plasmodium* lifecycle are being evaluated in preclinical and clinical studies [2]. While the level of functional cellular responses required to confer protection is unknown, humans living in endemic areas develop cellular immune responses to epitopes from these parasite proteins. Cellular immune responses may contribute to protective immunity by reducing the parasite load early after initial infection through recognition of multiple *P. falciparum* antigens. The development of vaccines against *P. falciparum* is complicated by antigenic variation between different isolates. Epitope-based vaccines represent a logical approach to this problem because they can be designed to focus immune responses on conserved epitopes. Vaccines composed of synthetic peptides can be produced when a small number (1-10 peptides) of epitopes are needed. However, presentation of a larger number of epitopes through a mixture of individual epitope peptides is complicated by practical production, formulation, and administration issues. One strategy for presenting a larger number of epitopes is through the expression of epitopes in a multiepitope DNA vaccine. Multiepitope DNA vaccines are undergoing evaluation as a method of eliciting protection against HIV, Hepatitis B, and a variety of other infectious agents including malaria [3-9].

The DNA plasmid vaccine designated EP-1300 was designed for use as a preventive vaccine against malaria caused by infection with *P. falciparum*. EP-1300 targets 4 different antigens in the pre-erythrocytic stage of parasite development that are thought to play a role in the initial establishment of infection. The vaccine was designed to induce cellular immune responses to *P. falciparum* proteins that are expressed predominantly in the liver stage of the malaria parasite life cycle, which occurs immediately after infection at a time when the overall numbers of organisms are limited. The design and development for this vaccine was based on the use of a vaccine immunogen composed of well-defined cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes derived from the circumsporozoite protein (CSP), sporozoite surface protein-2 or thrombospondin-related adhesive protein (SSP2/TRAP), liver-stage antigen-1 (LSA-1) and exported antigen-1 (EXP-1) of *P. falciparum*. A universal HTL epitope, PADRE, was also included as a part of this vaccine. Epitopes were included within a single gene DNA plasmid with epitopes linked using spacers and expressed as a single protein product that is processed in the body into individual epitopes.

Preliminary studies in HLA transgenic mice indicated that EP-1300 was immunogenic, eliciting responses to CTL and helper epitopes, prompting the advancement of this product to clinical trial (data not shown). In these studies, HLA transgenic and nontransgenic mice were immunized with 100 µg of the EP-1300 DNA vaccine in the tibialis anterior muscle, and responses measured using an IFN-γ ELISPOT assay. CTL responses were elicited against approximately 75% of the measurable CTL epitopes in the vaccine using this transgenic mouse model (unpublished data, Pharmexa Inc.).

Although DNA plasmid vaccines are highly immunogenic in many animal models, the induction of immune responses in humans has been largely suboptimal. A limiting factor is the amount of plasmid DNA that can be administered to humans compared to the doses (mg/kg) commonly administered in animal models. Since the total amount of plasmid DNA that can be delivered in any single dose is limited by product solubility, concentration and dose volume, improved methods of DNA vaccine delivery to increase cellular uptake are needed. To address this issue, the EP-1300 DNA vaccine was delivered to healthy volunteers using the process of *in vivo* electroporation to propagate threshold level electrical fields at the site of DNA vaccine administration to improve the effectiveness of DNA delivery. We conducted this Phase 1 randomized, controlled, dose-escalation trial of the EP-1300 polyepitope DNA vaccine against *P. falciparum* malaria administered to healthy volunteers by electroporation to determine the safety, reactogenicity, tolerability, and immunogenicity of this potential malaria vaccine.

2. Methods

2.1. Design and conduct of the clinical trial

Healthy men and non-pregnant women, ages 18 through 40 years of age (inclusive) were recruited to enroll in this prospective, placebo-controlled, single center, partially-blinded, dose-escalating, Phase 1 clinical vaccine trial (Clinical Trials Registration: NCT01169077). The study was conducted at Emory University after review and approval by the Emory University IRB. Inclusion criteria included no prior travel to a malaria-endemic area, no exposure to nor infection with malaria, and no prior participation as a subject in a malaria vaccine study. Subjects with seizures during the 5 years before enrollment, prior severe reactions to vaccination, with bleeding or clotting disorders, psychiatric illness, abnormal baseline clinical safety laboratory tests, reactive hepatitis B surface antigen, reactive hepatitis C antibodies reactive HIV antibodies, muscular/neuromuscular degenerative disorders, and implanted electronic stimulation devices were excluded. Subjects also could not report an acute illness or oral temperature 100.0 degrees Fahrenheit within the 3 days prior to enrollment. Subjects with childbearing capacity were required to have a negative pregnancy test on the date of enrollment and prior to each vaccination.

Subjects that met all enrollment criteria were randomized to vaccine or placebo. Subjects were randomized to receive either 3 doses of EP-1300 vaccine or placebo on Days 0, 28, and 56. Subjects were followed to Day 224 for safety.

The vaccine dose increased between groups from 0.25 mg EP-1300 in group 1 to 1.0 mg EP-1300 (group 2) to 4.0 mg EP-1300 (group 3). In each group, 10 subjects received vaccine

and 3 received placebo (normal saline). Progression to the next sequential group took place only after review of safety data compiled from all subjects from the day 42 timepoint (two weeks following the second DNA or saline control administration).

2.2 Study Product

The EP-1300 Vaccine was manufactured by Pharmexa-Epimmune Inc. (previously Pharmexa, Inc., San Diego, CA). The EP-1300 DNA vaccine encodes a gene composed of the 38 cytotoxic T lymphocyte (CTL) and 15 helper T lymphocyte (HTL) epitopes described in the previous section; a schematic of the DNA vaccine is shown in Figure 1. To identify potential CTL epitopes that were likely to bind to HLA-A1, -A2, -A3, -A24, B7 and -B44 in a supertype manner, the amino acid sequences of *P. falciparum* CSP, SSP2/TRAP, LSA-1 and EXP-1 were scanned for HLA Class I and HLA-DR1 and -DR3 epitope peptide binding motifs. This analysis was conducted using multiple field isolates and only highly conserved epitopes were selected. Epitope conservation was evaluated using sequence information from GenBank and a large number of field isolates for SSP2 (103), CSP (37), EXP1 (24) and LSA1 (27). Based on this analysis it was determined that 30 of the 38 CTL epitopes selected for the EP1300 DNA vaccine were 100% conserved and only 3 epitopes were variable being found in 50% of the sequences analyzed. Similarly, 12 of the 14 HTL vaccine epitopes were found in 89% of the isolates indicating a conserved nature. The majority of the HLA-A2, -A3, -B7 and HLA-DR restricted epitopes used in the EP1300 vaccine are known to be immunogenic in *P. falciparum*-infected subjects or sporozoite-challenged volunteers. To directly measure the immunogenicity of individual epitope peptides and the EP-1300 DNA vaccine studies were completed using HLA transgenic and non-transgenic mice as described above. A complete listing of CTL and HTL epitopes included in EP-1300 with associated references is included in Supplemental Tables 1 and 2. The vaccine design includes the use of selected amino acids inserted between epitopes to enhance processing of the multi-epitope protein into peptide epitopes. A consensus Ig κ signal sequence was fused to the 5' end of the vaccine to facilitate transport of the encoded gene product into the endoplasmic reticulum. The full-length vaccine insert was cloned into the Pst I and Bam HI sites of the pMB75.6 vector backbone. This vector backbone was chosen because of its well-documented history, derivation, and manufacturing capabilities; moreover, this is the same vector used in previous DNA HIV vaccine trials (EP HIV-1090; BB-IND 10445 and EP-1233; BB-IND 13243). Positive clones were identified by PCR screening and one clone confirmed by DNA sequence analysis. The resulting vaccine, EP-1300, is a single plasmid vaccine 6,709 nucleotide pairs (np) in length. EP-1300 was manufactured under cGMP conditions and provided dissolved in phosphate-buffered saline (PBS). Placebo was normal saline.

2.3 Vaccine Administration

Doses of 0.25, 1, and 4 mg, based on DNA content, of EP-1300 were evaluated. The EP-1300 DNA vaccine was administered by electroporation for all groups using the intramuscular TriGrid Delivery System (TDS-IM) device (Ichor Medical Systems, San Diego, CA). Vaccine preparation was performed by an unblinded research pharmacist, and administration was performed by blinded study personnel who had completed the TDS-IM training seminar conducted by Ichor Medical systems personnel. The administration site was

the medial head of the deltoid muscle. A skin fold measurement was performed prior to administration of the study product. The skin fold measurement was used to alter the depth setting on the Application Cartridge based upon the manufacturer's recommendations. Vaccination sites were alternated between the left and right deltoid. Subjects were able to choose initial site. If the subject had a tattoo overlying the proposed vaccination site, an alternative site was selected to ensure that local reaction could be accurately assessed.

2.4 Data Collection

Safety data included both solicited and unsolicited adverse events experienced by healthy adult volunteers after vaccination of EP-1300 DNA vaccine or normal saline placebo. Reactogenicity data were collected during a post-vaccination encounter with the subject and via recorded information in a "memory aid" diary for 7 days following each vaccination. Subjects who experienced ongoing adverse events at day 7 were asked to extend their record through day 14. Hematology, biochemistry and urinalysis results were collected at the screening visit and on study days 14, 28 (vaccination 2), 42, 56 (vaccination 3), and 70. At each vaccination, subjects were asked to respond to a brief vaccine tolerability questionnaire developed by the TDS-IM device manufacturer. Subjective responses to the questionnaire were independent of objective reactogenicity assessments. Following each administration, subjects were asked to rate the level of pain perceived at injection, immediately after electrical stimulation, and at 30 minutes post administration on a six point verbal scale (none, light, uncomfortable, intense, severe, excruciating). In addition, subjects were asked to rate the overall acceptability of the procedure for use in the delivery of a vaccine against a serious disease.

2.5. IFN- γ ELISPOT assay

Peripheral blood mononuclear cells (PBMCs), isolated from peripheral blood drawn in CPT tubes containing sodium citrate on Day 0, 42, and 70, were counted, aliquoted in cryovials at 1×10^7 cells/vial and frozen for later batch processing for all immunologic assays. Cryopreserved cells were assessed for the secretion of IFN- γ in response to stimulation with *P. falciparum* peptide pools. PBMCs were washed with RPMI media (Mediatech, Manassas, VA) supplemented with 10% Human AB serum (Mediatech, Manassas, VA), 1X penicillin/streptomycin (Mediatech, Manassas, VA) 1X glutamax (Gibco, Grand Island, NY), 1X MEM non-essential amino acids (Gibco), 1 mM sodium pyruvate (Mediatech, Manassas, VA) (HR10) containing 50 U/ml of benzonase (Novagen, San Diego, CA) at 300 $\times$ g for 10 minutes followed by two more washings with HR10 without benzonase. Cells were re-suspended in HR10 at a cell density of 2×10^6 cells/ml and incubated overnight in 50 ml tubes in a 37°C, 5% CO₂ incubator. Activated 96 well Elispot plates (Millipore, Cork, IRL) were coated with 0.5 μ g of capture antibody (anti-human IFN- γ mAb-D1K) (Mabtech, Mariemont, OH) by overnight incubation at 4°C. Plates were blocked with HR10 followed by addition of 2×10^5 cells/well in triplicates. Cells were stimulated with the addition of either peptide pools consisting of *P. falciparum* derived peptides (5 μ g/ml of each peptide), individual *P. falciparum* derived peptides or a pan-DR epitope, PADRE (NeoBioSci, Cambridge, MA and Anaspec, Fremont, CA). Each peptide pool contained peptides that bound specifically to only one of the following seven HLA supertypes: HLA-B7, HLA-B44, HLA-A1, HLA-A2, HLA-A3/A11, HLA-A24 and HLA-DR. CEF peptide pool (Mabtech,

Mariemont, OH, 2 µg/ml) or PHA (Sigma, St Louis, MO, 500 ng/ml) served as the positive controls while unstimulated cells or those stimulated with DMSO alone served as the negative controls. Plates were incubated in 37°C, 5% CO₂ incubator for 40 hours. Following washing with PBS/PBS-Tween, plates were incubated with 0.2 µg/100 µl of biotinylated detection antibody (7-B6-1, Mabtech, Mariemont, OH) for 2 hours at room temperature. After washing, streptavidin-HRP (0.2 µg/100 µl, BD Biosciences, San Diego, CA) was added to each well and plates were incubated at room temperature for 1 hour. Plates were washed with PBS-Tween/PBS and reaction developed with 100 µl of AEC substrate mix (BD Biosciences, San Diego, CA) for 5 minutes. Reactions were stopped by rinsing the plates with cold water and plates were completely dried before counting the spots on a CTL-ImmunoSpot analyzer (Shaker Heights, OH). Results were expressed as spot-forming cells (SFCs)/10⁶ live PBMC and a positive ELISPOT response was defined as >50 SFCs/million PBMC. Frequency of positive responses and response distributions were presented by treatment group, study day and peptide/peptide pool. The frequency of subjects in each cohort with at least one positive response was recorded.

2.6. Lymphoproliferation assay

PBMCs from day 0, 42, and 70 were assessed for *P. falciparum* derived peptide-specific activation induced proliferation. PBMCs were washed and cultured overnight. They were then suspended in RPMI media without any supplements at a cell density of 10×10⁶ live cells/ml and stained with 5 µM of CFDA SE (Molecular Probes, Eugene, OR). Following staining, cells were washed and suspended in HR10 at a cell density of 2×10⁶ cells/ml and plated into 96 well tissue culture plates (Corning, Corning, NY) at cell density of 2×10⁵ cells/well. Cells were stimulated either with individual *P. falciparum* derived HLA-DR-specific peptides (10 µg/ml), pan-DR epitope, PADRE (10 µg/ml), candida antigen (Greer Lab, 10 µg/ml) or the superantigen SEB (Toxin Technology, Sarasota, FL, 10 ng/ml). Peptides included in this analysis are outlined in Supplemental Table 5. Unstimulated cells or those stimulated with DMSO alone served as negative controls. Plates were incubated in 37°C, 5% CO₂ incubator for 6 days followed by staining with an antibody cocktail consisting of anti-human CD3-Alexa Fluor 700 (BD Biosciences), CD4-eFluor450 (eBioscience, San Diego, CA) and CD8-APC cy7 (BD Biosciences). Data was acquired on BD LSR-II flow cytometer and analyzed using Flowjo software (Treestar, Ashland, OR). The percentage of CD3+CD4+ and CD3+CD8+ cells in the proliferating gate were calculated.

2.7. CSP ELISA

The CSP repeat sequence NANPNVDPNANP was included as a helper T cell epitope in EP-1300, and potentially could have raised anti-CSP antibody responses. A CSP ELISA was performed to address this. Serum samples from the same immunogenicity time points were obtained from subjects in vaccination groups II and III for the performance of an ELISA for anti-circumsporozoite protein (CSP) antibody response (Geometric Mean Titer and individual log ELISA units). Anti-CSP repeat region antibodies were measured in serum samples acquired on days 0, 42, and 70 using a peptide-based ELISA and a protein-based ELISA, similar to previously published methods [10]. For the CSP protein coating, 10 ng of recombinant CSP protein 3D7 from Reagent Proteins was coated onto wells of Maxisorp

NUNC immunoplates) (Nalge Nunc International) in 100 µl of phosphate buffered saline (PBS). For peptide ELISA, 5 µg of NANP repeat peptide (obtained from V. Udhayakumar, CDC) in 100 µl of PBS was used to coat wells. Plates were blocked for one hour with 5% milk and .05% Tween 20 in PBS. Test sera were diluted 1:100 in blocking buffer and incubated for 1 hour prior to wash steps. HRP-conjugated anti-human IgG and TZMB substrate were utilized for color development and O.D. measured on a ThermoMax microplate reader (Molecular Devices). Positive control sera from malaria-immune patients were used to validate the assay prior to running test samples.

2.8. Statistical Analysis

This study was exploratory, designed to estimate event rates and patterns of immune responses rather than to test formal statistical hypotheses. Estimates were presented with their 95% confidence intervals. Descriptive approaches were used to meet the protocol objectives. Data from the subjects who received a saline placebo in place of the active vaccine served as a comparison to similar data collected from recipients of the active vaccine. The analysis variables consisted of baseline variables, safety variables, immunogenicity variables and laboratory data. Continuous variables were summarized with standard descriptive statistics and categorical variables were described by frequency tables. Primary safety analyses of the data were performed according to the participants' original vaccine assignment (i.e., intention-to-treat analyses) and the inclusion of all data from all participants randomized in the final analysis. Vaccine dose groups were compared for baseline characteristics including demographics and laboratory measurements using descriptive statistics.

Spontaneous adverse reports were coded into MedDRA preferred terms. The number and percentage of participants experiencing each specific adverse experience were tabulated by severity and by relationship to vaccine. For the calculations, each participant's adverse experience was counted once under the maximum severity or the strongest recorded causal relationship for each vaccine dose. Solicited local and systemic reactogenicity reports were analyzed similarly to spontaneously reported adverse events but were listed by specific symptom documented as well as categorized as local or systemic. Additional assessments of product safety included clinical observation by investigators or study staff and monitoring of hematological, chemical, and immunologic parameters.

Participants were monitored for *P. falciparum*-specific responses by IFN- γ ELISPOT assay, lymphoproliferation assay and by measuring antibodies to malarial CSA protein. Analyses were performed by tabulating the frequency of positive responses to each assay by each dose group. Response rates were presented with their corresponding 95% confidence interval estimates. Analyses were performed to evaluate whether responses at 70 days were more frequent (Cochran-Armitage test for linear trend) at the higher doses.

For continuous assay variables, geometric mean and 95% confidence interval or median and interquartile range were reported by dose group and study day. Overall differences between groups at a specific time point were tested by 2-sample t-test or Wilcoxon rank sum test.

3. Results

3.1. Study Participants

Enrollment of 39 subjects in dose escalation Groups 1, 2, and 3 occurred between September 23, 2010 and November 18, 2011 with the last subject visit completed on June 18, 2012. Overall, 17 men and 22 women were enrolled into this study. Baseline demographics are shown in Table 1. The mean age was 28 years (range 18 – 40 years) with the majority self-described as white (27/39, 69%). Subjects were generally well balanced among the different groups and placebo although more males (7/10) were vaccinated in the 1.0 mg vaccine group, and more individuals in the placebo group considered themselves multi-racial. Overall, 37 (95%) subjects completed all 3 scheduled study vaccinations. Two subjects (one subject in the placebo group after vaccine 1, one subject in the 0.25 mg EP-1300/EP after dose 2) choose to discontinue receiving subsequent vaccinations due to local pain at the site of vaccine administration with electroporation. Both subjects remained in the study for safety follow-up. The CONSORT diagram outlining study screening and enrollment details is shown in Figure 2.

3.2. Adverse Events

134 unsolicited adverse events were reported of which 115 (86%) were mild and 19 (14%) were moderate in severity (no severe adverse events were reported). Of these, 29 adverse events (24 mild and 5 moderate) were considered to be associated with the study product. The breakdown of adverse events by study group is provided in Table 2. The most common related adverse events included 14 events attributed to bruising at the vaccination site, and 5 events of redness and swelling observed following aborted procedure applications. Aborted procedures were due to device detection of improper needle deployment prior to electrode array activation. Improper electrode array deployment was detected by the TDS-IM pulse stimulator device, which displayed an error code prior to administration of the electrical pulse and the vaccine. The device in these cases was removed from contact with the participant's skin. In these instances, the vaccine application was then performed on the opposite arm. Notably, the instances of aborted applications occurred early in the study and diminished following further training of nursing staff with the device. Other associated adverse events included a mild elevation in the CPK (2 subjects), myalgia (2 subjects), dizziness (2 subjects), feeling cold (1 subject), influenza-like illness (1 subject), muscle weakness (1 subject), and paresthesia (1 subject). Thirteen associated adverse events were reported by subjects in the 0.25 mg group, 6 in the 1.0 mg group, 4 in the 4.0 mg group, and 6 events in placebo recipients. No serious adverse events were observed during the study. For a listing of the adverse events solicited and graded, refer to Supplemental Table 3 (reactogenicity) and Supplemental Table 4 (laboratory abnormalities).

3.3. Vaccine Reactogenicity

Nine (23%) subjects reported a moderate severity for the measurement of erythema after the first vaccination. Pain of moderate severity was reported by 10/39 (26%) subjects after dose 1; 12/38 (32%) subjects after dose 2; and 14/37 (38%) subjects after dose 3. Tenderness of moderate severity was reported by 7/39 (18%) subjects after dose 1; 9/38 (24%) subjects after dose 2, and by 7/37 (19%) of subjects after dose 3. A single subject (4.0 mg group)

experienced severe (Grade 3) pain and malaise on the day of the first vaccine dose. The pain resolved one day after vaccination and the malaise resolved after 3 days. This subject completed the remaining vaccinations and reported mild to moderate symptoms with subsequent vaccinations. One subject (1.0 mg group) reported an oral temperature to 102° Fahrenheit (Grade 3 severity) on the evening of second dose of vaccine; the subject's oral temperature was within normal range the following day.

3.4. Clinical Laboratory Evaluations

The most frequently abnormal laboratory measure was non-fasting glucose (1 subject in the 0.25 mg group; 3 subjects in the 1.0 mg group; 3 subjects in the 4.0 mg group and 3 subjects in the placebo group). Seven subjects developed mild-moderate anemia during the study (4 in the 0.25 mg group, 3 in the 4.0 mg group including one that was moderate). Mild elevations of the ALT were noted in two subjects in the 1.0 mg group. Grade 3 hypoglycemia was observed in one (4.0 mg dose group) on day 42; the subject's glucose returned to normal on days 56 and 70. No other grade 3 laboratory abnormalities were reported. For a complete explanation of grading for clinical laboratory measures, please refer to Supplemental Table 2.

3.5. Electroporation Tolerability

A total of 114 administrations were performed over the course of the study. At the time of injection, subjects reported their pain as “none” or “light” for 78% of procedure applications, 21% as “uncomfortable” and 1% as “intense”. Immediately after electrical stimulation, subjects reported pain as “none” or “light”, for 13% of administrations, 80% were reported as “uncomfortable” or “intense”, and 7% reported as “severe” or above. At the 30 minute timepoint, subjects reported their pain as “none” or “light” for 61% of procedure applications, 35% as “uncomfortable”, and 3% as “intense” or above. Two subjects (one in the 0.25 mg group and one in the placebo group) reported experiencing “excruciating” discomfort immediately following the electroporation procedure. The subject in the placebo group declined further vaccination but completed follow-up visits for safety evaluation. Despite the discomfort associated with the procedure, subjects indicated that the procedure would be acceptable for delivery of a preventative vaccine against a serious disease following a significant majority of procedure administrations 109/114 (96%).

3.6. Immunogenicity Results

IFN- γ ELISPOT assay was performed on samples obtained on study days 0 (pre-vaccination 1); 42 (14 days post-vaccination 2); and 70 (14 days post-vaccination 3). ELISPOT responses for each subject at each time point were calculated as the geometric mean of the mock-subtracted triplicate values (spot-forming cells (SFC) /million PBMC). Results were derived from duplicate rather than triplicate values for one subject (1.0 mg group) on Day 42, where volume of cells was insufficient for the assay to be performed in triplicate. Analyses excluded the data points that were obtained after a missed vaccination by one subject in the 0.25 mg group on Day 70 and by one subject in the placebo group on Days 42 and 70.

The frequency of positive responses (>50 SFCs/million PBMC) among any peptide pool on Day 0 (pre-vaccination) was 0% in the 0.25 mg group; 30% in the 1.0 mg group; 10% in the 4.0 mg group; and 11% in the placebo group. Positive responses at Day 70 did not change from Day 0 (Figure 3A and 3B). Median ELISPOT responses (SFC/million PBMC) among all peptide pools on Day 0 was 1.1 in the 0.25 mg group; 1.8 in the 1.0 mg group; 1.4 in the 4.0 mg group; and 1.7 in the placebo group. Results were similar on Days 42 and 70 and did not substantially vary by peptide pool (Figure 3). Additional ELISPOT assays and lymphoproliferative assays (LPA) conducted with three individual HLA-DR specific malarial epitopes and a pan-HLA-DR specific epitope did not show any substantial differences in the median ELISPOT response and median LPA response (percent of cells in the proliferating gate) respectively between day 0, day 42 and day 70 (not shown).

Antibody responses to anti-circumsporozoite protein (CSP) (Geometric Mean Titer and individual log OD units) were similar between the EP-1300 groups and did not differ from placebo (Figure 4), while control serum samples from *P. falciparum*-exposed individuals from an unrelated study were highly positive (not shown). Together, these results demonstrate that little or no specific immune responses to malarial antigens were raised by this DNA vaccine.

4. Discussion

This study evaluated the safety, tolerability, and immunogenicity of a multiepitope DNA vaccine designed to elicit cellular immunity against proteins produced predominantly in the pre-erythrocytic phase of *P. falciparum* infection. This was the first-in-humans study of the EP-1300 vaccine, and employed electroporation with the TDSIM device. The vaccine was safe but subjects reported significant discomfort with the administration procedure, most notably at the time of electric field application. However, overall feedback from the subjects on the acceptability of the procedure suggests that it could be used for certain prophylactic indications.

Electroporation is a promising modality for the administration of DNA vaccines. In general, DNA vaccines administered by needle and syringe have been poorly immunogenic, prompting the development of alternative modes of delivery including electroporation [7, 11, 12]. Electroporation has been shown to enhance antigen production and immunogenicity in a variety of animal models and is under evaluation in human phase I clinical trials.

Electroporation was employed in a phase I HIV vaccine study of an HIV clade B and C *gag*, *env*, *pol*, and *nef-tat* DNA plasmid using the same TDS-IM device as was employed in the current trial [13, 14]. These investigators performed a randomized comparison of needle and syringe IM injection of plasmid DNA to electroporation and thus were able to directly evaluate the potential benefit of the technique. Notably, cellular responses to HIV were markedly enhanced by electroporation, with 13- to 70-fold increases in magnitude of responses as measured by IFN- γ ELISPOT assay. A second HIV DNA vaccine trial utilized a different device (CELLECTRA Device, Inovio) to deliver an HIV *gag*, *env*, and *pol* genes together with a plasmid expressing IL-12 [15]. Electroporation in this HIV DNA vaccine trial enhanced immunogenicity as compared with standard intramuscular delivery of the same DNA vaccine in a previous study, with 89% of participants developing measurable

CD4+ or CD8+ measured by ICS following three immunizations. These and a number of other clinical trials have established that electroporation can enhance immune responses to plasmid DNA vaccines in humans (reviewed in [16]).

The success of electroporation in enhancing immunogenicity of plasmid DNA vaccines was not observed in our trial, where essentially no cellular or humoral immune responses were observed. Based on the robust immunogenicity observed in preclinical studies following electroporation-mediated delivery of EP-1300 (unpublished) and other epitope-based DNA vaccine candidates in HLA transgenic mice [17], the failure of the EP-1300 plasmid DNA approach to elicit responses was contrary to expected results. We initially performed IFN- γ ELISPOT assays using pools of peptides representing the *P. falciparum* epitopes within the plasmid vaccine. When these proved to be negative, we re-synthesized the individual peptides representing all of the HLA-DR epitopes in the vaccine, including the universal pan-DR helper epitope PADRE [18], and repeated the ELISPOT assays on all remaining samples. In addition, we developed a CFSE-based lymphoproliferation assay to identify any CD4+ responses. These results were also negative, while the assay controls were robustly positive. Therefore, we do not believe insufficient rigor in conducting the assays of cellular immunity could account for the negative results.

One difference in the design of the EP-1300 plasmid as compared with many of the more successful DNA vaccine trials is in the use of a “string” of epitopes expressed as a single open reading frame rather than expression of epitopes within a larger gene encoding a functional protein or the use of minigenes in which multiple open reading frames are employed for epitope expression. We note that a similar construct (EP HIV-1090) [19] was a DNA poly-epitope vaccine consisting of 21 CTL epitopes together with the pan-DR PADRE helper epitope. This vaccine was administered in a Phase I trial of 36 healthy volunteers in a dose-escalating fashion, with an additional six participants receiving placebo injections with normal saline. Similar to the results presented here, immunogenicity of this vaccine was minimal. Low and transient IFN- γ ELISPOT responses were detected in two vaccine recipients, one of whom had detectable ELISPOT responses to the PADRE epitope and one who was a placebo recipient [19]. Given the prior experience of EP HIV-1090 and similarly poor immunogenicity of EP-1300 in this study, we suggest that the polyepitope plasmid DNA vaccine design may not be the optimal mode of generating immune responses, as compared with those DNA vaccine approaches expressing intact proteins or protein subunits from the targeted pathogen. However, we were unable to find published reports of polyepitope DNA vaccines compared head-to-head with DNA vaccines expressing intact genes in human trials. Epitopes that are embedded within protein scaffolds appear promising, as illustrated by a recent study in which melanoma epitopes embedded in an IgG (Immunobody) scaffold have elicited immune responses and some clinical responses in a human trial [20].

Our study provides useful information regarding the acceptability of the electroporation procedure. Similar to other published reports in human trials, the volunteers in this study had a range of responses to the electrical pulse itself. Two participants rated the pain at the time of electrical stimulation as excruciating, and one refused further vaccination. Others reported that at the time of the electrical stimulation there was a sensation of being “punched” in the

muscle that was variably painful. Most participants reported only local discomfort that was mild or moderate at the time of the pulse, with mild or moderate discomfort during the first 30 minutes that resolved by day 2 or 3 post-vaccination. Clearly the use of electroporation elicits more discomfort than a typical intramuscular injection, so immunogenicity benefits must outweigh the diminished tolerability of the mode of administration. We note that other than local discomfort, there were no remarkable adverse events and no serious adverse events associated with either administration of this vaccine.

In conclusion, we found that the EP-1300 DNA polyepitope vaccine delivered by electroporation was safe, while tolerability of the administration procedure was fair but clearly diminished from that observed with standard needle and syringe administration. Despite delivery via electroporation, immunogenicity of this DNA vaccine was poor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the vaccine volunteers and all the VTEU faculty and staff who assisted in this clinical trial, including Laila Hussein, Harry Keyserling, and Katie Casper. We thank Xuemin Chen for assistance with the CSP ELISA. We recognize Walter Jones, Steve Rosenthal, and Lee Hall at the Division of Microbiology and Infectious Diseases (DMID) of NIAID, NIH, for their significant contributions to the design and conduct of the study.

Funding

This project was supported by the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID) of NIH through the Vaccine and Treatment Evaluation Units (VTEU), and the US Department of Health and Human Services under contracts HHSN272200800005C (Emory University) and HHSN272200800013C (EMMES Corporation). Additional support was provided by the Georgia Research Alliance, Children's Healthcare of Atlanta, and the National Center for Advancing Translational Sciences of the National Institutes of Health under awards UL1TR000454 and UL1TR000442. The authors and participating faculty and staff were compensated for their work on this project through the US government contracts to their institutions listed above. The vaccine was provided by the National Institutes of Health.

References

1. Organization WH. World Malaria Report 2014. 2014 2014.
2. Schwartz L, Brown GV, Genton B, Moorthy VS. A review of malaria vaccine clinical projects based on the WHO rainbow table. *Malaria journal*. 2012; 11:11. [PubMed: 22230255]
3. Depla E, Van der Aa A, Livingston BD, Crimi C, Allosery K, De Brabandere V, et al. Rational design of a multiepitope vaccine encoding T-lymphocyte epitopes for treatment of chronic hepatitis B virus infections. *Journal of virology*. Jan; 2008 82(1):435–50. [PubMed: 17942551]
4. Keating SM, Bejon P, Berthoud T, Vuola JM, Todryk S, Webster DP, et al. Durable human memory T cells quantifiable by cultured enzyme-linked immunospot assays are induced by heterologous prime boost immunization and correlate with protection against malaria. *Journal of immunology*. Nov 1; 2005 175(9):5675–80.
5. Li C, Shen Z, Li X, Bai J, Zeng L, Tian M, et al. Protection against SHIV-KB9 infection by combining rDNA and rFPV vaccines based on HIV multiepitope and p24 protein in Chinese rhesus macaques. *Clinical & developmental immunology*. 2012; 2012:958404. [PubMed: 22474488]
6. Livingston BD, Newman M, Crimi C, McKinney D, Chesnut R, Sette A. Optimization of epitope processing enhances immunogenicity of multiepitope DNA vaccines. *Vaccine*. Sep 14; 2001 19(32): 4652–60. [PubMed: 11535313]

7. Schalk JA, Mooi FR, Berbers GA, van Aerts LA, Ovelgonne H, Kimman TG. Preclinical and clinical safety studies on DNA vaccines. *Human vaccines*. Mar-Apr;2006 2(2):45–53. [PubMed: 17012886]
8. Subbramanian RA, Kuroda MJ, Charini WA, Barouch DH, Costantino C, Santra S, et al. Magnitude and diversity of cytotoxic-T-lymphocyte responses elicited by multiepitope DNA vaccination in rhesus monkeys. *Journal of virology*. Sep; 2003 77(18):10113–8. [PubMed: 12941922]
9. Vuola JM, Keating S, Webster DP, Berthoud T, Dunachie S, Gilbert SC, et al. Differential immunogenicity of various heterologous prime-boost vaccine regimens using DNA and viral vectors in healthy volunteers. *Journal of immunology*. Jan 1; 2005 174(1):449–55.
10. Udhayakumar V, Saekhou A, Fang S, Jue D, Wohlhueter RM, Lal AA. Immunogenicity of *Plasmodium falciparum* and *Plasmodium vivax* circumsporozoite protein repeat multiple antigen constructs (MAC). *Vaccine*. May-Jun;1998 16(9-10):982–8. [PubMed: 9682348]
11. Sallberg M, Frelin L, Ahlen G, Sallberg-Chen M. Electroporation for therapeutic DNA vaccination in patients. *Medical microbiology and immunology*. Feb; 2015 204(1):131–5. [PubMed: 25535102]
12. Sardesai NY, Weiner DB. Electroporation delivery of DNA vaccines: prospects for success. *Current opinion in immunology*. Jun; 2011 23(3):421–9. [PubMed: 21530212]
13. Kopycinski J, Cheeseman H, Ashraf A, Gill D, Hayes P, Hannaman D, et al. A DNA-based candidate HIV vaccine delivered via in vivo electroporation induces CD4 responses toward the alpha4beta7-binding V2 loop of HIV gp120 in healthy volunteers. *Clinical and vaccine immunology : CVI*. Sep; 2012 19(9):1557–9. [PubMed: 22837097]
14. Vasan S, Hurley A, Schlesinger SJ, Hannaman D, Gardiner DF, Dugin DP, et al. In vivo electroporation enhances the immunogenicity of an HIV-1 DNA vaccine candidate in healthy volunteers. *PloS one*. 2011; 6(5):e19252. [PubMed: 21603651]
15. Kalams SA, Parker SD, Elizaga M, Metch B, Edupuganti S, Hural J, et al. Safety and comparative immunogenicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. *The Journal of infectious diseases*. Sep 1; 2013 208(5):818–29. [PubMed: 23840043]
16. Heller R, Heller LC. Gene electrotransfer clinical trials. *Advances in genetics*. 2015; 89:235–62. [PubMed: 25620013]
17. Alexander J, Bilsel P, del Guercio MF, Stewart S, Marinkovic-Petrovic A, Southwood S, et al. Universal influenza DNA vaccine encoding conserved CD4+ T cell epitopes protects against lethal viral challenge in HLA-DR transgenic mice. *Vaccine*. Jan 8; 2010 28(3):664–72. [PubMed: 19895924]
18. del Guercio MF, Alexander J, Kubo RT, Arrhenius T, Maewal A, Appella E, et al. Potent immunogenic short linear peptide constructs composed of B cell epitopes and Pan DR T helper epitopes (PADRE) for antibody responses in vivo. *Vaccine*. Mar; 1997 15(4):441–8. [PubMed: 9141216]
19. Gorse GJ, Baden LR, Wecker M, Newman MJ, Ferrari G, Weinhold KJ, et al. Safety and immunogenicity of cytotoxic T-lymphocyte poly-epitope, DNA plasmid (EP HIV-1090) vaccine in healthy, human immunodeficiency virus type 1 (HIV-1)-uninfected adults. *Vaccine*. Jan 10; 2008 26(2):215–23. [PubMed: 18055072]
20. Patel, PM.; Durrant, LG.; Ottensmeier, C.; Mulatero, C.; Lorigan, P.; Plummer, R., et al. A phase 1/2 trial of SCIB1 Immunobody in melanoma patients. *American Society of Clinical Oncology*; Chicago, IL: 2014.

Fig. 1. Schematic Depiction of EP-1300 DNA Vaccine Plasmid. Epitopes are shown separated by spacer peptides, with HLA restriction indicated below each epitope. The vaccine insert was cloned into the backbone plasmid pMB75.6. The functional elements of the expression plasmid are also indicated. Note that each epitope included in the vaccine plasmid is detailed in Supplemental Tables 1 and 2 for ease of reference.

Fig. 2.
CONSORT diagram for the EP-1300 trial.

Fig. 3.

A) IFN- γ ELISPOT Responses by HLA peptide pool. Peptides were pooled by HLA restriction and evaluated in triplicate wells. Shown are the median responses for participants by dosage group vs. placebo, after correcting for background levels. Dashed black line indicates placebo recipients, and day is shown on the x-axis. ALL represents median responses for all HLA supertypes combined. **B)** IFN- γ ELISPOT Responses, all peptide pools. Shown are results of ELISPOT from 3 time points by dosage group vs. placebo. Box plots indicate median and 25th (lower box line) and 75th (upper box line) percentiles, with whiskers indicating 5th and 95th percentiles.

Fig. 4. Anti-CSP ELISA Responses. Geometric means of the optical density (OD) for participants in the 1.0 mg and 4.0 mg groups compared to placebo are shown.

Table 1

Baseline demographics of study participants.

	All Groups N = 39 (%)	0.25 mg EP-1300 N= 10 (%)	1.0 mg EP-1300 N= 10 (%)	4.0 mg EP-1300 N= 10 (%)	Placebo N= 9 (%)
Gender					
Male	17 (44)	5 (50)	7 (70)	2 (20)	3 (33)
Female	22 (56)	5 (50)	3 (30)	8 (80)	6 (67)
Ethnicity					
Non-Hispanic	38 (97)	10 (100)	10 (100)	10 (100)	8 (89)
Hispanic	1 (3)	0	0	0	1 (11)
Race					
Asian	1 (3)	0	1 (10)	0	0
African American	8 (21)	2 (20)	2 (20)	4 (40)	0
White	27 (69)	8 (80)	7 (70)	6 (60)	6 (67)
Multi-Racial	3 (8)	0	0	0	3 (33)
Age (years)					
Mean (STD)	28.3 (6.2)	27.5 (4.4)	28.4 (6.7)	29.8 (6.1)	27.4 (7.9)
Range	(18.7 – 40.1)	(20.1 – 36.0)	(20.6 – 40.1)	(19.3 – 39.3)	(18.7 – 38.9)

Table 2

Frequency of adverse events post-vaccination and through 224 days follow-up.

	All Groups N = 39 (%)	0.25 mg EP-1300 N = 10 (%)	1.0 mg EP-1300 N = 10 (%)	4.0 mg EP-1300 N = 10 (%)	Placebo N = 9 (%)
Solicited AEs					
Mild	39 (100)	10 (100)	10 (100)	10 (100)	9 (100)
Moderate	29 (74.4)	6 (60)	6 (60)	9 (90)	8 (88.9)
Severe	2 (5.1)	0 (0)	1 (10)	1 (10)	0 (0)
Unsolicited AEs					
Mild	36 (92.3)	10 (100)	8 (80.0)	9 (90.0)	9 (100)
Moderate	13 (33.3)	2 (20.0)	2 (20.0)	6 (60.0)	3 (33.3)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

n = number of subjects who experienced one or more AEs of the severity shown.