

Research Paper

The candidate tuberculosis vaccine Mtb72F/AS02A

Tolerability and immunogenicity in humans

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Abbreviations: AE, adverse event; BCG, bacillus calmette-guérin; CD40-L, CD40-ligand; CMI, cell-mediated immune; DLT, dose-limiting toxicity; DSM, Data Safety Monitor; ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; GMT, geometric mean titers; GSK, GlaxoSmithKline; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ICS, intracellular cytokine staining; IFN γ , interferon-gamma; IL-2, interleukin-2; MPL, monophosphoryl lipid A; PBMC, peripheral blood mononuclear cells; PPD, tuberculin purified protein derivative; TB, tuberculosis; TNF α , tumour necrosis factor-alpha

Key words: tuberculosis vaccine, clinical trial, PPD-negative, Mtb39a, Mtb32a, Mtb72F, AS02A, safety, immunogenicity, first-time-in-human

Tuberculosis (TB) remains uncontrolled in many parts of the world and the development of an effective vaccine against TB represents a high priority unmet medical need. Healthy PPD (tuberculin purified protein derivative)-negative adult volunteers, aged 18–40 years received three doses of the candidate Mtb72F/AS02A vaccine according to a 0-1-2 months schedule in an open-label Phase I study (NCT00730795). Solicited, unsolicited and serious adverse events (AEs), hematological and biochemical laboratory parameters were assessed. Mtb72F-specific humoral responses were assessed by ELISA and cell-mediated immune (CMI) responses by intracellular cytokine staining (ICS) and short-term ELISPOT assays. CMI responses to the component peptides (Mtb39a and the Mtb32a C- and N-terminal antigen domains, Mtb32C and Mtb32N) were also assessed by ICS. The Mtb72F/AS02A vaccine appeared to be mainly locally reactogenic but this was considered acceptable, since these AEs were usually transient and resolved within 1–2 days. Most AEs reported were mild in intensity, no serious AEs occurred, no medically significant biochemical or hematological abnormalities related to vaccination were measured and all AEs resolved without sequelae. The vaccine induced statistically significant changes in humoral and CMI response measures. The Mtb72F antigen induced good production of IL-2 and IFN γ in the ELISPOT assay and CD4⁺ T cells expressing at least two activation markers (mainly CD40-L and IL-2) were observed with ICS. A similar CMI profile was observed with Mtb39a and Mtb32N. The induced CMI responses persisted for at least 6 months post-vaccination. All subjects were seropositive for anti-Mtb72F

antibodies one month post-dose 2 and 6 months post-dose 3. This first trial in humans found Mtb72F/AS02A to have an acceptable tolerability, to be immunogenic in healthy adults and warrants further development of the vaccine.

Introduction

Despite the widespread use of the BCG vaccine, with vaccination rates of over 80%,¹ TB remains one of the leading infectious causes of mortality worldwide. Although the incidence of severe childhood TB has been reduced by neonatal administration of BCG, it is clear that vaccination with BCG does not have a similar magnitude of effect in prophylaxis of adult-onset TB and pulmonary TB in children.² Even though not all infected individuals will develop active disease, any prevalence of active disease (in particular, readily transmitted pulmonary TB) poses a community risk to individuals with reduced immunity. TB represents the highest risk of death in HIV/AIDS infected people.³ The situation is compounded by the emergence of multi-drug resistant strains of *M. tuberculosis*. Therefore, the development of an effective vaccine against the pulmonary form of TB is a high priority unmet medical need.

GSK Biologicals candidate TB vaccine Mtb72F/AS02A is being developed to boost specific, pre-existing immunity induced by BCG and/or *M. tuberculosis*. Mtb72F is a recombinant protein comprising two antigens (Mtb39a and Mtb32a), which are expressed in *M. tuberculosis* and in BCG but not in other mycobacteria.⁴ AS02A is a GSK proprietary Adjuvant System inducing humoral responses and type 1 T cell responses.⁵ Mtb39a and Mtb32a were selected by T cell antigen screening based on their ability to (1) restimulate, in vitro, peripheral blood mononuclear cells (PBMCs) from healthy PPD-positive individuals, (2) induce Th1 responses in mice and (3) induce protection in animal models of TB. Mtb72F/AS02A was shown to be well tolerated and protective in preclinical studies of mice, guinea pigs, rabbits⁶⁻⁸ and non-human primates.⁹

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In this first-time-in-human study, the safety and immunogenicity of Mtb72F/AS02A was evaluated in TB naïve volunteers in the US.

Results

Participant flow. The participant flow is presented in Figure 1. A total of 47 volunteers consented and were screened for study entry. Ten subjects were enrolled. One subject was withdrawn by the investigator after dose 1 for grade 3 injection site pain, which according to protocol was a DLT. He was not replaced. Another subject was withdrawn after dose 1 for elevated diastolic blood pressure linked to pre-existing hypertension and was replaced. A third subject withdrew from further vaccination after dose 2 and was replaced. Thus, a total of 12 subjects received at least one dose of the vaccine. All 12 subjects completed the study through to the final visit 6 months post-dose 3 (Day 224).

Baseline data. Of the 12 enrolled subjects (four females and eight males), ten subjects were Caucasian, one was Asian and one was Hispanic/Latino. Subjects ranged in age from 19 to 40 years, with a median age of 27 years.

Numbers analyzed. All 12 enrolled subjects received at least one vaccine dose and were analyzed for safety. Nine subjects who met the inclusion/exclusion criteria, received all three vaccinations, had no protocol violations that could affect immune response endpoints and returned to the clinic for the final safety and immunogenicity assessment visit at Day 224, were included in the immunogenicity analyses. For CMI, data from eight subjects were available.

Safety and reactogenicity. All subjects vaccinated in this trial reported at least one local AE. No serious or life-threatening AEs were reported during the study. The majority (74%) of AEs (solicited and unsolicited) reported after all 31 doses were graded as 1 (mild) and there were no clinically significant changes in biochemistry and hematology values in this trial. The frequencies of AEs with possible, probable and definite relationship to vaccination after all doses were 27%, 35% and 22%, respectively and all resolved within the 7-day follow-up period.

Nine subjects experienced grade 3 AEs. Of these, seven reported erythema and/or swelling at the injection site. These local reactions mostly occurred after dose 2 and/or 3, were transient, usually reducing in intensity or resolving within two days. One subject, who had not disclosed pre-existing hypertension at screening, experienced grade 3 elevated diastolic blood pressure after dose 1 (unrelated to vaccination); the subject was withdrawn from vaccination and referred to his personal physician for further evaluation. One subject reported grade 3 injection site pain after dose 1 described by the investigator as resulting in “limited arm abduction,” which lasted for four days and grade 3 nausea lasting one day. Based on the DSM review, this subject experienced a DLT. All grade 3 AEs resolved without sequelae.

The most common related solicited AEs were transient injection site reactions (pain, swelling and redness), and “flu-like” symptoms (headache, fatigue, malaise and myalgia) Figure 2. All solicited AEs resolved without sequelae.

The most common unsolicited AEs graded as possibly related to vaccination by the investigator were increases in eosinophil counts

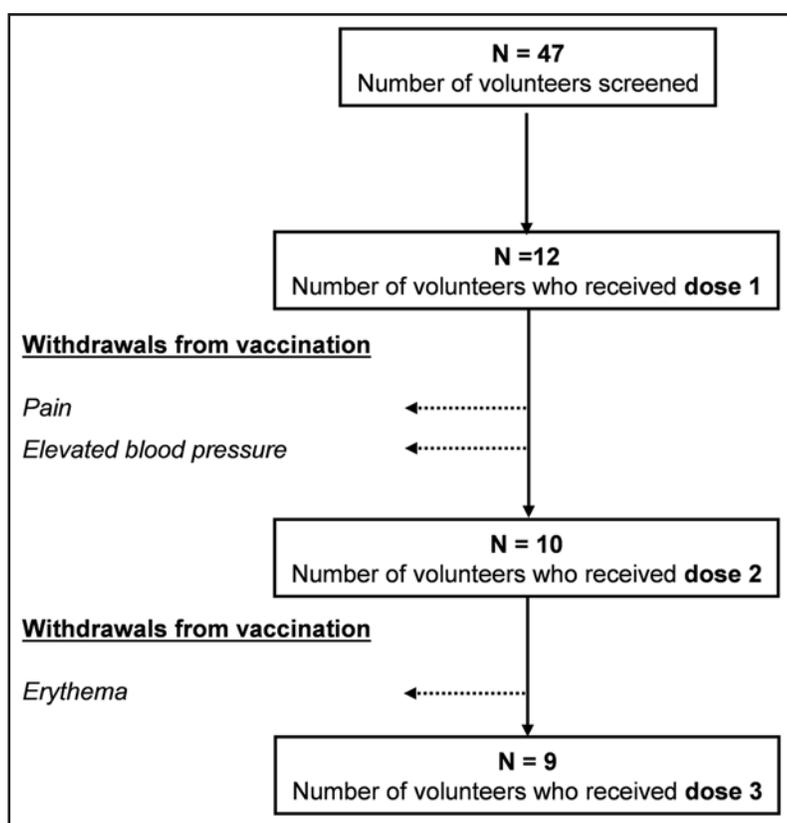


Figure 1. Participant flow.

(eight subjects) without any associated symptomatology such as pulmonary or allergic manifestations and decreases in hemoglobin counts (seven subjects). All other unsolicited AEs (rigor, blood pressure or respiratory rate fluctuations, congested nose and back pain) possibly, probably or definitely related to vaccination were reported each usually by 1 or 2 subjects.

For the eight subjects reporting eosinophilia, six reported grade 1 eosinophilia (range 5.1%–9.7%). Grade 1 eosinophilia was reported once by 4 subjects (3 reports on samples taken prior to the third vaccination and 1 report one month post dose 3). In samples taken just prior to dose 2, two other subjects reported grade 1 eosinophilia, which remained mildly elevated until one month post dose 3 in one subject and until study end in the other subject. Two subjects reported grade 2 eosinophilia (10.4% and 12.2%). One subject with a history of hay fever in Spring had mildly elevated levels from 2 days post dose 1 until study end except for the day of dose 3 and 7 days later, when this was graded as a moderate elevation. The investigator reported this as likely secondary to environmental allergen exposure. The second subject recorded elevated eosinophilia at screening which upon repeated tests had normalized (4.5%) on day of vaccination. He recorded mild elevations at each subsequent time point tested (up until 1 month post dose 3) except for the day of and 2 days post dose 3 when he had moderate elevation. All reports of decreased hemoglobin except one were considered to be mild in intensity (11.6–13.8 g/dL). The female subject reporting moderate decrease in hemoglobin had a consistently low level of hemoglobin from prior to dose 2 (10.8 g/dL) until 1 week post dose 3 (11.5 g/dL). She was

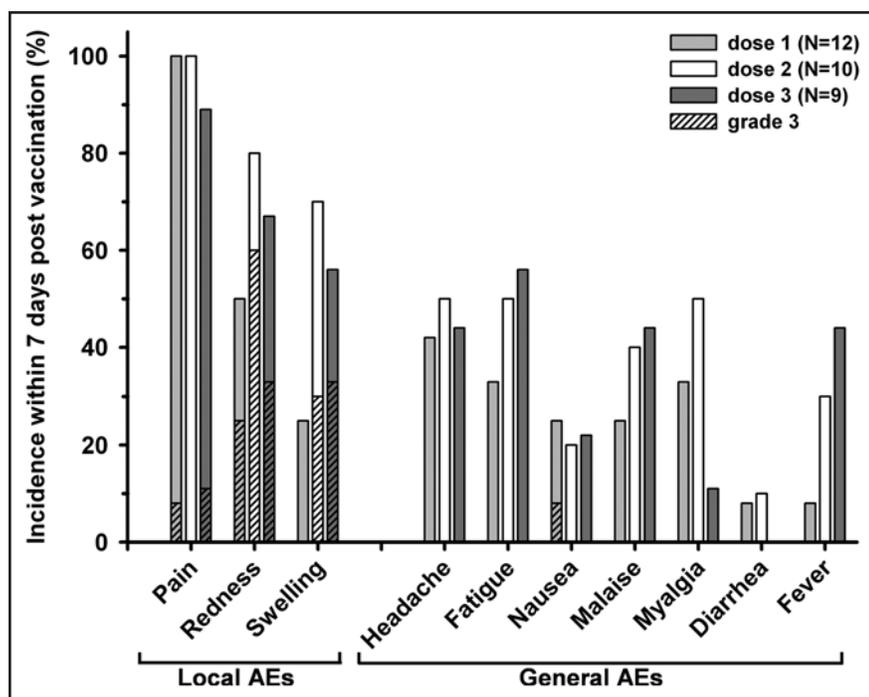


Figure 2. Summary of solicited local and general adverse events. Grouped columns represent incidence of specified solicited AEs occurring within 7 days post vaccination with Mtb72F/AS02A (doses 1, 2 and 3). Grade 3 AEs are presented by hatched bars.

found to have significant iron deficiency anemia and responded to treatment with ferritin.

At study end (6 months after the last vaccination), no late onset safety issues were identified. All subjects remained PPD-negative and no abnormal chest X-ray findings were observed.

Immunogenicity. T cell response using intracellular cytokine staining (ICS). One month post-dose 1, antigen-specific CD4⁺ T cells expressing at least two cytokines/activation markers (IFN γ and/or IL-2 and/or TNF α and/or CD40-L) specific to Mtb72F, Mtb39a and Mtb32N were observed (Fig. 3). These specific immune responses increased vigorously from dose 1 to dose 2. After the third dose, median levels of Mtb72F- and Mtb39a-specific CD4⁺ T cells increased slightly. Although the magnitude of responses to Mtb72F, Mtb39a and Mtb32N decreased with time, they persisted at 6 months post-dose 3. Only a weak response to Mtb32C stimulation was observed at all times.

Mainly CD40-L and IL-2 were expressed and to a lesser extent TNF α and IFN γ in response to stimulation with Mtb72F (Fig. 4) and Mtb72F-derived peptides (data not shown). No vaccine-induced CD8⁺ T cell counts above baseline were detected by ICS using this short-term in vitro stimulation (data not shown).

T cell response using ELISPOT. Using the ELISPOT technique to enumerate IL-2 and IFN γ secreting CD4⁺ T cells in vitro, a good induction of Mtb72F-specific cells producing IL-2 and IFN γ was observed after one dose of Mtb72F/AS02A. A second dose enhanced the magnitude of the response. The third dose did not appear to further increase this response (Fig. 5).

Anti-Mtb72F IgG response. All subjects except one were seropositive for anti-Mtb72F antibodies at one month after the first dose (Fig. 6). One month after the second dose of Mtb72F/AS02A, all subjects had seroconverted and a geometric mean titers (GMT) 793.78 EU/mL was measured. One month post-dose 3, titers

had increased moderately (1126.24 EU/mL). All subjects remained seropositive for anti-Mtb72F antibodies at 6 months post dose 3, with a decreased GMT of 163.39 EU/mL.

Discussion

We present here the first clinical data with the Mtb72F/AS02A vaccine. Mtb72F/AS02A showed an acceptable safety profile and elicited good humoral and cellular immune responses in healthy adults with no history of exposure to or vaccination against *M. tuberculosis*.

The overall reactogenicity pattern was considered acceptable since no serious AEs occurred, most AEs reported were mild in intensity and transient, and all resolved without sequelae. The vaccine was locally reactogenic with pain being predominantly reported. Although all subjects reported pain immediately after vaccination severe grade 3 pain was infrequent and almost all resolved or decreased in intensity within 24 hours. For erythema and swelling at the injection site, these usually occurred independent of each other or pain. For the solicited general AEs, there were no severe cases reported except for one subject who reported grade 3 nausea. There was only 1 grade 3 unsolicited AE reported which was not related to vaccination. Two subjects were withdrawn from vaccination by the investigator for AEs after dose 1, with one AE (injection site pain) linked to vaccination. The injection site pain was graded as 3 because the volunteer had difficulty combing her hair with that hand. Given that this was the first time that Mtb72F/AS02A was given to humans, very strict stopping criteria were applied and it is recognized that this AE may not have motivated withdrawal from a study conducted in the context of a more fully established reactogenicity profile. A second subject was withdrawn because of grade 3 elevated diastolic blood pressure (not related to vaccination) after dose 1. A third subject withdrew from vaccination after dose 2. At the time of his voluntary withdrawal, the only vaccine related AE he had reported was grade 3 injection site erythema. This AE had been reviewed by the DSM and was found not to preclude him from further vaccination. All AEs resolved without sequelae.

No clinically significant changes in laboratory biochemistry and/or hematology were observed. The study was conducted during a particularly strong season for environmental allergens at the trial site and this may have contributed to the eight cases of eosinophilia that were evaluated as possibly related to vaccination. Data on these subjects were reviewed by the Sponsor, the DSM and the FDA. Following this safety review the conclusion was that the increases in eosinophil counts were not indicative of a potential safety issue and in each case permitted the administration of the next dose of vaccine. With one exception, the decreased levels in hemoglobin were mild and explained by phlebotomy. One case of anemia of moderate intensity was recorded and was found to be the result of iron deficiency. This AE resolved once iron treatment had been initiated. Additional safety data collected at the 6-month post vaccination follow-up visit did not indicate any late onset safety issues.

ICS results showed that Mtb72F-specific CD4⁺ T cells were induced by vaccination with Mtb72F/AS02A. The responses were mostly detected against the Mtb72F components Mtb39a and

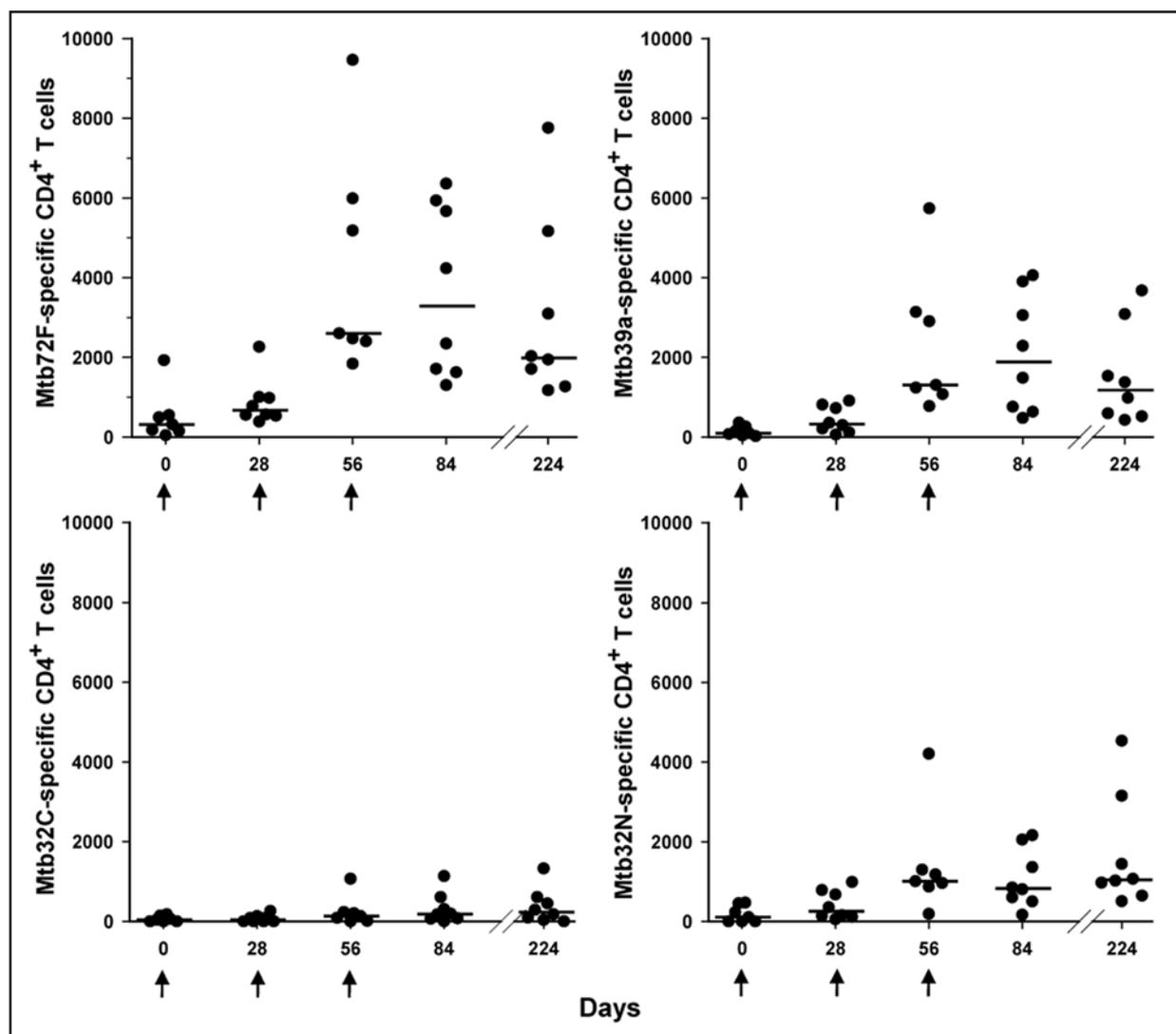


Figure 3. Frequency of CD4⁺ T cells (per million CD4⁺ T cells) expressing at least two different cytokines/activation markers* after short-term in vitro stimulation with Mtb72F and Mtb72F-derived peptides (Mtb39a, Mtb32 N-terminus, Mtb32 C-terminus). Time points for vaccination are indicated with an arrow. Sampling time points were Days 0, 28, 56, 84 and 224. Individual data (by dots) and medians (by a line) are shown. *CD40-L, IL-2, TNF α , IFN γ .

Mtb32N. Similar observations were made using a lymphoproliferation assay (data not shown).

The cytokines/activation markers expressed by Mtb72F-specific CD4⁺ T cells, identified by ICS, were mainly CD40-L and IL-2 and to a lesser extent TNF α and IFN γ . IL-2 and CD40-L are reported to be key factors in the induction of memory T cell responses. CD40-L has been identified as a reliable marker for antigen-specific T cells.^{10,11} This observation is consistent with previously published scientific literature indicating that vaccination with protein antigens generally induces a CD4⁺ T cell memory response biased toward IL-2 production.¹² IFN γ and TNF α have been extensively described as effector cytokines of the immune system and are potent anti-intracellular pathogen factors.¹³⁻¹⁵

Mtb72F-specific IFN γ and IL-2 spot forming cells were detected in a comparable manner by ELISPOT assay. With the ICS assay a higher number of CD4⁺ T cells expressed IL-2 as compared to IFN γ . This is not unexpected as cells other than CD4⁺ T cells could express IFN γ as a bystander effect. These CD4-negative T cells would be detected by ELISPOT whereas the ICS focused on the

IFN γ and IL-2 expression analysis by CD4⁺ and CD8⁺ T cells only. Furthermore the stimulation periods may have had an impact on secretion of these two cytokines; in vitro stimulation lasted for one day in ELISPOT as compared to a 2 hour stimulation followed by an overnight incubation with cytokine secretion blocker (Brefeldin A) to accumulate the cytokines produced within the cells in the ICS assay. Finally the ELISPOT and ICS data are not directly comparable but complementary since the latter assay identified the cytokine expression within CD4⁺ T cells whilst ELISPOT recorded any cell secreting IL-2 or IFN γ in total white cells plated in culture wells.

All immune responses persisted at 6 months after the last vaccine dose.

Under the conditions of the short-term in vitro ICS assay, no vaccine-induced CD8⁺ T cell responses were detectable. A long-term stimulation ICS assay may be required to further evaluate the CD8⁺ response.

A vigorous induction of Mtb72F-specific humoral immune response was observed, with 100% seroconversion at one month post-dose 2. All study participants remained seropositive for

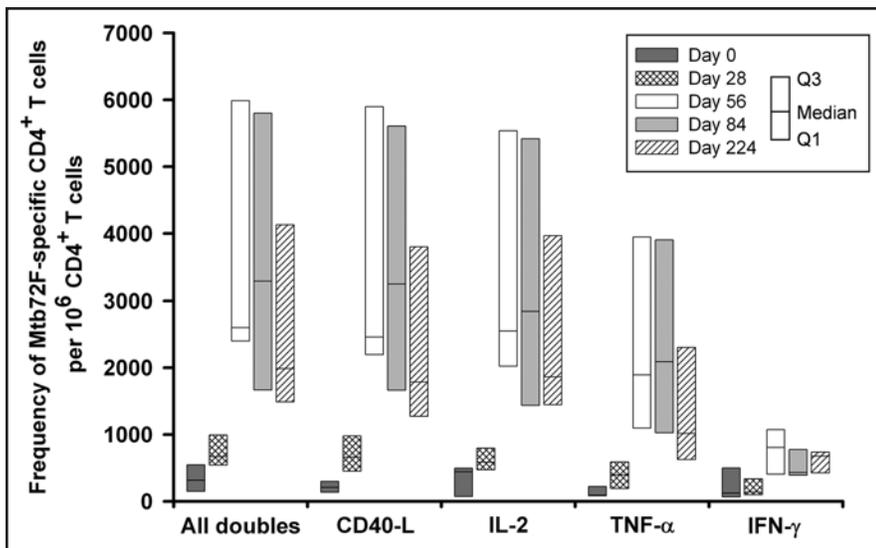


Figure 4. Functional characterization of CD4⁺ T cells after stimulation with Mtb72F. Sampling time points were Days 0, 28, 56, 84 and 224. Median, Q1 and Q3 are shown.

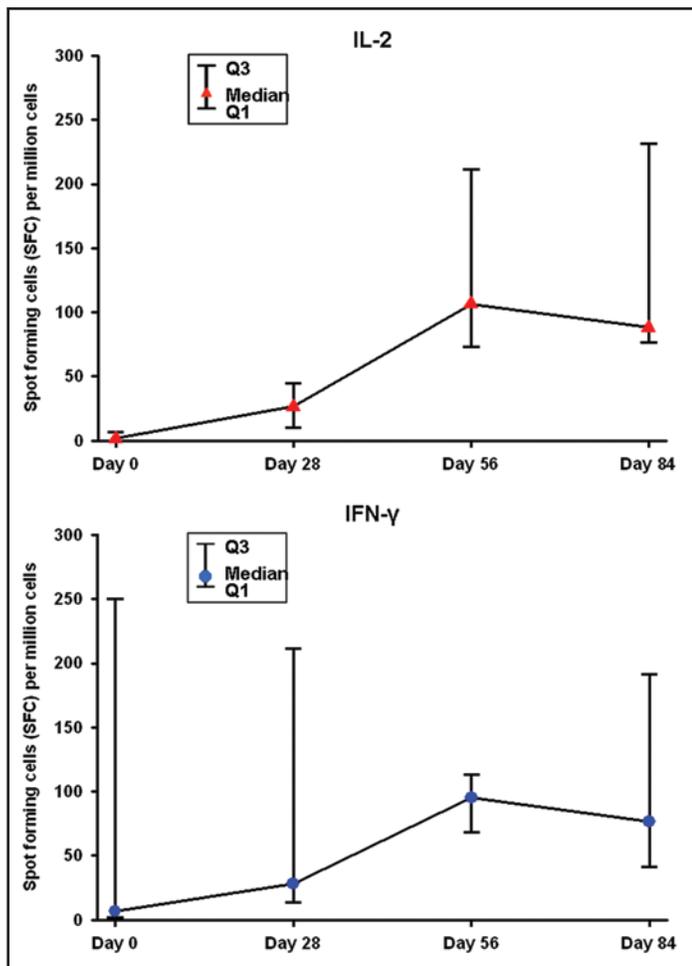


Figure 5. Enumeration of IL-2 and IFN γ secreting cells using short-term ELISPOT. Data were expressed as number of spot forming cells (SFC) per million cells. Median, Q1 and Q3 are shown.

anti-Mtb72F antibodies until the end of the study, at 6 months post-dose 3. Although the role of antibody-mediated immunity to *M. tuberculosis* remains uncertain, there is sufficient evidence in the literature to conclude that antibody-mediated immunity may alter the course of infection in certain situations.¹⁶

The third dose of Mtb72F/AS02A did not significantly increase either the CMI or the humoral immune responses beyond dose 2. This is perhaps an indication that two doses of this vaccine are sufficient to attain maximum immunity.

All subjects remained PPD-negative after completion of the vaccination course indicating that administration of the Mtb72F/AS02A vaccine does not have an impact on the PPD status. This implies that the PPD skin test, which remains a powerful tool for the early diagnosis of TB infection, could discriminate between *M. tuberculosis*-infected and *M. tuberculosis*-naïve individuals in a Mtb72F/AS02A vaccinated population. This

is an interesting observation, given that in several countries prophylactic vaccination with BCG is not recommended due to its induction of PPD skin test conversion.

The data presented here show that three doses of Mtb72F/AS02A vaccine are locally reactogenic, but acceptably tolerated by healthy adults who have never been exposed to *M. tuberculosis*. The results presented also show that the vaccine is immunogenic, stimulating both cellular and humoral immune responses, and that the Mtb39a and Mtb32N components add the most to the immunogenicity of the antigen. These observations support the further evaluation of this promising TB vaccine candidate.

Methods

Participants. This open-labeled Phase I trial with Mtb72F/AS02A was conducted at a Phase I/II testing facility (Northwest Kinetics, Inc., Tacoma, WA, currently Charles River Clinical Services). Adults aged 18 to 40 years, who were in good general health with clinically normal screening laboratory values and having negative results at screening for PPD skin test, hepatitis B surface antigen (HBsAg), human immunodeficiency virus-1 and -2 (HIV 1/2) and hepatitis C virus (HCV) antibodies were eligible for the trial.

Subjects were excluded if they had a history of documented exposure to *M. tuberculosis* or to experimental products containing any of the vaccine components, previous administration of BCG or other experimental *M. tuberculosis* vaccination, or if they were under current therapy or had received prophylaxis for TB. Females who were pregnant or planning to become pregnant or to discontinue contraceptive precautions were also excluded.

This study was approved by an Independent Institutional Review Board and was conducted under a US FDA IND in compliance with the Declaration of Helsinki and with Good Clinical Practice. Written informed consent was obtained from each subject prior to the performance of any study-specific procedures. The trial is registered with the ClinicalTrials.gov registry (NCT00730795).

Study vaccine. The Mtb72F antigen was manufactured at Corixa Corporation (now GSK Biologicals) and was supplied as a lyophilized cake for reconstitution with the proprietary AS02A Adjuvant System.

AS02A is an oil-in-water emulsion with two immunostimulants, MPL and *Quillaja saponaria* fraction 21 (QS21).¹⁷ Each dose (0.5 mL) of the reconstituted vaccine Mtb72F/AS02A, containing 10 µg Mtb72F and 50 µg each of MPL and QS21, was administered by slow intramuscular injection in the deltoid regions of alternating arms according to a 0, 1, 2 months vaccination schedule. It was planned initially to assess 2 antigen dose levels (10 µg and 40 µg) but the protocol was amended to evaluate only the 10 µg antigen dose. The 40 µg antigen dose has subsequently been assessed in another study and a manuscript is in preparation.

Objectives. The primary objective of the trial was to evaluate the safety of Mtb72F/AS02A given as three monthly intramuscular injections. Secondary objectives were to evaluate the immunogenicity of the vaccine by measuring humoral immune response to Mtb72F and CMI responses to Mtb72F and its components, Mtb39a and the Mtb32a C- and N-terminal antigen domains, Mtb32C and Mtb32N.

Safety and reactogenicity evaluation. Safety was assessed based on occurrence of adverse events (AEs) and serious AEs, changes in biochemical and hematological parameters, changes in resting vital signs, chest X-ray findings and PPD skin test reactivity. Solicited local AEs were injection site pain, redness and swelling. Solicited general (systemic) AEs were fever, headache, fatigue, nausea, malaise, myalgia, joint pain, diarrhea and vomiting. AEs were recorded during a 7-day follow-up period after each vaccine dose for solicited AEs, during a 1-month follow-up period after each vaccine dose for unsolicited AEs and up to 6 months after dose 3 (Day 224) for serious AEs. The intensity of solicited AEs was evaluated according to a specific pre-agreed grading system (Table 1). All other AEs were graded in accordance with the NCI Common Toxicity Criteria Version 3.¹⁸ PPD skin tests and chest X-rays were performed at screening and at the end of the study (6 months post-dose 3).

An independent DSM was appointed to review the safety data for all subjects after each vaccination and was authorized to withdraw individual subjects from further vaccination or suspend vaccination for all subjects. Stopping rules were defined in the protocol and were evaluated by the DSM via periodic in-depth reviews of each individual's AEs. Subjects who were withdrawn from subsequent vaccination were counted as having experienced a DLT. Stopping rules were as follows: laboratory parameters graded as 2 and persisting for at least 48 hours or graded as 3; grade 3 erythema and/or swelling beginning within two days after vaccination and accompanied by another AE which prevents normal activity for at least 24 hours; grade 3 injection site pain; any grade 3 or life threatening diarrhea or vomiting; other general AEs beginning within two days after vaccination and persisting for at least 24 hours.

The study allowed for replacement of subjects who withdrew from vaccination up until dose 3, provided they did not experience a DLT.

Immunogenicity evaluation. Blood samples for immunogenicity evaluation were taken at baseline, one month after each vaccination and 6 months post-dose 3. CMI response was evaluated using ICS and IFN γ /IL-2 ELISPOT assays. Humoral response was assessed by ELISA (enzyme-linked immunosorbent assay).

Intracellular cytokine staining assay. The ICS method used was adapted from Maecker et al.^{19,20} To measure the frequencies of antigen-specific CD4⁺ or CD8⁺ T cells expressing two or more of

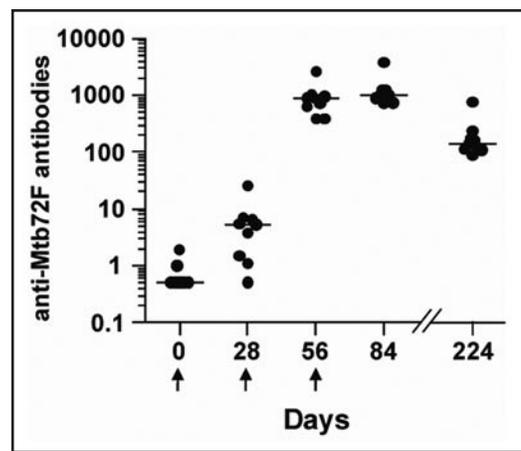


Figure 6. Anti-Mtb72F IgG responses. Time points for vaccination are indicated with an arrow. Sampling time points were Days 0, 28, 56, 84 and 224. Individual data (by dots) and GMTs (by a line) are shown.

the four quantified cytokines/immune response markers (CD40-L, IL-2, TNF α and IFN γ), PBMCs were stimulated with the recombinant protein Mtb72F or pools of 15-mer peptides overlapping by 11 amino acids and covering the sequence of the three Mtb72F components: (1) Mtb39a, (2) Mtb32C and (3) Mtb32N. After two hours, intracellular block (Brefeldin A) was added to accumulate the cytokines produced within the cells. After overnight restimulation in vitro, cells were harvested, stained for surface markers (CD4 and CD8) and then fixed. The fixed cells were permeabilized and stained with labelled anti-immune marker-specific antibodies, washed, resuspended in PBS and analyzed for antigen-specific responses by flow cytometry.

ELISPOT. The ELISPOT technique applied was a 96-well plate method where PBMCs were stimulated in vitro for one day with the Mtb72F protein before the cells were transferred to the ELISPOT nitrocellulose plate. The cytokines secreted by antigen-specific T cells were captured by immobilized antibody onto the nitrocellulose plates and subsequently detected by a colorimetric substrate as localized spots on the membrane-coated well.²¹ The results obtained were expressed as number of spot forming cells (SFC) per million cells.

Anti-Mtb72F response. Mtb72F-specific antibody levels were determined by evaluating antibody (IgG) responses using ELISA. Mtb72F antigen was pre-coated onto a 96-well plate at 0.5 µg/mL in a 0.05 M bicarbonate solution and incubated at 4°C for 16 hours. After blocking (PBS/Tween 0.2%/Skimmed milk 10 g/mL) the plates, eight serial dilutions (1:2.25) in blocking buffer of pre-diluted plasma samples (1:50) were added directly to the plate followed by a washing step (PBS/Tween 20 0.1%). A secondary polyclonal rabbit anti-human antibody conjugated with horseradish peroxidase diluted in blocking buffer (1:8,000) was added for 30 minutes at room temperature (RT). The addition of TMB (3,3',5,5'-tetramethylbenzidine substrate) substrate for 5 min at RT provided a means of detecting the plasma antibody that is specific for the Mtb72F antigen. A positive control and calibrator (pool of plasma collected from Mtb72F vaccinated subjects) were run on each plate in similar dilutions in order to assess the relative titer of each test sample. Negative controls (AB-serum from CELLECT, ICN) were also run on each plate to ensure specificity. The plates were read in

Table 1 Study-specific solicited adverse event grading

| Adverse event | Grade 0 | Grade 1 | Grade 2 | Grade 3 |
|--|--------------------------------|--|--|---|
| Injection site redness | Greatest surface Φ : 0 mm | Greatest surface Φ : 0–20 mm | Greatest surface Φ : >20–50 mm | Greatest surface Φ : >50 mm |
| Injection site swelling | Greatest surface Φ : 0 mm | Greatest surface Φ : 0–20 mm | Greatest surface Φ : >20–50 mm | Greatest surface Φ : >50 mm |
| Fever | <37.5°C | 37.5–38°C | >38–39.5°C | >39.5°C |
| Diarrhea | ≤1 loose stool in 24 h | 2–3 loose stools in 24 h | 4–5 loose stools in 24 h | ≥6 loose stools in 24 h |
| Vomiting | none | 1–2 episodes in 24 h | >2 episodes in 24 h | Requires IV hydration |
| Fatigue, Nausea, Malaise, Myalgia, Joint pain, Injection site pain, Headache | normal | Mild, no interference with normal activity | Moderate, some interference with normal activity | Severe, significant, prevents normal activity |

an ELISA reader at 450 nm. The titers were calculated from the standard curve with the software SoftmaxPro™ (version 3.1.1), using an unweighted four parameter logistic function (4PL) equation.^{22,23} The cut-off for the anti-Mtb72F ELISA was chosen as equal to LOQ (limit of quantification) and set at 1 EU/mL.

Intradermal PPD skin test. At least 14 days before administration of dose 1, two PPD skin tests were performed (with a 7 to 10-day interval) using a commercial kit and following the manufacturer's instructions (TUBERSOL®, Aventis Pasteur). PPD skin testing was repeated 6 months after the last vaccination (Day 224). Skin test results were read at 48–72 hours after administration of PPD. For this study, any area of induration greater than 0 mm was considered a positive test.

Sample size. The sample size of 10 was chosen to obtain an initial safety profile for the Mtb72F/AS02A vaccine and to perform preliminary evaluation of immunogenicity specific to Mtb72F/AS02A in healthy PPD-negative adults.

Statistical methods. Demographics, reactogenicity and safety evaluation were performed on all subjects who received at least one vaccine dose. The number (%) of subjects reporting grade 3 AEs or a DLT were summarized. Serious AEs were described and changes in serum chemistry and hematology measurements were evaluated. The number (%) of subjects who changed from PPD-negative at screening to PPD-positive at 6 months post-dose 3 or showed a change in chest X-ray images was summarized.

The ICS results were presented as number of antigen (Mtb72F, Mtb39a, Mtb32C and Mtb32N)-specific CD4⁺ or CD8⁺ T cells expressing CD40-L and/or IL-2 and/or TNF α and/or IFN γ , respectively, per million of CD4⁺ or CD8⁺ T cells. Specific responses were measured by subtracting the responses obtained for non-stimulated cells (blank cells) from the responses obtained in stimulated cells.

The IFN γ and IL-2 ELISPOT assay data were scored as number of SFC/10⁶ cells expressing the relevant cytokine. The results were presented as the difference between the mean of three wells with and without antigen.

The anti-Mtb72F antibody titers were summarized using GMT with 95% confidence interval (CI). A seropositive subject was a subject whose anti-Mtb72F titer was greater than or equal to the cut-off value of 1 ELISA Unit (EU)/mL.

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and Joe Cohen are present employees of GlaxoSmithKline. Els De Kock and W. Ripley Ballou were employed by GSK at the time of the study. Royce Morrison, Madeleine Braun and Daniel Cain have declared that no competing interests exist.

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