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# placebo-controlled phase 2b trial

in infants previously vaccinated with BCG: a randomised,

Safety and efficacy of MVA85A, a new tuberculosis vaccine,

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# Summary

**Background** BCG vaccination provides incomplete protection against tuberculosis in infants. A new vaccine, modified Vaccinia Ankara virus expressing antigen 85A (MVA85A), was designed to enhance the protective efficacy of BCG. We aimed to assess safety, immunogenicity, and efficacy of MVA85A against tuberculosis and *Mycobacterium tuberculosis* infection in infants.

Methods In our double-blind, randomised, placebo-controlled phase 2b trial, we enrolled healthy infants (aged 4–6 months) without HIV infection who had previously received BCG vaccination. We randomly allocated infants (1:1), according to an independently generated sequence with block sizes of four, to receive one intradermal dose of MVA85A or an equal volume of *Candida* skin test antigen as placebo at a clinical facility in a rural region near Cape Town, South Africa. We actively followed up infants every 3 months for up to 37 months. The primary study outcome was safety (incidence of adverse and serious adverse events) in all vaccinated participants, but we also assessed efficacy in a protocol-defined group of participants who received at least one dose of allocated vaccine. The primary efficacy endpoint was incident tuberculosis incorporating microbiological, radiological, and clinical criteria, and the secondary efficacy endpoint was *M tuberculosis* infection according to QuantiFERON TB Gold In-tube conversion (Cellestis, Australia). This trial was registered with the South African National Clinical Trials Register (DOH-27-0109-2654) and with ClinicalTrials.gov on July 31, 2009, number NCT00953927

Findings Between July 15, 2009, and May 4, 2011, we enrolled 2797 infants (1399 allocated MVA85A and 1398 allocated placebo). Median follow-up in the per-protocol population was  $24 \cdot 6$  months (IQR  $19 \cdot 2-28 \cdot 1$ ), and did not differ between groups. More infants who received MVA85A than controls had at least one local adverse event (1251 [89%] of 1399 MVA85A recipients and 628 [45%] of 1396 controls who received the allocated intervention) but the numbers of infants with systemic adverse events (1120 [80%] and 1059 [76%]) or serious adverse events (257 [18%] and 258 (18%) did not differ between groups. None of the 648 serious adverse events in these 515 infants was related to MVA85A. 32 (2%) of 1399 MVA85A recipients met the primary efficacy endpoint (tuberculosis incidence of  $1 \cdot 15$  per 100 person-years [95% CI  $0 \cdot 79$  to  $1 \cdot 62$ ]; with conversion in 178 [13%] of 1398 infants [95% CI  $11 \cdot 0$  to  $14 \cdot 6$ ]) as did 39 (3%) of 1395 controls ( $1 \cdot 39$  per 100 person-years [ $1 \cdot 00$  to  $1 \cdot 91$ ]; with conversion in 171 [12%] of 1394 infants [ $10 \cdot 6$  to  $14 \cdot 1$ ]). Efficacy against tuberculosis was  $17 \cdot 3\%$  (95% CI  $-31 \cdot 9$  to  $48 \cdot 2$ ) and against *M tuberculosis* infection was  $-3 \cdot 8\%$  ( $-28 \cdot 1$  to  $15 \cdot 9$ ).

Interpretation MVA85A was well tolerated and induced modest cell-mediated immune responses. Reasons for the absence of MVA85A efficacy against tuberculosis or *M tuberculosis* infection in infants need exploration.

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# Introduction

Tuberculosis is a major global health problem, with an estimated 8.7 million cases and 1.4 million deaths in 2011.<sup>1</sup> The Stop TB Partnership developed the *Global Plan to Stop TB: 2006–2015*, with a goal of tuberculosis elimination by 2050.<sup>2</sup> One of the long-term strategies essential for control of the epidemic is effective vaccination. The existing BCG vaccine protects against disseminated tuberculosis in young children,<sup>34</sup> but protection against pulmonary tuberculosis is very variable.<sup>4-6</sup> Efficacy against infection with *Mycobacterium tuberculosis* has only been reported in observational studies in low-burden settings.<sup>7</sup> In endemic countries such as South Africa, the incidence of tuberculosis in infants and young children is very high despite

high BCG coverage.<sup>89</sup> An improved infant tuberculosis vaccination regimen is urgently needed.

12 candidate vaccines are being tested in clinical trials.<sup>10</sup> MVA85A is a recombinant strain of modified Vaccinia Ankara virus expressing the immunodominant *M tuberculosis* protein, antigen 85A.<sup>11</sup> MVA85A has been developed as a heterologous boost for BCG.<sup>11</sup> Boosting BCG with MVA85A improved BCG-induced protection against mycobacterial challenge in animals.<sup>12-15</sup> MVA85A was well tolerated in clinical trials in infants.<sup>11.16.17</sup> Furthermore, a BCG prime-MVA85A boost immunisation regimen in infants induced antigen-specific Th1 and Th17 cells,<sup>16</sup> which are regarded as important in protection against tuberculosis.<sup>18,19</sup>

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Prof Helen McShane, University of Oxford, Jenner Institute, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, UK helen.mcShane@ndm.ox.ac.uk We aimed to further assess safety of MVA85A in HIV-negative infants who were previously vaccinated with BCG. As secondary endpoints, we also aimed to assess efficacy of MVA85A against tuberculosis and *M tuberculosis* infection beyond that of BCG alone, assess immunogenicity of MVA85A, and identify correlates of protection. To our knowledge, our investigation was the first infant efficacy trial of a new tuberculosis vaccine since BCG was last assessed in infants as part of the Chingleput-Madras trial that started in 1968.<sup>20</sup>

## Methods

or

# Study design and participants

We undertook a parallel-group, randomised, placebocontrolled, double-blind phase 2b trial at the South African Tuberculosis Vaccine Initiative (SATVI) site in a rural region near Cape Town, South Africa. The region has a population of about 290 000 people and an annual birth cohort of about 7000 babies. The overall incidence of tuberculosis in South Africa in 2011 was estimated to be almost 1% (993 per 100 000 individuals).<sup>1</sup> The incidence of tuberculosis in children younger than 2 years was about 3% at our trial site.<sup>21</sup>

Parents of recently born infants were approached at local immunisation clinics or at home about study participation. We enrolled healthy infants, aged 4–6 months and who had received BCG (Danish 1331, Statens Serum Institut, Denmark) within 7 days of birth. Infants had to have received all age-appropriate routine immunisations, and two doses of pneumococcal conjugate vaccine at least 28 days before study vaccination (amended to 14 days during enrolment). All infants had to be HIV ELISA negative, QuantiFERON-TB Gold In-tube test (QFT; Cellestis, Australia) negative, and have had no substantial exposure to a patient with known tuberculosis. The appendix contains the study protocol.

See Online for appendix

The trial was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee, Oxford University Tropical Research Ethics Committee, and the Medicines Control Council of South Africa. Parents or legal guardians provided written, informed consent.

## Randomisation and masking

We randomly allocated infants in a 1:1 ratio, with a block size of four, by use of an interactive voice/online response system to receive one intradermal dose of MVA85A ( $1\times10^8$  plaque-forming units in 0.06 mL) or an equal volume of *Candida* skin test antigen (Candin, AllerMed, USA) as placebo. Doses were prepared and labelled in masked syringes by an unmasked study pharmacist. An independent statistician prepared the randomisation schedule. The parents or legal guardians of study participants, study staff administering vaccinations or undertaking follow-up clinic assessments, and laboratory staff were masked to intervention group assignment.

## Procedures

The study design included specific cohorts for specialised analyses, but all participants were followed up for assessment of efficacy and incidence of serious adverse events. Peripheral blood for routine haematological and biochemical tests was taken at screening and on day 7 and day 28 after vaccination in an initial safety cohort of at least 330 infants (study group 1). We assessed immunogenicity in three subsequent cohorts of up to 60 participants with an enzyme-linked immunosorbent spot analysis (study group 2), an intracellular cytokine staining (ICS) assay for peripheral blood mononuclear cell (PBMCs) counts (study group 3), and a whole blood ICS assay (study group 4). We enrolled remaining infants into a fifth cohort (study group 5). PBMCs obtained from all infants before and after vaccination were cryopreserved for future correlates analyses. We did OFT testing at screening, day 336, at the end of study visit, and for infants admitted to a dedicated study ward for investigation for tuberculosis.21

We obtained data for incidence of solicited and unsolicited local (injection site) and systemic adverse events reported by parents or guardians on diary cards for 7 days after vaccination and by direct questioning by study staff for 28 days after vaccination. We also obtained data for serious adverse events throughout follow-up by active surveillance. Adverse events were assessed by the trial investigators and serious adverse events were assessed by the trial investigators and a local medical monitor, acting on behalf of the sponsor, to determine relation to vaccination. The trial investigators and local medical monitor were masked to intervention group throughout the trial. The safety monitoring committee (SMC) did not determine the association or severity of the adverse events. When the last infant in the safety cohort completed day 84, the SMC reviewed unmasked safety data to determine if a pattern of adverse events related to MVA85A or other safety concerns existed so as to advise on further enrolments. The SMC also conducted a second unmasked analysis-by-group safety and risk review after the 1000th infant completed their visit at study day 84.

We actively followed up infants every 3 months to identify any signs, symptoms, or exposure that merited further investigation. Participants who had a persistent cough, failure to thrive, weight loss crossing a major centile band, QFT or tuberculin skin test conversion, household tuberculosis contact, or any other condition causing investigator concern were admitted to the study ward. Standardised investigations involved assessments with chest radiography, tuberculin skin test, QFT, HIV-ELISA, two consecutive early morning gastric lavage samples, and two induced sputa. Gastric lavage and sputum samples underwent auramine smear microscopy, GeneXpert MTB/RIF (Cepheid, USA; routinely from January, 2011, onwards), and MGIT (Becton Dickinson, Sparks, USA) liquid culture and sensitivity testing. Positive samples were speciated by PCR. We developed a hierarchy of three disease endpoint definitions. Endpoint 1 (panel 1) and endpoint 2 (appendix p 49) were based on the presence of specific clinical, radiological, and microbiological findings.<sup>22</sup> Endpoint 2 (which included all infants who met endpoint 1 criteria) had marginally less stringent criteria to define tuberculosis infection and household exposure. Endpoint 3 included all participants placed on treatment for tuberculosis by a health professional. This approach allowed objective case classification without the need for an adjudication committee.

The endpoint of infection with *M* tuberculosis was defined as conversion to a positive QFT test at any time during follow-up. We assessed rates of QFT conversion 1 year after vaccination and at end of study in those participants not previously started on anti-tuberculous treatment.

We measured immunological sensitisation to *M* tuberculosis antigens, suggesting *M* tuberculosis infection, by QFT during screening, 1 year after vaccination, and at the close-out visit. We obtained blood samples from study groups 2–4 for immunogenicity analyses 7 days before vaccination and 7 days or 28 days after vaccination. We assessed immunogenicity with an ex-vivo interferon  $\gamma$  enzyme-linked immunosorbent spot assay, together with PBMC and whole blood ICS assays done as previously described.<sup>23</sup> Further details of the methods are available in the appendix.

## Statistical analyses

The primary study outcome was safety in all vaccinated participants (safety population), including all solicited, unsolicited, and serious adverse events. We compared the proportion of participants with at least one such adverse event in the placebo and MVA85A groups with Fisher's exact test, and we calculated two-sided exact 95% CIs for proportions of individual events within treatment groups. We did immunogenicity analyses for all vaccinated participants enrolled in study groups 2–4. Statistical analyses were prespecified in a statistical analysis plan, signed off prior to study database lock and unmasking of data (appendix).

The primary efficacy outcome was incidence of endpoint 1 and the secondary efficacy outcome was infection with *M tuberculosis*. Endpoints 2 and 3 were exploratory efficacy outcomes. All efficacy analyses were based on the per-protocol population, consisting of all randomly allocated participants who received at least one dose of study vaccine as randomised, and who had no major protocol deviations.

The primary statistical method for analysis of endpoint 1 was vaccine efficacy, defined as 1 minus the estimated hazard ratio based on a Cox regression analysis of time to first diagnosis of endpoint 1. The Cox model contained one indicator variable for treatment group. To investigate the potential effect of variable follow-up times, we also did this analysis with a predefined cutoff of 2 years after vaccination. Analysis of endpoint 1 also included time (months) to initial tuberculosis diagnosis from day of vaccination in each treatment group with the Kaplan-Meier estimate of the survival function by treatment group, and the exact binomial method to estimate vaccine efficacy and its corresponding 95% CI (Clopper-Pearson with mid-p adjustment) conditional on the total number of events. We included participants with more than one diagnosis (eg, a diagnosis of tuberculosis endpoint 2 that was subsequently diagnosed as endpoint 1) in analyses separately for each diagnostic level. For the analysis of secondary and exploratory efficacy endpoints, no adjustment for multiplicity was done. We regarded a twosided p value of less than 0.05 as significant. Summaries were presented for all cases reported during the study, and also, all cases with a diagnosis during the first 2 years of individual follow-up.

For efficacy analyses, we based the sample size calculation on the primary efficacy endpoint of tuberculosis (endpoint 1). We assumed a cumulative tuberculosis incidence of 3% after a median of 18 months' follow-up

#### Panel 1: Definition of endpoint 1

## Any of the following criteria:

- Isolation of Mycobacterium tuberculosis from any site
- Identification of M tuberculosis by an approved molecular diagnostic technique from any site
- Histopathology diagnostic for tuberculosis disease (eg, caseating granulomas)
- Choroidal tubercle diagnosed by an ophthalmologist
- Miliary pattern on chest radiograph in an HIV-negative infant
- Clinical diagnosis of tuberculous meningitis (cerebrospinal fluid protein concentrations >0.6 g/L and pleocytosis of >50 cells per μL with >50% mononuclear cells) with features of basal meningeal enhancement and hydrocephalus on head CT
  Vertebral spondylosis
- One smear or histology specimen positive for auramine-positive bacilli from a normally sterile body site
- One of each of the following:
  - Evidence of mycobacterial infection defined as two acid-fast positive smears (each from a separate collection) that were morphologically consistent with mycobacteria from either sputum or gastric aspirate that were not found to be non-tuberculous mycobacteria bacteria on culture; QuantiFERON-TB Gold In-tube test conversion from negative to positive; or tuberculin skin test ≥15 mm

#### and

- Radiographic findings compatible with tuberculosis defined as ≥1 of the following factors identified independently by at least two of three paediatric radiologists serving on a masked review panel: calcified Ghon focus, pulmonary cavity, hilar or mediastinal adenopathy, pleural effusion, or airspace opacification and
- Clinical manifestations compatible with tuberculosis defined as cough without improvement for >2 weeks; weight loss of >10% of bodyweight for >2 months; or failure to thrive, defined as crossing >1 complete major centile band (<97th–90th, <90th–75th, <75th–50th, <50th–25th, <25th–10th, and <10th–3rd weight-for-age centiles) downward for >2 months

in the placebo group,<sup>21</sup> with an estimated 7.5% loss to follow-up.<sup>24</sup> Thus, 1392 participants per intervention group would provide a 90% chance of detection of a 60% reduction between the intervention and control groups based on a two-sided log-rank test at a significance level of 0.05. We implemented a 6 month extension to the planned follow-up to achieve the target case accrual.

For safety analyses, the sample size of 1392 participants receiving MVA85A would provide a greater than 75% chance of observing an adverse event that had an approximately one in 1000 actual rate of occurrence.



#### Figure 1: Trial profile

\*One infant developed gastroenteritis that precluded inclusion and one infant became ineligible after a randomisation error. QFT=QuantiFERON-TB Gold In-tube.

	Placebo (n=1395)	MVA85A (n=1399)	Overall (n=2794)
Age, days	145.7 (13.5)	146-6 (14-3)	146-2 (13-9)
Sex, male	714 (51%)	708 (51%)	1422 (51%)
Ethnic group			
Black	267 (19%)	287 (21%)	554 (20%)
Mixed race	1126 (81%)	1107 (79%)	2233 (80%)
Asian	1(<1%)	3 (<1%)	4 (<1%)
White	1(<1%)	2 (<1%)	3 (<1%)
Weight			
Infants assessed	1389 (>99%)	1394 (>99%)	2783 (>99%)
Mean, kg	6.47 (0.98)	6.45 (0.99)	6.46 (0.98)
Full-term birth (≥38 weeks)	983 (70%)	1031 (74%)	2014 (72%)
Data are mean (SD) or n (%).			

Table 1: Demographics and baseline characteristics of the per-protocol population

The trial was registered with the South African National Clinical Trials Register on Nov, 4, 2008 (DOH-27-0109-2654), and with ClinicalTrials.gov on July 31, 2009, number NCT00953927.

## Role of the funding source

Aeras was the trial sponsor. Aeras and the Oxford-Emergent Tuberculosis Consortium (OETC) contributed to study design, data interpretation, and writing of the manuscript. MDT, MH, BSL, TJS, MAS, SL, HM, and HMcS had complete access to the data. HMcS had final responsibility for the decision to submit for publication.

## Results

Between July 15, 2009, and May 4, 2011, we obtained consent for 4754 infants. We enrolled 2797 infants who had completed screening when the enrolment target of 2784 was met (figure 1). Reasons for screening failure have been reported elsewhere.<sup>22</sup> 363 infants were entered into study group 1 (initial safety cohort; 182 in MVA85A group and 181 in the placebo group); 54 into group 2 (27 and 27), 54 into group 3 (27 and 27), and 39 into group 4 (19 and 20; immunogenicity groups); and 2287 in group 5 (1144 and 1143; correlates of protection). Follow-up was completed in October, 2012. The perprotocol population was 2794, excluding three participants from the intention-to-treat population (figure 1). The intention-to-treat analysis is not reported.

Demographic and baseline clinical characteristics of the study participants were much the same between groups (table 1). In the per-protocol population, median followup for 1399 recipients of MVA85A was 24·6 months (range 0.2-37.3; IQR 19.2-27.8) and for 1395 controls was 24·6 months (0.3-37.3; 19.2-30.1). The number of participants discontinuing the study did not differ between the two treatment groups (figure 1). 126 infants (5%) were lost to follow-up, 11 died (<1%), and 62 (2%) had consent withdrawn.

At least one local adverse event was reported in 628 (45%) of 1396 controls who received the allocated intervention and 1251 (89%) of 1399 recipients of MVA85A. At least one systemic adverse event was reported in 1059 (76%) controls and 1120 (80%) of recipients of MVA85A. At least one serious adverse event was reported in 258 (18%) controls and 257 (18%) recipients of MVA85A (appendix). No serious adverse events related to vaccine were reported in the MVA85A group, but one serious adverse event regarded as related to placebo occurred in the placebo group (short admission to hospital for fever 4 days after vaccination). 417 (64%) of 648 serious adverse events were acute lower-respiratory-tract infections or gastroenteritis (appendix). Seven (1%) infants died in the vaccine group (two from kwashiorkor, two from nontuberculous meningitis, one from gastroenteritis, one from asphyxia due to drowning, and one from sudden death) and four (<1%) infants died in the placebo group



Figure 2: Vaccine immunogenicity

(A) Frequencies of Ag85A-specific T cells measured by interferon- $\gamma$  enzyme-linked immunosorbent spot assay in infants in study group 2 (27 infants in the MVA85A group and 27 infants in the placebo group) before administration of placebo or MVA85A (day 0) and 7 days after vaccination. (B) Frequencies of cytokine-expressing Ag85A-specific Th1 (CD4-positive T cells expressing IFN- $\gamma$ , TNF $\alpha$ , or interleukin 2) and (C) frequencies of Ag85A-specific Th17 (CD4-positive T cells expressing interleukin 17) cells, measured by whole blood intracellular cytokine staining 28 days after administration of placebo or MVA85A to infants in study group four (17 infants in the MVA85A group and 19 infants in the placebo group). SFC=spot-forming cells. PBMC=peripheral blood mononuclear cell.

(two from gastroenteritis, one from encephalitis, and one from a lower-respiratory-tract infection). During follow-up, 510 (37%) of 1395 recipients of placebo and 507 (36%) of 1399 recipients of MVA85A were admitted to the study ward for investigation.

MVA85A induced an Ag85-specific T-cell response as measured by ex-vivo interferon  $\gamma$  enzyme-linked immunosorbent spot (median 136 spot-forming cells per million PBMCs, IQR 87–362; figure 2). Whole blood ICS showed that these cells were CD4-positive T cells predominantly expressing interferon  $\gamma$ , TNF $\alpha$ , and interleukin 2 (figure 2). We also detected CD4-positive interleukin 17-positive T cells (figure 2), some of which co-expressed Th1 cytokines (data not shown). These responses were not detected in recipients of placebo. No CD8-positive T-cell responses were detectable and no responses were detected with ICS completed on cryopreserved PBMCs (data not shown).

Table 2 shows vaccine efficacy and numbers of infants who met endpoints 1, 2, or 3 by intervention group. For analysis with follow-up data truncated at 2 years after vaccination, vaccine efficacy was 23.9% (95% CI –27.9 to 54.7) for endpoint 1, –0.7% (–52.3 to 33.4) for endpoint 2, and –3.6% (–29.0 to 16.8) for endpoint 3. A post-hoc review of case distribution in the first year showed 16 recipients of placebo met endpoint 1 as did ten MVA85A recipients. Figure 3 shows the Kaplan-Meier survival analysis for endpoint 1.

39 (3%) of 1395 infants assessed in the placebo group had incident tuberculosis (1.39 per 100 person-years [95% CI 1.00 to 1.91]) as did 32 (2%) of 1399 infants in the MVA85A group (1.15 per 100 person-years

	Placebo (n=1395)	MVA85A (n=1399)	Vaccine efficacy
Endpoint 1 (primary efficacy endpoint)	39 (3%)	32 (2%)	17·3% (-31·9 to 48·2)
Endpoint 2 (exploratory efficacy endpoint)	52 (4%)	55 (4%)	-6·9% (-56·1 to 26·9)
Endpoint 3 (exploratory efficacy endpoint)	177 (13%)	196 (14%)	-12·1% (-37·4 to 8·5)

Data are n (%) or % (95% CI). Participants with more than one diagnosis were analysed in each level of diagnosis attained. Vaccine efficacy and corresponding 95% CI was estimated with the Cox regression model (1–estimated hazard ratio).

Table 2: Primary and secondary efficacy endpoints



Figure 3: Cumulative incidence of diagnosis of tuberculosis endpoint 1

[0.79 to 1.62]). 171 (12% [95% CI 10.6 to 14.1]) infants assessed in the placebo group and 178 (13% [95% CI 11.0 to 14.6]) infants in the MVA85A group became

infected with *M* tuberculosis as defined by QFT conversion during the course of the study. Vaccine efficacy against infection was -3.8% (95% CI -28.1 to 15.9). Efficacy was much the same when the comparison was restricted to QFT conversion at day 336 and end of study visit (data not shown).

# Discussion

We report completion of a phase 2b safety and efficacy trial for infants with a new tuberculosis vaccine strategy (panel 2). In this trial, MVA85A was well tolerated and immunogenic in healthy infants who had previously been vaccinated with BCG, with a safety and immunogenicity profile consistent with that reported in other studies of infants.<sup>16,17</sup> However, we noted no significant efficacy against tuberculosis or *M tuberculosis* infection.

This absence of efficacy was not consistent with findings from studies in animals, which suggested potential for efficacy,<sup>12-15</sup> and evidence of immunogenicity in previous clinical trials<sup>16,17,23</sup> that measured immune responses regarded as important for protection.<sup>18,19</sup> Our results suggest that the CD4-positive T cells induced by MVA85A—at least at the modest frequencies noted in this trial—do not correlate with protection against tuberculosis or *M tuberculosis* infection. Frequencies of antigen-specific Th1 cells observed in infants with MVA85A were up to a tenth of the frequencies noted in adults.<sup>16,25</sup>

Our efficacy trial was undertaken in infants. However, this group is not responsible for most transmission of *M tuberculosis*. Thus, MVA85A could potentially protect adolescents or adults against pulmonary tuberculosis, in view of the fact that immunologically immature infants do not respond as well to this vaccine as adults do. MVA85A could also potentially have high efficacy in people of all ages against severe forms of tuberculosis, including pulmonary tuberculosis, without preventing infection or mild forms of disease. A high efficacy against severe disease could be masked in a trial that predominantly detects mild forms of tuberculosis. The sample size of a

#### Panel 2: Research in context

#### Systematic review

To our knowledge, our trial is the first efficacy study of a novel BCG booster tuberculosis vaccine in infants. A systematic review is not applicable.

#### Interpretation

The safety of MVA85A reported in our large cohort is an important finding for tuberculosis vaccine development. However the absence of efficacy noted, despite studies in animals suggesting potential for efficacy and evidence of immunogenicity in previous clinical trials, was unexpected and suggests that the present parameters for selection of tuberculosis vaccine candidates might be inadequate. The relatively weak immunogenicity we noted in this study makes it difficult to conclude whether a higher magnitude response (ie, one that is qualitatively different or a completely new immunological mechanism) will be required for a protective vaccine. Lessons learnt from this trial, including trial design, execution, and vaccine selection, will be of enormous importance to the broader specialty of vaccine development.

trial powered to detect only severe or disseminated disease would be prohibitively large. The safety and immunogenicity of MVA85A alone in infants exposed to HIV is currently being assessed.26 BCG-specific Th1 and Th17 responses were recently shown not to correlate with risk of tuberculosis in infants after BCG vaccination.27 Whether a substantially greater magnitude of response, a response that is qualitatively different, or a completely new immunological response would be necessary for protection is unclear. In our study, frequencies of BCGprimed Ag85A-specific T cells detected before MVA85A vaccination were very low or undetectable (figure 2). Conversely, adults and adolescents have significantly higher Ag85A-specific responses before vaccination,16 which might be an important factor in the stronger responses induced by MVA85A in older individuals. MVA85A was designed to boost BCG-primed responses, and the low frequencies of BCG-induced cells in infants might restrict the immunogenicity, and potentially the efficacy, of MVA85A in this age group. Ongoing assessment of study samples for potential correlates of risk might also yield important insights into why MVA85A did not confer protection in this trial and could add to the design and assessment of the next generation of tuberculosis vaccine candidates. Identification of immune correlates of protection would greatly aid vaccine design and assessment. However such correlates can only be identified in trials in which efficacy was shown. Identification and optimisation of animal models that accurately predict efficacy in human beings is also needed. Other efficacy trials of new HIV and malaria vaccines have reported early but waning efficacy.28,29 In this trial, a posthoc analysis of distribution of case accrual in the first year suggested a possible early effect on disease that merits further study of route of administration, regimen, and dosing strategies with MVA85A and other vaccines.

Despite concerns about potential immunopathology induced by new tuberculosis vaccines,<sup>30</sup> we noted no evidence for this effect. The high incidence of respiratory and gastrointestinal serious adverse events recorded in this trial reflects the known burden of childhood morbidity in this community.<sup>24</sup> High numbers of unrelated serious adverse events should be expected in clinical trials in infant populations in developing countries. The high frequency of mild, self-limiting local reactions in MVA85A recipients is consistent with previous studies.<sup>16,17</sup> These local reactions were only partially controlled for by Candin, a placebo selected for its local reactogenicity profile. The overall safety profile supports modified Vaccinia Ankara virus as a suitable vector for infant vaccination strategies.

The high incidence of disease noted in our study was comparable to the high rates noted in previous trials.<sup>21,24</sup> We noted no confirmed cases of disseminated tuberculosis (two cases of tuberculous meningitis met the definition for endpoint 2) and no deaths from tuberculosis, supporting our previous observation that disseminated and severe

tuberculosis are uncommon in a setting of modern trials with active surveillance, effective isoniazid prophylaxis, and effective anti-tuberculous treatment.<sup>21</sup> The high overall rate of *M tuberculosis* infection noted in this trial (349 [13%] of 2792) suggests a high level of exposure and transmission in this community. This infection burden suggests that M tuberculosis infection might be a suitable endpoint for future trials of new tuberculosis vaccines that aim to prevent infection and subsequent disease. Because BCG is regarded as less effective for prevention of infection than prevention of disease, our finding that MVA85A did not prevent infection is unsurprising and should be interpreted separately from the findings about efficacy against disease. We recognise that QFT has not been validated as a diagnostic test for M tuberculosis infection in infants and young children; however, a previous study<sup>31</sup> done by our group showed good correlation between QFT and the tuberculin skin test.

Our study showed that a large efficacy trial of a new tuberculosis vaccine in a high-burden setting is feasible with a stringent and objective case definition that incorporated the primary elements proposed in a recent consensus statement.<sup>32</sup> We have also shown that standardised investigation for tuberculosis with multiple respiratory sampling, microbiological confirmation of disease, and masked expert panel review of digital radiograph images is feasible in a developing country setting where tuberculosis vaccine efficacy trials are likely to be done. We recognise that there is no gold standard definition of childhood tuberculosis,<sup>33</sup> but we believe that the hierarchal endpoint definition used in this trial is robust and might be suitable for future tuberculosis vaccine trials.

Cohort retention was very high in this trial, and no evidence was noted that the rate of loss to follow-up had a differential effect on case accrual. Similarly, exclusion of three enrolled infants in the per-protocol analysis did not affect the results.

In conclusion, MVA85A was well tolerated, modestly immunogenic but unable to confer significant protection against tuberculosis or *M tuberculosis* infection. The information gained from the successful execution of this study will aid the planning of future trials and vaccination strategies. Substantial global efforts to develop an improved vaccine against tuberculosis must continue.

#### Contributors

All authors, on behalf of the MVA85A 020 Trial Study Team, contributed to study design, data analysis and interpretation, and writing and approval of the manuscript. MDT, MH, TJS, and HM contributed to the implementation of the study and supervision at the study site. MDT, GDH, and HM were the principal investigators. MAS designed and led the statistical analysis.

# The MVA85A 020 Trial Study Team

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#### Conflicts of interest

SL and JES are employees of Emergent BioSolutions and own shares and stock options in the company. HMcS is a shareholder in the Oxford-Emergent Tuberculosis Consortium (a joint venture between Emergent BioSolutions and the University of Oxford). All other authors declare that they have no conflicts of interest.

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