ORIGINAL ARTICLE

Final Analysis of a Trial of M72/AS01$_E$ Vaccine to Prevent Tuberculosis

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ABSTRACT

BACKGROUND

Results of an earlier analysis of a trial of the M72/AS01$_E$ candidate vaccine against *Mycobacterium tuberculosis* showed that in infected adults, the vaccine provided 54.0% protection against active pulmonary tuberculosis disease, without evident safety concerns. We now report the results of the 3-year final analysis of efficacy, safety, and immunogenicity.

METHODS

From August 2014 through November 2015, we enrolled adults 18 to 50 years of age with *M. tuberculosis* infection (defined by positive results on interferon-γ release assay) without evidence of active tuberculosis disease at centers in Kenya, South Africa, and Zambia. Participants were randomly assigned in a 1:1 ratio to receive two doses of either M72/AS01$_E$ or placebo, administered 1 month apart. The primary objective was to evaluate the efficacy of M72/AS01$_E$ to prevent active pulmonary tuberculosis disease according to the first case definition (bacteriologically confirmed pulmonary tuberculosis not associated with human immunodeficiency virus infection). Participants were followed for 3 years after the second dose. Participants with clinical suspicion of tuberculosis provided sputum samples for polymerase-chain-reaction assay, mycobacterial culture, or both. Humoral and cell-mediated immune responses were evaluated until month 36 in a subgroup of 300 participants. Safety was assessed in all participants who received at least one dose of M72/AS01$_E$ or placebo.

RESULTS

A total of 3575 participants underwent randomization, of whom 3573 received at least one dose of M72/AS01$_E$ or placebo, and 3330 received both planned doses. Among the 3289 participants in the according-to-protocol efficacy cohort, 13 of the 1626 participants in the M72/AS01$_E$ group, as compared with 26 of the 1663 participants in the placebo group, had cases of tuberculosis that met the first case definition (incidence, 0.3 vs. 0.6 cases per 100 person-years). The vaccine efficacy at month 36 was 49.7% (90% confidence interval [CI], 12.1 to 71.2; 95% CI, 2.1 to 74.2). Among participants in the M72/AS01$_E$ group, the concentrations of M72-specific antibodies and the frequencies of M72-specific CD4+ T cells increased after the first dose and were sustained throughout the follow-up period. Serious adverse events, potential immune-mediated diseases, and deaths occurred with similar frequencies in the two groups.

CONCLUSIONS

Among adults infected with *M. tuberculosis*, vaccination with M72/AS01$_E$ elicited an immune response and provided protection against progression to pulmonary tuberculosis disease for at least 3 years. (Funded by GlaxoSmithKline Biologicals and Aeras; ClinicalTrials.gov number, NCT01755598.)

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According to the World Health Organization (WHO), tuberculosis remains the leading cause of death from a single pathogen globally, and safe, effective tuberculosis vaccines will be key in ending the epidemic.[1,2] According to the Preferred Product Characteristics published by the WHO, new tuberculosis vaccines for adolescents and adults with or without *Mycobacterium tuberculosis* infection should have at least 50% efficacy against bacteriologically confirmed tuberculosis, and this efficacy should be sustained for at least 2 (and ideally 10) years.[3,4]

The M72/AS01$_E$ candidate vaccine (GlaxoSmithKline) contains a recombinant fusion protein derived from two *M. tuberculosis* antigens (Mtb32A and Mtb39A), combined with the AS01$_E$ adjuvant system. A previously reported proof-of-concept, placebo-controlled, phase 2b trial showed efficacy of two doses of M72/AS01$_E$ in preventing bacteriologically confirmed pulmonary tuberculosis in human immunodeficiency virus (HIV)–negative adults with latent *M. tuberculosis* infection (defined by a positive result on interferon-γ release assay) who had no evidence of active tuberculosis disease.[5] The analysis of the primary trial objective was performed when all participants had completed at least 2 years of follow-up. The vaccine efficacy against bacteriologically confirmed active pulmonary tuberculosis (confirmed with the use of sputum specimens obtained before the commencement of treatment for tuberculosis) was significant (vaccine efficacy, 54.0%; 90% confidence interval [CI], 13.9 to 75.4; 95% CI, 2.9 to 78.2; P=0.04). Injection-site reactions and influenza-like symptoms occurred more frequently in the M72/AS01$_E$ group than in the placebo group, but serious adverse events, potential immune-mediated diseases, and deaths occurred with similar frequencies in the two groups. None of the deaths were considered by the investigators to be related to the trial regimen. We now present the final results of the efficacy and safety analyses after 3 years of follow-up and the results regarding M72-specific humoral and cell-mediated immunogenicity.

Methods

Trial Design, Oversight, and Population

The trial methods have been described in detail previously.[5] Information regarding the composition of the vaccine and the placebo is provided in the Supplementary Appendix and the protocol (both available with the full text of this article at NEJM.org). In brief, this double-blind, randomized, placebo-controlled trial was conducted in three African countries in which tuberculosis is endemic (Kenya, South Africa, and Zambia). The trial was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. The protocol was approved by the ethics committees and regulatory authorities in each participating country. The trial was funded by GlaxoSmithKline Biologicals and Aeras. Authors who are employees of GlaxoSmithKline and Aeras were involved in the conception and design of the trial and the collection, analysis, and interpretation of data, and some of them were part of the core writing team (see the Supplementary Appendix for a list of authors' contributions). All the participants provided written or witnessed oral informed consent.

The trial population consisted of HIV-negative adults 18 to 50 years of age with *M. tuberculosis* infection, as determined by a positive result on interferon-γ release assay (QuantiFERON-TB Gold In-Tube assay [QFT, Qiagen]), who had no signs or symptoms of tuberculosis disease and who had a sputum specimen that was negative for *M. tuberculosis* on a polymerase-chain-reaction (PCR) assay (GeneXpert MTB/RIF, Cepheid) at baseline.

Objectives and Follow-up

The primary objective was to evaluate the efficacy of M72/AS01$_E$ to prevent active pulmonary tuberculosis disease according to the first case definition (bacteriologically confirmed pulmonary tuberculosis not associated with HIV infection and diagnosed with sputum obtained before initiation of treatment for tuberculosis). Cases that occurred from day 30 after the second dose of M72/AS01$_E$ or placebo until month 36 were included in the analyses. A secondary trial objective was to evaluate vaccine efficacy with respect to other case definitions: definite PCR-positive pulmonary tuberculosis disease not associated with HIV infection, diagnosed with sputum obtained before initiation of tuberculosis treatment (second case definition); definite pulmonary tuberculosis disease not associated with HIV infection, diagnosed with sputum obtained up to 4 weeks after initiation of tuberculosis treatment (third case definition); microbiologically confirmed pulmonary tuberculosis disease, diagnosed with

sputum obtained up to 4 weeks after initiation of tuberculosis treatment (fourth case definition); clinical tuberculosis (fifth case definition); and clinical tuberculosis not associated with HIV infection (modified fifth case definition) (Table S1 in the Supplementary Appendix). An additional trial objective was to assess immunogenicity. Data regarding secondary reactogenicity and safety objectives (including potential immune-mediated diseases and serious adverse events) recorded up to month 6 after the second dose were reported previously[5]; data recorded up to month 36 are reported here. Serious adverse events that were considered by the site investigators to be related to the trial regimen and deaths from any cause were recorded until the end of the follow-up period.

Participants were followed for incident tuberculosis by means of visits, telephone calls, text messages, and participant reports for 3 years after the second dose of M72/AS01$_E$ or placebo. In some cases, when participants could not be contacted despite several attempts, the site staff also contacted close family members or friends to obtain new contact details and mitigate the risk of missing important safety information. Participants with clinical suspicion of pulmonary tuberculosis were asked to provide three sputum specimens, which were collected over a period of 1 week, for PCR assay and liquid culture by Mycobacterial Growth Indicator Tube. Specimens obtained before initiation of tuberculosis treatment were acceptable for assessment of the first and second case definitions, and specimens obtained up to 4 weeks after initiation of treatment could be considered for the third and fourth case definitions. Treatment decisions were made by physicians who were not otherwise involved in the trial. Participants with confirmed tuberculosis underwent retesting for HIV, and their glycated hemoglobin levels were measured to screen for diabetes. All participants were retested for HIV at the final trial visit.

EVALUATION OF IMMUNOGENICITY

The immunogenicity cohort included the first 150 participants enrolled at the Kenya Medical Research Institute and the first 150 enrolled in the South African Tuberculosis Vaccine Initiative. Blood samples were obtained before administration of the first dose of M72/AS01$_E$ or placebo, at 1 month after the second dose, and annually until year 3. The total IgG antibodies against the M72 fusion protein were measured with the use of an enzyme-linked immunosorbent assay (ELISA), as described previously[6]; seropositivity was defined as a geometric mean concentration of anti-M72 IgG antibodies of 2.8 or more ELISA units per milliliter. Cell-mediated immunity was assessed on the basis of M72-specific T-cell responses, which were evaluated with the use of a whole-blood intracellular cytokine staining assay, as described previously.[7] In brief, whole-blood specimens were incubated for 2 hours at 37°C with a pool of overlapping peptides covering the M72 protein sequence or with stimulation controls, in the presence of anti-CD28 and anti-CD49d antibodies. A cytokine secretion inhibitor (brefeldin A) was added for an additional 18 hours. After red-cell lysis and cell fixation, an intracellular cytokine staining assay was used to determine CD4+ and CD8+ T-cell expression of CD40L, interleukin-2, tumor necrosis factor α (TNF-α), and interferon-γ. Results are reported as the frequency of CD4+ or CD8+ T cells expressing at least two immune markers (defined as polypositive T cells) or expressing any combination of markers per million CD4+ or CD8+ T cells. A response to the vaccine was defined as a polypositive T-cell frequency that was higher than the 95th percentile of the frequencies in all participants before administration of the first dose.

STATISTICAL ANALYSIS

When the previously reported primary analysis was performed, the members of the trial team were unaware of the trial-group assignments. In contrast, this analysis was performed in a fully unblinded manner. The primary analysis of vaccine efficacy was performed in the according-to-protocol efficacy cohort with the use of Cox proportional-hazards regression models (vaccine efficacy = 1 − hazard ratio), with 90% confidence intervals and P values for Wald tests. Descriptive post hoc 95% confidence intervals are also reported. We used a Cox model with time-dependent covariates (piecewise Cox model) to assess vaccine efficacy according to year of follow-up.

Efficacy was also assessed in the total efficacy cohort, which consisted of all participants who received at least one dose of M72/AS01$_E$ or placebo with the exception of seven participants who were found to have had active tuberculosis disease at baseline or a medical history of active tuberculosis. Immunogenicity was assessed in the according-to-protocol immunogenicity cohort.

Table 1. Vaccine Efficacy of M72/AS01$_E$ as Compared with Placebo against Pulmonary Tuberculosis in Adults with Evidence of Tuberculosis Infection.*

Cohort and Case Definition	M72/AS01$_E$			Placebo			Vaccine Efficacy	
	No. of Participants†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	No. of Participants†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	% (90% CI)	% (95% CI)
According-to-protocol efficacy cohort								
First definition	13	4427.62	0.3 (0.2 to 0.5)	26	4463.06	0.6 (0.4 to 0.8)	49.7 (12.1 to 71.2)	49.7 (2.1 to 74.2)
First definition: sensitivity analysis‡	7	4429.29	0.2 (0.1 to 0.3)	22	4467.51	0.5 (0.3 to 0.7)	68.0 (34.7 to 84.3)	68.0 (25.1 to 86.3)
Second definition	8	4429.69	0.2 (0.1 to 0.3)	21	4467.51	0.5 (0.3 to 0.7)	61.7 (24.1 to 80.6)	61.7 (13.5 to 83.0)
Third definition	19	4427.62	0.4 (0.3 to 0.6)	30	4463.06	0.7 (0.5 to 1.0)	36.3 (–3.2 to 60.7)	36.3 (–13.2 to 64.1)
Fourth definition§	19	4427.62	0.4 (0.3 to 0.6)	32	4463.06	0.7 (0.5 to 1.0)	40.3 (3.8 to 62.9)	40.3 (–5.4 to 66.1)
Fifth definition§	26	4434.21	0.6 (0.4 to 0.8)	38	4472.91	0.9 (0.7 to 1.1)	30.5 (–5.6 to 54.3)	30.5 (–14.4 to 57.8)
Modified fifth definition	25	4434.21	0.6 (0.4 to 0.8)	36	4471.56	0.8 (0.6 to 1.1)	29.5 (–8.2 to 54.1)	29.5 (–17.4 to 57.7)
Total efficacy cohort								
First definition	13	5055.30	0.3 (0.2 to 0.4)	28	5005.18	0.6 (0.4 to 0.8)	54.1 (20.3 to 73.6)	54.1 (11.5 to 76.2)
Second definition	8	5057.38	0.2 (0.1 to 0.3)	22	5011.28	0.4 (0.3 to 0.6)	64.1 (29.1 to 81.8)	64.1 (19.3 to 84.0)
Third definition	20	5055.30	0.4 (0.3 to 0.6)	32	5005.18	0.6 (0.5 to 0.9)	38.2 (1.3 to 61.4)	38.2 (–8.0 to 64.7)
Fourth definition	20	5055.30	0.4 (0.3 to 0.6)	34	5005.18	0.7 (0.5 to 0.9)	41.9 (7.6 to 63.4)	41.9 (–1.0 to 66.5)
Fifth definition	28	5061.90	0.6 (0.4 to 0.8)	38	5016.93	0.8 (0.6 to 1.0)	26.5 (–10.8 to 51.2)	26.5 (–19.8 to 54.9)
Modified fifth definition	27	5061.90	0.5 (0.4 to 0.7)	36	5015.58	0.7 (0.5 to 0.9)	25.2 (–13.8 to 50.8)	25.2 (–23.3 to 54.6)

* The analyses were performed with an unadjusted Cox regression model. The according-to-protocol efficacy cohort included 1626 participants in the M72/AS01$_E$ group and 1663 in the placebo group. The total efficacy cohort included 1783 participants in the M72/AS01$_E$ group and 1783 in the placebo group. Follow-up in the according-to-protocol efficacy cohort started 30 days after the second dose of M72/AS01$_E$ or placebo; in the total efficacy cohort, follow-up began on the day of the first dose. Follow-up in both cohorts ended at the time of the first occurrence of pulmonary tuberculosis that met a case definition. If criteria for a case definition were not met, follow-up ended either at month 36 or at the last contact date or the date of a positive laboratory result for tuberculosis, whichever occurred first. Descriptions of the case definitions are provided in Table S1.

† Shown is the number of participants who met the criteria for the case definition.

‡ The prespecified sensitivity analysis was restricted to participants who met the criteria for the first case definition and presented with tuberculosis that was confirmed by at least two bacteriologic tests.

§ One participant in the M72/AS01$_E$ group who met the criteria for the fifth case definition was positive for the human immunodeficiency virus (HIV), and 2 participants in the placebo group who met the criteria for both the fourth and fifth case definitions were positive for HIV.

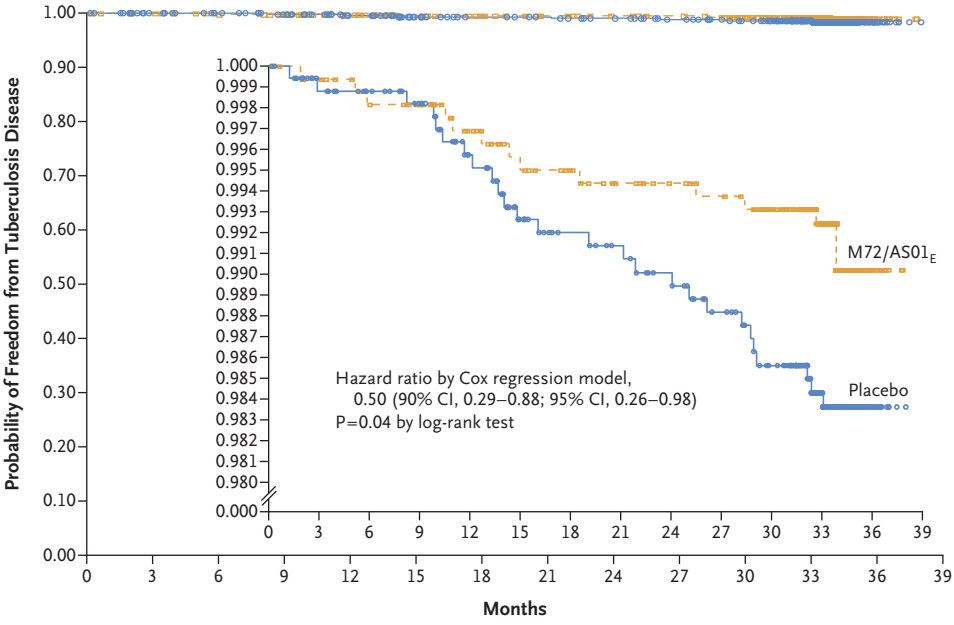


Figure 1. Kaplan–Meier Estimate of Definite Pulmonary Tuberculosis According to the First Case Definition.

Shown is the Kaplan–Meier estimate of the probability of freedom from tuberculosis disease according to the first case definition (bacteriologically confirmed pulmonary tuberculosis not associated with HIV infection, diagnosed before the initiation of treatment for tuberculosis). The analysis was conducted in the according-to-protocol efficacy cohort (3289 participants — 1626 in the M72/AS01$_E$ group and 1663 in the placebo group). The time shown is the time from the beginning of follow-up (i.e., 30 days after dose 2). The inset shows the same data on an enlarged y axis.

Statistical analyses were performed with SAS software, version 9.2 or higher, on the SAS Drug Development system.

RESULTS

TRIAL POPULATION

A total of 3575 participants underwent randomization; 3573 participants received at least one dose of M72/AS01$_E$ or placebo and were included in the total vaccinated cohort, and 3330 of these participants received both planned doses. Demographic characteristics were balanced between the groups (Table S2). The distribution of participants in the cohorts and the reasons for withdrawal from the trial are described in Figure S1. HIV seroconversion occurred in 113 participants during the trial: in 61 of 1462 participants (4.2%; 95% CI, 3.2 to 5.3) in the M72/AS01$_E$ group and in 52 of 1456 participants (3.6%; 95% CI, 2.7 to 4.7) in the placebo group.

VACCINE EFFICACY

The according-to-protocol efficacy cohort included 3289 participants (1626 in the M72/AS01$_E$ group and 1663 in the placebo group). After a mean (±SD) follow-up of 2.7±0.4 years in the M72/AS01$_E$ group and 2.7±0.5 years in the placebo group (median, 2.8 years), 13 cases of active pulmonary tuberculosis in the M72/AS01$_E$ group and 26 cases in the placebo group met the first case definition (Table 1). The incidence of cases of pulmonary tuberculosis that met the first case definition was unchanged from the previous analysis (0.3 cases per 100 person-years in the M72/AS01$_E$ group and 0.6 cases per 100 person-years in the placebo group). The overall vaccine efficacy at month 36 (analyzed with the use of an unadjusted Cox regression model) was 49.7% (90% CI, 12.1 to 71.2; 95% CI, 2.1 to 74.2). Kaplan–Meier curves for the first case definition are shown in Figure 1. The vaccine efficacy estimates according to year were 27.4% for year 1

Table 2. Vaccine Efficacy against Definite Pulmonary Tuberculosis Disease Not Associated with HIV Infection (First Case Definition).*

Covariate and Group	No. of Participants/ Total No.†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	Vaccine Efficacy	
				% (90% CI)	% (95% CI)
Overall					
M72/AS01$_E$	13/1626	4427.62	0.3 (0.2 to 0.5)	49.7 (12.1 to 71.2)	49.7 (2.1 to 74.2)
Placebo	26/1663	4463.06	0.6 (0.4 to 0.8)		
Diabetes					
No					
M72/AS01$_E$	13/1618	4407.23	0.3 (0.2 to 0.5)	49.6 (12.0 to 71.2)	49.6 (2.0 to 74.1)
Placebo	26/1658	4449.07	0.6 (0.4 to 0.8)		
Yes					
M72/AS01$_E$	0/7	17.68	0	Undefined	Undefined
Placebo	0/5	14.00	0		
Sex					
Female					
M72/AS01$_E$	7/680	1857.13	0.4 (0.2 to 0.7)	34.0 (–46.1 to 70.2)	34.0 (–70.2 to 74.4)
Placebo	11/710	1925.22	0.6 (0.3 to 0.9)		
Male					
M72/AS01$_E$	6/946	2570.49	0.2 (0.1 to 0.5)	60.7 (12.9 to 82.2)	60.7 (–1.4 to 84.7)
Placebo	15/953	2537.84	0.6 (0.4 to 0.9)		
Country					
Kenya					
M72/AS01$_E$	2/245	675.16	0.3 (0.1 to 0.9)	–100.8 (–1405.4 to 73.2)	–100.8 (–2114.4 to 81.8)
Placebo	1/248	675.73	0.1 (0.0 to 0.8)		
South Africa					
M72/AS01$_E$	11/1307	3548.10	0.3 (0.2 to 0.5)	53.7 (15.6 to 74.5)	53.7 (5.4 to 77.3)
Placebo	24/1344	3598.50	0.7 (0.5 to 0.9)		
Zambia					
M72/AS01$_E$	0/74	204.35	0	Undefined	Undefined
Placebo	1/71	188.84	0.5 (0.1 to 2.7)		
Current smoker					
Yes					
M72/AS01$_E$	10/831	2247.12	0.4 (0.3 to 0.7)	44.9 (–5.4 to 71.2)	44.9 (–19.4 to 74.6)
Placebo	18/844	2236.74	0.8 (0.5 to 1.2)		
No					
M72/AS01$_E$	3/794	2177.78	0.1 (0.1 to 0.4)	61.7 (–16.6 to 87.4)	61.7 (–44.3 to 89.8)
Placebo	8/819	2226.32	0.4 (0.2 to 0.6)		
Age					
≤25 years					
M72/AS01$_E$	3/706	1911.17	0.2 (0.1 to 0.4)	81.1 (46.9 to 93.3)	81.1 (35.3 to 94.5)
Placebo	16/724	1928.26	0.8 (0.6 to 1.3)		
>25 years					
M72/AS01$_E$	10/920	2516.45	0.4 (0.2 to 0.7)	–0.6 (–109.9 to 51.8)	–0.6 (–141.7 to 58.1)
Placebo	10/939	2534.80	0.4 (0.2 to 0.7)		
≤30 years‡					
M72/AS01$_E$	9/1045	2838.94	0.3 (0.2 to 0.5)	50.0 (2.2 to 74.5)	50.0 (–11.3 to 77.5)
Placebo	18/1069	2847.15	0.6 (0.4 to 0.9)		
>30 years‡					
M72/AS01$_E$	4/581	1588.68	0.3 (0.1 to 0.6)	49.0 (–39.7 to 81.4)	49.0 (–69.4 to 84.6)
Placebo	8/594	1615.91	0.5 (0.3 to 0.9)		

Table 2. (Continued.)

Covariate and Group	No. of Participants/ Total No.†	Person-yr of Follow-up	Rate per 100 Person-yr (90% CI)	Vaccine Efficacy	
				% (90% CI)	% (95% CI)
BCG vaccination§					
No					
M72/AS01$_E$	1/136	372.22	0.3 (0.1 to 1.4)	Undefined	Undefined
Placebo	0/149	402.43	0		
Yes					
M72/AS01$_E$	10/1244	3382.25	0.3 (0.2 to 0.5)	52.8 (11.3 to 74.9)	52.8 (–0.1 to 77.8)
Placebo	21/1250	3358.44	0.6 (0.4 to 0.9)		
Unknown					
M72/AS01$_E$	2/245	670.43	0.3 (0.1 to 1.0)	58.1 (–65.9 to 89.4)	58.1 (–115.9 to 91.9)
Placebo	5/264	702.18	0.7 (0.3 to 1.5)		
Baseline QFT level — IU/ml‡¶					
<4					
M72/AS01$_E$	5/692	1886.81	0.3 (0.1 to 0.6)	56.2 (–6.4 to 82)	56.2 (–26.1 to 84.8)
Placebo	11/680	1820.62	0.6 (0.4 to 1.0)		
≥4					
M72/AS01$_E$	8/934	2540.81	0.3 (0.2 to 0.6)	44.7 (–13.7 to 73.1)	44.7 (–30.5 to 76.5)
Placebo	15/983	2642.44	0.6 (0.4 to 0.9)		
Body-mass index‡‖					
≤25					
M72/AS01$_E$	12/1130	3057.08	0.4 (0.2 to 0.6)	42.1 (–5.6 to 68.2)	42.1 (–18.5 to 71.7)
Placebo	20/1113	2960.81	0.7 (0.5 to 1.0)		
>25					
M72/AS01$_E$	1/495	1367.67	0.1 (0.0 to 0.4)	81.8 (–7.6 to 96.9)	81.8 (–51.2 to 97.8)
Placebo	6/546	1491.10	0.4 (0.2 to 0.8)		

* The analysis was conducted with an unadjusted Cox regression model in the according-to-protocol efficacy cohort.
† Shown is the number of participants who met the criteria for the first case definition and the total number of participants.
‡ The analysis was a post hoc analysis.
§ Bacille Calmette–Guérin (BCG) vaccination indicates documentation of previous BCG vaccination or the presence of a BCG scar.
¶ QFT denotes QuantiFERON-TB Gold In-Tube assay.
‖ The body-mass index is the weight in kilograms divided by the square of the height in meters.

(90% CI, –90.2 to 72.3; 95% CI, –128.8 to 77.0), 55.2% for year 2 (90% CI, –20.2 to 83.3; 95% CI, –45.3 to 86.2), and 60.2% for year 3 (90% CI, –5.4 to 84.9; 95% CI, –27.0 to 87.5).

An analysis of vaccine efficacy at month 36 that used a Cox regression model with adjustment for country (Kenya, South Africa, or Zambia), sex, diabetes status, age (≤25 or >25 years), current smoking status (yes or no), and previous bacille Calmette–Guérin vaccination (yes, no, or unknown) yielded nearly identical results to those of the primary analysis (vaccine efficacy, 49.6%; 90% CI, 11.8 to 71.02; 95% CI, 1.8 to 74.1). We performed a prespecified sensitivity analysis, described previously, that was restricted to participants who presented with cases of tuberculosis that met the first case definition and that were confirmed by at least two bacteriologic tests. The analysis included 7 participants in the M72/AS01$_E$ group and 22 participants in the placebo group; the vaccine efficacy was 68.0% (90% CI, 34.7 to 84.3; 95% CI, 25.1 to 86.3). The vaccine efficacy among participants who met the criteria for the second case definition was 61.7% (90% CI, 24.1 to 80.6; 95% CI, 13.5 to 83.0). Among participants who met the criteria for the other protocol-specified case definitions, the efficacy ranged from 29.5 to 40.3% (Table 1).

Analyses performed in the total efficacy cohort yielded results that were similar to those in the according-to-protocol efficacy cohort. In the total efficacy cohort, the incidence of pulmonary

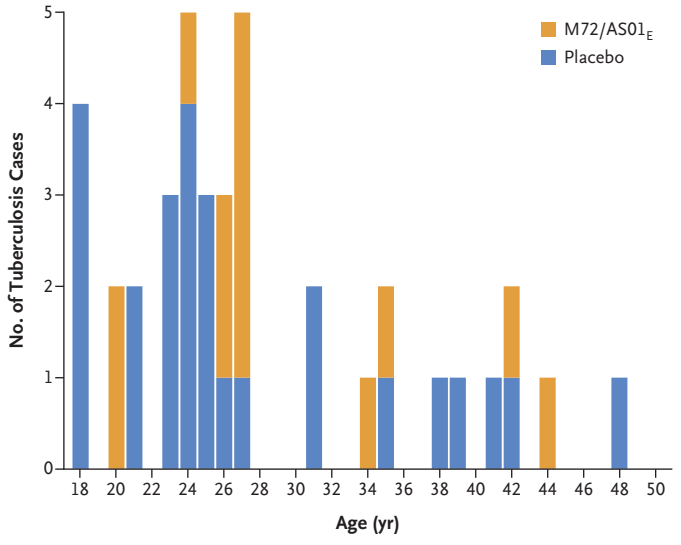


Figure 2. Tuberculosis Cases According to Age at Enrollment (According-to-Protocol Efficacy Cohort).

Shown is a post hoc assessment of the distribution of the 39 cases of tuberculosis that met the first case definition, according to the age of the participant.

Figure 3 (facing page). Immunogenicity Analyses.

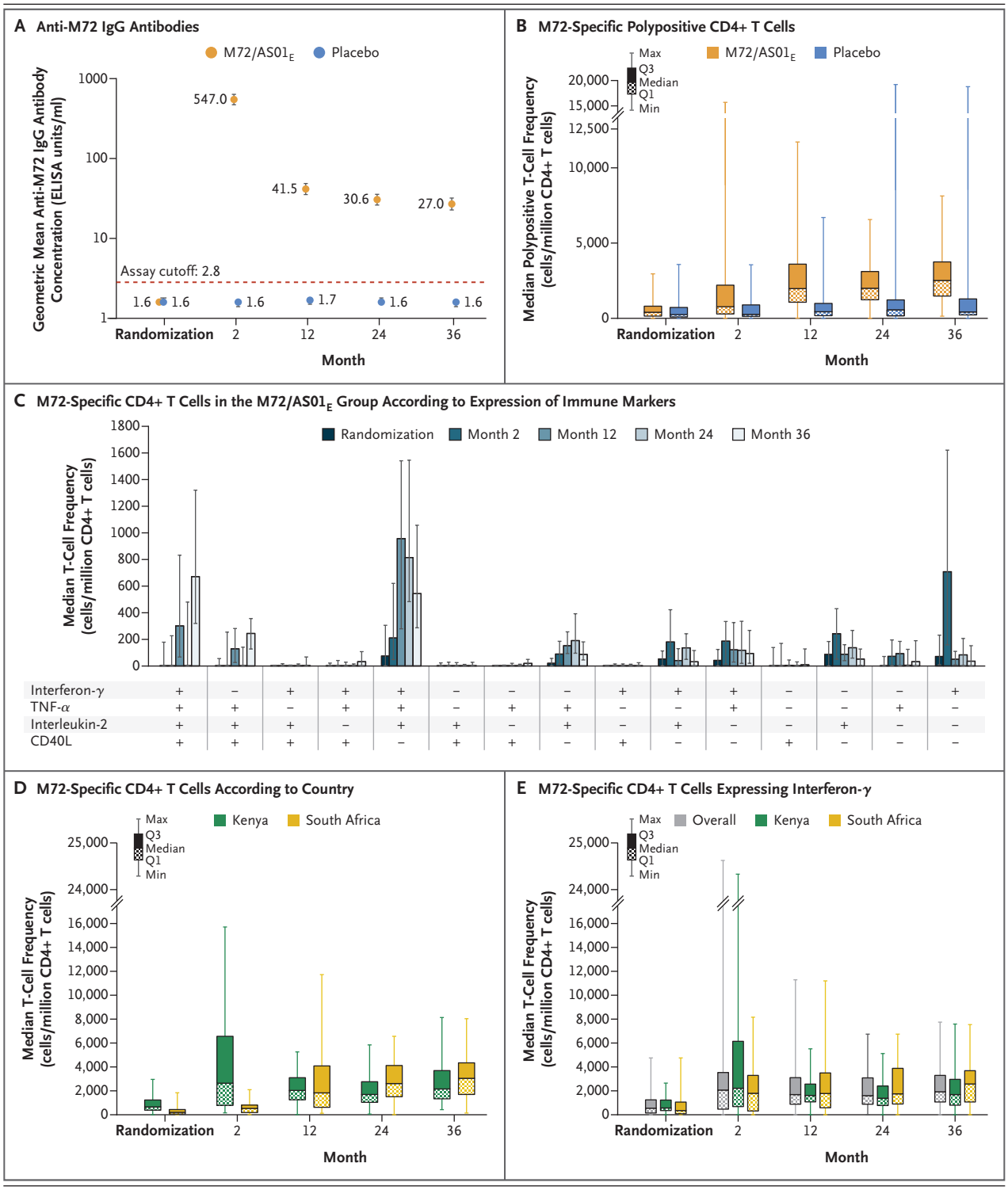
Immunogenicity was assessed in the according-to-protocol immunogenicity cohort (120 participants in the M72/AS01$_E$ group and 124 participants in the placebo group) from the time of randomization (before the first dose of M72/AS01$_E$ or placebo) until month 36. Polypositive CD4+ T cells were defined as those that expressed at least two immune markers (interferon-γ, interleukin-2, tumor necrosis factor α [TNF-α], or CD40L). Panel A shows the geometric mean concentrations of anti-M72 IgG antibodies in the M72/AS01$_E$ group and the placebo group in enzyme-linked immunosorbent assay (ELISA) units per milliliter. The dashed line indicates the assay cutoff to determine seropositivity. For descriptive purposes, all participants who had a value below the assay cutoff were assigned a value of half the cutoff. I bars indicate 95% confidence intervals. Panel B shows the median frequencies of M72-specific CD4+ T cells in the two groups according to expression of immune markers and month. Panel C shows the median frequencies of M72-specific CD4+ T cells expressing any one or any combination of immune markers among participants in the M72/AS01$_E$ group. The I bars indicate the interquartile range. Panel D shows the median frequencies of polypositive M72-specific CD4+ T cells according to country (post hoc analysis). Panel E shows the median frequencies of M72-specific CD4+ T cells expressing interferon-γ overall and according to country (post hoc analysis). Q1 denotes the first quartile, and Q3 the third quartile.

tuberculosis that met the first case definition was 0.3 cases per 100 person-years in the M72/AS01$_E$ group and 0.6 cases per 100 person-years in the placebo group, and the vaccine efficacy was 54.1% (90% CI, 20.3 to 73.6; 95% CI, 11.5 to 76.2) (Table 1).

Results of prespecified subgroup analyses among participants who met the criteria for the first case definition indicated a higher vaccine efficacy among participants who were 25 years of age or younger than among participants who were older than 25 years of age (vaccine efficacy, 81.1% vs. −0.6%) and showed a significant interaction for the difference between the age groups (P=0.02, unadjusted for multiplicity) (Table 2). However, the age distribution among participants who had tuberculosis cases that met the first case definition was unevenly clustered around the cutoff of 25 years (Fig. 2). When we repeated the subgroup analysis with an age cutoff above that cluster (i.e., 30 years), we observed a vaccine efficacy of 50.0% (90% CI, 2.2 to 74.5; 95% CI, −11.3 to 77.5) among participants 30 years of age or younger and 49.0% (90% CI, −39.7 to 81.4; 95% CI, −69.4 to 84.6) among participants older than 30 years of age (Table 2). In addition, a post hoc analysis did not show a significant interaction between age as a continuous variable and group assignment with respect to efficacy. The distribution of QFT levels in positive and negative cases of tuberculosis is provided in Figure S2.

IMMUNOGENICITY

The according-to-protocol immunogenicity cohort included 244 participants (120 in the M72/AS01$_E$ group and 124 in the placebo group) (Table S4). All participants in this cohort were from Kenya or South Africa, and the demographic characteristics were similar to those of the total vaccinated cohort. All participants in the M72/AS01$_E$ group were seropositive by month 2 and remained positive until month 36 (Fig. 3A). Results of post hoc analyses of the geometric mean concentrations of anti-M72 IgG antibodies (stratified according to sex, baseline QFT level [<4 IU per milliliter or ≥4 IU per milliliter], age [≤25 years or >25 years], and country [Kenya or South Africa]) are shown in Figure S3. All 95% confidence intervals overlap between groups, with the exception of the geometric mean concentration of anti-M72 IgG antibodies among participants in South Africa (670.9 ELISA units per milliliter; 95% CI,



527.9 to 852.5) and Kenya (440.4 ELISA units per milliliter; 95% CI, 375.2 to 516.8) at month 2.

Among participants in the M72/AS01$_E$ group, the frequencies of polypositive M72-specific CD4+ T cells increased substantially after administration of the vaccine and persisted through month 36, with no evidence of waning (Fig. 3B). Polypositive M72-specific CD4+ T cells predominantly

expressed interferon-γ, interleukin-2, or TNF-α, or any combination of the three, and CD40L expression was low at all time points (Fig. 3C). The percentage of participants with a response to the vaccine was 23.5% (95% CI, 12.8 to 37.5) at month 2 and 53.7% (95% CI, 39.6 to 67.4) at month 36.

The frequencies of polypositive M72-specific CD4+ T cells were lower at month 2 than at subsequent time points, with a wide interquartile range (Fig. 3B) that coincided with a peak of T cells expressing only interferon-γ (Fig. 3C). The frequency of polypositive T cells was approximately 5 times as high among participants in Kenya as among those in South Africa (median at month 2, 2636.0 vs. 529.0 per million CD4+ T cells) (Fig. 3D), whereas there was little difference between the two countries in the frequencies of T cells that expressed interferon-γ alone or interferon-γ in addition to other immune markers (Fig. 3E). The median frequencies of CD4+ T cells at month 2 that expressed interferon-γ alone were 1134.0 and 450.0 per million CD4+ T cells in South Africa and Kenya, respectively. Results of a post hoc analysis of the frequency of M72-specific CD4+ T cells expressing interferon-γ alone or interferon-γ in addition to other immune markers at month 2 — stratified according to sex, QFT level at baseline (<4 IU per milliliter or ≥4 IU per milliliter), and age (≤25 years or >25 years) — showed no obvious differences among these subgroups (Fig. S4).

There was no significant change in polypositive M72-specific CD4+ T-cell frequencies after administration of placebo at any time point. No CD8+ T-cell responses could be detected in either group.

SAFETY

Two serious adverse events were considered by the investigators to be related to the trial regimen: one case of pyrexia in the M72/AS01$_E$ group (with onset on the day of dose 2) and one case of hypertensive encephalopathy in the placebo group (with onset on the day of dose 1). There were 47 deaths during the trial period: 19 deaths among 1786 participants (1.1%) in the M72/AS01$_E$ group and 28 among 1787 participants (1.6%) in the placebo group (relative risk, 0.68; 95% CI, 0.36 to 1.26; P=0.24). No deaths were determined by the investigators to be related to the trial regimen. The most common cause of death was trauma (in 28 participants). (All serious adverse events reported until month 6 after the second dose are listed in Table S5, all serious adverse events reported throughout the entire trial period in Table S6, and all deaths in Table S7.) Potential immune-mediated diseases were reported in 2 participants in the M72/AS01$_E$ group and in 6 in the placebo group and affected a variety of organ classes (Table S8).

DISCUSSION

The adjuvanted recombinant protein vaccine M72/AS01$_E$, which contains two *M. tuberculosis* antigens, provided approximately 50% protection against progression to active pulmonary tuberculosis for 3 years in *M. tuberculosis*–infected, HIV-negative adults. This protection was observed among participants who met the criteria for the first and second case definitions and was also observed in the sensitivity analysis. These results support further evaluation of M72/AS01$_E$ as a tool for global tuberculosis control and represent progress toward a vaccine that meets the attributes recommended by the WHO for new tuberculosis vaccines targeted at adolescents and adults.[3]

In our previous report, an exploratory subgroup analysis indicated higher percentage estimates for vaccine efficacy among participants 25 years of age or younger than among participants older than 25 years of age.[5] Although this result is still apparent in the final analysis, graphical representation of the distribution of tuberculosis cases according to age at enrollment reveals clustering between 23 and 27 years and suggests that the result is a chance finding (Fig. 2). A post hoc analysis that used age as a continuous variable did not suggest any age effect on vaccine efficacy. Our final data set does not support the hypothesis of differential vaccine efficacy according to age. Similarly, other covariates, such as sex, were not associated with differences in vaccine efficacy in exploratory interaction tests (Table 2).

Humoral responses were consistent with previous experience with M72/AS01$_E$,[8-11] and the responses were sustained until month 36. The persistence of humoral and polypositive cellular responses is noteworthy. These results were generated from a limited subgroup of participants in whom no cases of tuberculosis occurred, and no analysis of associations between the immune response and protection can be made. However,

the majority of participants consented to biobanking of blood specimens for evaluation in a substudy to discover potential immune correlates of risk or protection (ClinicalTrials.gov number, NCT02097095).

We observed lower-than-expected frequencies of polypositive CD4+ T cells at month 2. This was attributable largely to the CD4+ T-cell responses among participants in South Africa, whose CD4+ T-cell responses were characterized by an almost exclusive expression of interferon-γ at month 2 (with no detectable expression of CD40L), and so were not captured within the polypositive T-cell population. This observation of lower-than-expected frequencies of polypositive CD4+ T cells at month 2 is reflected in the overall response rate of 23.5% at that time point. We believe this response rate is artificially low because of the conservative definition of a response (a polypositive T-cell frequency higher than the 95th percentile of the frequencies in all participants before administration of the first dose), and this result contrasts with the 100% seropositivity rate for anti-M72 antibodies at month 2.

By month 12, the cells that had been expressing only interferon-γ had been replaced by cells with polypositive profiles. This predominance of CD4+ T cells producing only interferon-γ at month 2 was not observed in previous analyses involving South African participants.[8,9] In-depth analysis of quality controls did not identify technical problems during collection, processing, or testing of samples obtained at month 2. Although we cannot exclude the possibility of a technical artifact, the different patterns of CD4+ T-cell expression observed between participants in Kenya and those in South Africa may correspond to various states of cellular differentiation resulting from a combination of infection-related factors, vaccine-induced factors, and ethnic-group factors.

In previous studies, M72-specific CD8+ T-cell responses were detected at low levels in blood samples obtained 7 days after the first immunization.[8,9] This time point was not included in the analyses in the current trial, and therefore we do not know whether CD8+ T-cell responses have a role in protection.

The findings during the extended follow-up are consistent with the previously reported safety profile of M72/AS01$_E$. No patterns were evident with respect to the occurrence or the nature of serious adverse events, fatal events, or potential immune-mediated diseases over the trial period.

These results show that vaccine efficacy of M72/AS01$_E$ against pulmonary tuberculosis and vaccine-induced immune responses were sustained for 3 years. These results need confirmation in larger and longer studies conducted in a broader range of populations, including persons who have negative results on interferon-γ release assay, who are of various ethnic backgrounds, who live in various geographic locations, and who are of various age groups.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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REFERENCES

1. Global tuberculosis report 2019. Geneva: World Health Organization, 2019 (https://www.who.int/tb/publications/global_report/en/).
2. Schrager LK, Harris RC, Vekemans J. Research and development of new tuberculosis vaccines: a review. F1000Res 2018;7:1732.
3. Schrager LK, Chandrasekaran P, Fritzell BH, et al. WHO preferred product characteristics for new vaccines against tuberculosis. Lancet Infect Dis 2018;18:828-9.
4. WHO preferred product characteristics for new tuberculosis vaccines. Geneva: World Health Organization, 2018 (http://apps.who.int/iris/bitstream/handle/10665/273089/WHO-IVB-18.06-eng.pdf?ua=1).
5. Van Der Meeren O, Hatherill M, Nduba V, et al. Phase 2b controlled trial of M72/AS01$_E$ vaccine to prevent tuberculosis. N Engl J Med 2018;379:1621-34.
6. Leroux-Roels I, Forgus S, De Boever F, et al. Improved CD4+ T cell responses to Mycobacterium tuberculosis in PPD-negative adults by M72/AS01 as compared to the M72/AS02 and Mtb72F/AS02 tuberculosis candidate vaccine formulations: a randomized trial. Vaccine 2013;31:2196-206.
7. Idoko OT, Owolabi OA, Owiafe PK, et al. Safety and immunogenicity of the M72/AS01 candidate tuberculosis vaccine when given as a booster to BCG in Gambian infants: an open-label randomized controlled trial. Tuberculosis (Edinb) 2014;94:564-78.
8. Penn-Nicholson A, Geldenhuys H, Burny W, et al. Safety and immunogenicity of candidate vaccine M72/AS01E in adolescents in a TB endemic setting. Vaccine 2015;33:4025-34.
9. Day CL, Tameris M, Mansoor N, et al. Induction and regulation of T-cell immunity by the novel tuberculosis vaccine M72/AS01 in South African adults. Am J Respir Crit Care Med 2013;188:492-502.
10. Kumarasamy N, Poongulali S, Beulah FE, et al. Long-term safety and immunogenicity of the M72/AS01E candidate tuberculosis vaccine in HIV-positive and -negative Indian adults: results from a phase II randomized controlled trial. Medicine (Baltimore) 2018;97(45):e13120.
11. Kumarasamy N, Poongulali S, Bollaerts A, et al. A randomized, controlled safety, and immunogenicity trial of the M72/AS01 candidate tuberculosis vaccine in HIV-positive Indian adults. Medicine (Baltimore) 2016;95(3):e2459.