

The novel tuberculosis vaccine, AERAS-402, is safe in healthy infants previously vaccinated with BCG, and induces dose-dependent CD4 and CD8T cell responses



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ARTICLE INFO

Article history:

Received 29 April 2014

Received in revised form 29 August 2014

Accepted 1 September 2014

Available online 10 September 2014

Keywords:

Tuberculosis vaccines

BCG

AERAS-402

Vaccine-induced T cell immunity

Infants

South Africa

ABSTRACT

Background: Efforts to reduce risk of tuberculosis disease in children include development of effective vaccines. Our aim was to test safety and immunogenicity of the new adenovirus 35-vectored tuberculosis vaccine candidate AERAS-402 in infants, administered as a boost following a prime with the Bacille Calmette-Guerin vaccine.

Methods: In a phase 1 randomised, double-blind, placebo-controlled, dose-escalation trial, BCG-vaccinated infants aged 6–9 months were sequentially assigned to four study groups, then randomized to receive an increasing dose-strength of AERAS-402, or placebo. The highest dose group received a second dose of vaccine or placebo 56 days after the first. The primary study outcome was safety. Whole blood intracellular cytokine staining assessed immunogenicity.

Results: Forty-two infants received AERAS-402 and 15 infants received placebo. During follow-up of 182 days, an acceptable safety profile was shown with no serious adverse events or discontinuations related to the vaccine. AERAS-402 induced a specific T cell response. A single dose of AERAS-402 induced CD4T cells predominantly expressing single IFN- γ whereas two doses induced CD4T cells predominantly expressing IFN- γ , TNF- α and IL-2 together. CD8T cells were induced and were more likely to be present after 2 doses of AERAS-402.

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Conclusions: AERAS-402 was safe and immunogenic in healthy infants previously vaccinated with BCG at birth. Administration of the highest dose twice may be the most optimal vaccination strategy, based on the induced immunity. Multiple differences in T cell responses when infants are compared with adults vaccinated with AERAS-402, in the same setting and using the same whole blood intracellular cytokine assay, suggest specific strategies may be important for vaccination for each population.

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1. Introduction

Tuberculosis (TB) remains a major pandemic. In 2012, 8.6 million new cases of TB disease were recorded, with 1.3 million deaths [1]. Modelling studies show that early diagnosis, optimal treatment and effective vaccines may significantly impact the pandemic [2,3]. Among these, effective TB vaccine is the most sustainable intervention [2,4].

AERAS-402, a novel TB vaccine designed to boost immunity induced by Bacille Calmette-Guerin (BCG), is a live replication-deficient, recombinant adenovirus serotype 35 (rAd35), expressing the immunodominant antigens Ag85A, Ag85B and TB10.4 of *Mycobacterium tuberculosis* (*M.tb*) and BCG. AERAS-402-induced immunity was shown to confer protection from TB in murine models [5]. In early phase trials of human adults, AERAS-402 was shown to be safe and induced robust CD4 and CD8T cell responses [6,7].

Children bear 6% of the global TB burden [1]. Infants under 1 year of age have up to 50% risk of developing TB disease within 2 years of *M.tb* infection, which is much higher than the ~5% risk in newly infected adults [8]. This vulnerability may be explained, in part, by relative immaturity of the immune system; the latter may also manifest with lower vaccine-induced T cell responses, compared with adolescents or adults [9–12]. CD4T cell immunity, and in particular capacity of these cells to make type 1 cytokines is necessary for protection against TB [13]. CD8T cells may also contribute to protection [14].

We aimed to assess the safety of AERAS-402 in infants and to assess whether the vaccine induce T cell responses following a BCG prime.

2. Materials and methods

2.1. Study design, setting and regulatory approvals

We conducted a phase I, randomized, double-blinded, placebo-controlled study at the South African Tuberculosis Vaccine Initiative (SATVI) clinical trial site near Cape Town, South Africa (SA). The area has high infant TB incidence, exceeding 1% per year [15]. The study protocol was approved by the Medicines Control Council of SA and the Human Research Ethics Committee of Faculty of Health Sciences of the University of Cape Town, and was registered with the SA National Clinical Trial Register (DOH-27-0209-2655).

2.2. Enrolment and vaccination

Infants were eligible if: aged between 6 and 9 months old; weight and length between 10th and 90th centile for age; proof of BCG vaccination at birth; good health on history and examination including no history or evidence of active TB and absence of HIV and hepatitis B infection; not from a residence where an individual had active TB; no history of anti-TB treatment; normal haematological and biochemistry indices.

Infants were sequentially allocated to four groups: group 1 were randomized to receive one intramuscular (thigh) dose of 1.5×10^8 viral particles (vp) of AERAS-402, or placebo, on study day 0, group 2 received 1.5×10^9 vp or placebo once, group 3,

1.5×10^{10} vp or placebo once, and group 4, 1.0×10^{11} vp or placebo twice, on days 0 and 56 (second dose in the contralateral thigh). The placebo in group 1 was a heptavalent pneumococcal vaccine (Prevenar®), whereas normal saline was used in subsequent groups, due to an AERAS-402 formulation change and an introduction of routine pneumococcal vaccination in SA, after group 1 enrolment.

2.3. Follow-up and safety evaluation

Follow-up visits occurred on days 7, 14, 28, 56, 84 and 182; infants in group 4 were also seen on days 63 and 70. Safety testing occurred on days 0 and 7 for all subjects, after vaccination. Investigators recorded AEs up to 28 days after each vaccination, and serious AEs throughout the follow-up period. All AEs were assessed for causality and severity, using predefined criteria. After completion of the study visits, all study participants were offered the varicella vaccine (Varilix®).

2.4. Whole blood intracellular cytokine staining (WB ICS) assay

Frequencies of specific T cells on study days 0, 28, 84 (group 4 only) and 182 was determined as previously described [16]. Briefly, 1 mL whole blood was incubated with either peptide pool of Ag85A/b (entire Ag85A pool together with dissimilar peptides of Ag85B), or with peptide pool of TB10.4 (peptide pools were each used at a concentration of 2 µg/mL) or with PHA (positive control, at a concentration of 5 µg/mL), or left unstimulated (negative control). The co-stimulatory antibodies of anti-CD28 and anti-CD49d (both from BD Biosciences, 0.5 µg/mL) were included in all assay conditions. Later, cells were stained with conjugated antibodies against the following human molecules: CD3 (Pacific Blue, BD Biosciences, MOPC-21), CD4 (QDot605, Invitrogen, S3.5), CD8 (Cy5.5PerCP, BD Biosciences, SK1), Ki67 (PE, BD Biosciences, B1), IFN-γ (Alexa 700, BD Biosciences, B27), TNF-α (B27, TeBioscience, MAb11, IL-2) (FITC, BD Biosciences, 5344.111) and IL-17 (Alexa 647, eBioscience, eBio64CAP17). Stained cells were acquired on a LSRII flow cytometer (BD Biosciences).

2.5. PBMC ICS assay

PBMCs were thawed, counted and rested overnight. Stimulation occurred in duplicate with peptide pools (Ag85A, Ag85B, and TB10.4), (each peptide at a concentration of 1.0 or 2.5 µg/mL), DMSO (negative control), or *Staphylococcal enterotoxin A* and *B* (SEA/SEB), (Sigma, St. Louis, MO; positive control, at a concentration of 3 µg/mL) for 6 h in the presence of anti-CD28 and anti-CD49d (both from BD Biosciences, San Jose, CA, 0.2 µg/mL) and Brefeldin-A (BD Biosciences, 1:200 v:v). Later, cells were stained with LIVE/DEAD aqua viability dye (Life Technologies), and antibodies against the following human molecules: CD3-APC-Cy7 (Clone SK7), CD4-V450 (Clone RPA-T4), CD8-PE-Cy5 (Clone HIT8a), IFN-γ-APC (Clone B27), TNF-FITC (Clone MAb11), and IL-2-PE (Clone MQ1-17H12) all from BD Biosciences. Stained cells were acquired on a BD LSR II flow cytometer.

2.6. Data analysis

Results from placebo recipients were combined into one group.

For safety evaluation, the percentage of AEs and total number of AEs were presented, with between-treatment group comparisons. The total number of subjects with at least one AE reported per preferred term was performed using Fisher's exact test (Stata v.11, MP-Parallel Edition, StataCorp).

For immunogenicity, flow cytometry data were generated using FlowJo, v9.4.11 (Treestar). Cytokine-producing cells were first identified (Supplementary Figs. 1A and B). Frequencies of vaccine-specific T cells were defined by subtracting frequencies of cytokine-expressing T cells in the negative control from frequencies of Ag85A/b- and TB10.4-peptide pool incubated blood. Test sample was excluded if: (a) the frequencies of the PHA-induced total cytokine-expressing CD4T cells in a sample (positive control) was less than the value obtained from this calculation: median of the frequencies of total cytokine-expressing CD4T cells in all unstimulated (negative controls) samples + 3 median absolute deviations of the frequencies of total cytokine-expressing CD4T cells in all unstimulated samples and (b) the PHA-stimulated total cytokine-expressing CD4T cell response and/or the BCG-induced CD4T cell response was lower than that of the negative control of the same participant. All samples satisfied the inclusion criteria. Therefore, all samples were included for the final analyses.

Statistical analysis was performed with Prism, v.5 (GraphPad Software, Inc). Differences between AERAS-402 and placebo vaccinated infants from more than two groups were assessed using the Kruskal–Wallis test. The Friedman repeated-measures analysis of variance was used to examine post-vaccination differences within a group. If these overall tests showed statistical significance ($p < 0.05$), post-hoc comparisons were performed to compare responses between individual groups using a Mann–Whitney U test, or pre-vaccination vs post-vaccination responses within-group comparisons using a Wilcoxon matched-pairs test. The Spearman test was used to assess associations between pre-vaccination and post-vaccination vaccine-induced T cell response. Fold change (FC) was calculated by dividing the response post-vaccination with the response at day 0. Within each group, the Wilcoxon matched-paired test was used to compare median fold change in the frequencies of total cytokine-expressing T cells at each individual time point. A p -value of <0.05 was considered significant.

3. Results

3.1. Participants

Between March and October 2009, we obtained consent for participation of 179 infants. Following screening, 57 were enrolled. Fig. 1 and Supplementary Table 1 contain data of initial inclusion, randomization, follow-up and inclusion or exclusion from analysis.

Table 1
Demographics of 54 participants whose results were analysed.

	Placebo (N = 14)	Group 1 1.5×10^8 vp (N = 10)	Group 2 1.5×10^9 vp (N = 10)	Group 3 1.5×10^{10} vp (N = 10)	Group 4 1.5×10^{11} vp twice (N = 10)
Male, n (%)	6 (43%)	5 (50%)	4 (40%)	7 (70%)	5 (50%)
Median age in days (range)	225 (179–263)	209 (179–244)	193 (175–243)	219 (189–242)	220 (184–258)
Race, n (%)					
Black	2 (14.3%)	4 (40%)	1 (10%)	1 (10%)	0 (0%)
Mixed race	12 (85.7%)	6 (60%)	9 (90%)	9 (90%)	10 (100%)
Median weight for age z score (range)	−0.25 (−1.15 to 1.57)	0.38 (−1.23 to 1.03)	−0.075 (−1.6 to 0.75)	0.61 (−2.42 to 1.1)	−0.43 (−1.05 to 1.05)

There were no statistically significant differences between the five groups (data not shown).

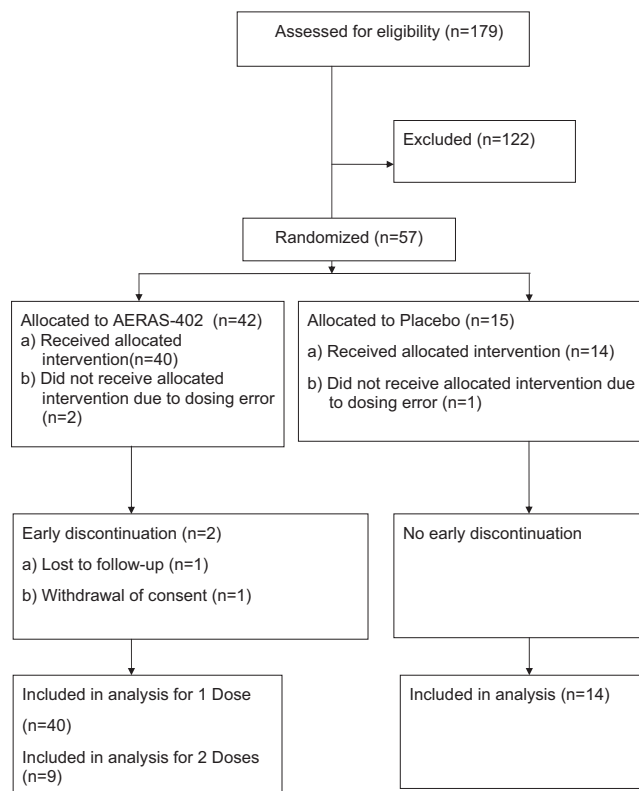


Fig. 1. Consort diagram of the participants assessed for eligibility, enrolled and randomized, and included and excluded in the final analyses.

Three infants from group 4 inadvertently received half the intended volume of the study vaccine at day 56, resulting in exclusion. The excluded infants completed follow-up as per the study protocol, but were replaced following screening of 10 additional infants in February 2010. Two infants from group 4 did not receive the second vaccination on day 56: one was withdrawn by the legal guardian and one failed to meet eligibility criteria (weight below the 10th centile on the vaccination day). One infant from group 2 did not complete the day 182 visit due to relocation. Demographic characteristics of the 54 infants whose results were analysed are shown in Table 1.

3.2. Safety

At least one AE, solicited or unsolicited, was reported in 39 (92.9%) of the 42 AERAS-402 recipients, and by 12 (80.0%) of the 14 infants who received placebo (Table 2 and Supplementary Table 2). All solicited AEs were mild or moderate in severity. The most frequently reported local solicited AE after the first vaccination with AERAS-402 was tenderness at injection site [$n = 13$ (32.5%)]; this AE

Table 2
Adverse events recorded within 28 days of vaccination.

	Placebo (N = 14)	Group 1 1.5 × 10 ⁸ vp (N = 10)	Group 2 1.5 × 10 ⁹ vp (N = 10)	Group 3 1.5 × 10 ¹⁰ vp (N = 10)	Group 4 1.0 × 10 ¹¹ vp twice: after 1st dose (N = 10)	Group 4 1.0 × 10 ¹¹ vp twice: after 2nd dose (N = 9)	Total adverse events (n)
Number of adverse events recorded in group, n (% of total number of adverse events, n)	30 (20.1%)	15 (10.1%)	25 (16.8%)	26 (17.4%)	35 (23.5%)	18 (12.1%)	149
Number of participants in group experiencing adverse events, n (% of total number of participants in group, N)	12 (85.7%)	10 (100%)	9 (90.0%)	9 (90.0%)	9 (90.0%)	6 (66.7%)	55
Severity, n (% of total number of adverse events recorded in group)							
Mild	24 (80.0%)	14 (93.3%)	22 (88.0%)	21 (80.7%)	28 (80.0%)	12 (66.7%)	121
Moderate	6 (20.0%)	1 (6.7%)	3 (12.0%)	2 (7.7%)	6 (17.1%)	5 (27.8%)	23
Severe	0	0	0	3 (11.5%) ^a	1 (2.9%)	1 (5.6%)	5
Relatedness, n (% of total number of adverse events recorded in group)							
Definitely	2 (6.7%)	0	4 (16%)	2 (7.7%)	8 (22.8%)	4 (22.2%)	20
Probably	3 (10%)	0	2 (8%)	0	5 (14.3%)	3 (16.7%)	13
Possibly	13 (43.3%)	8 (53.3%)	10 (40%)	9 (34.6%)	9 (25.7%)	9 (50%)	58
Unlikely	5 (16.7%)	5 (33.3%)	4 (16%)	11 (42.3%) ^b	3 (8.6%)	1 (5.5%)	29
Not	7 (23.3%)	2 (13.3%)	5 (20%)	4 (15.4%)	10 (28.6%)	1 (5.5%)	29

^a Placebo vs. group 3: $p = 0.08$.

^b Placebo vs. group 3: $p = 0.043$.

was not present in the placebo group. The severity of injection site tenderness increased with increasing dose of AERAS-402: experienced by 0 (0%), 4 (40.0%), 2 (20.0%), and 7 (70.0%) participants in groups 1, 2, 3 and 4, respectively. However, all cases were mild and self-limited. No increase in solicited AEs was reported in infants who received a second dose of AERAS-402 (group 4): after the second dose, 4 (44.4%) participants reported at least one AE, compared with 7 (70.0%) of these participants after the first dose.

The most common systemic solicited AE in all the AERAS-402 groups was rhinitis ($n = 25$, 59.5%), compared with 4 (28.6%) events in the placebo group. Ten episodes of fever were reported in 9 (21.4%) participants who received AERAS-402 and in 2 (14.3%) who received placebo. This event was most common in infants who received the highest dose (group 4); however, only one participant experienced fever after both doses. Fever was mild (38.0–38.4 °C) or moderate (38.5–40.0 °C) and resolved within 1 to 3 days of onset (Supplementary Table 2).

After vaccination on day 0, at least 1 unsolicited AE was reported in 34 (81.0%) participants who received AERAS-402 and in 11 (73.3%) who received placebo (Supplementary Table 2). Malaise, invariably mild and self-limited, was more common in group 4 ($n = 7$, 58.3%) than in any other group. In group 4 participants, no increase in unsolicited AEs was demonstrated after the second dose of AERAS-402. Rates of laboratory abnormalities were similar between AERAS-402 and placebo participants, and were mostly mild and transient (Supplementary Table 2). One infant in group 2 showed a mildly decreased neutrophil count while in group 4, a 195-day-old participant, had a decreased neutrophil count (0.47×10^9 cells/L) 7 days after the second dose of AERAS-402. The event resolved within 8 days of onset, with no treatment, at which time the neutrophil count had risen to 3.12×10^9 cells/L.

Five AERAS-402 recipients had a total of seven serious adverse events (SAEs), all requiring hospitalisation, but none considered related to the vaccine. In group 1, the SAEs were upper respiratory tract infection and post-infectious peripheral neuropathy, while group 3 had three episodes of hospitalisation for gastroenteritis and two for urinary tract infection. All SAEs occurred in the AERAS-402 recipients and none in Placebo group. The respiratory tract infection was recorded 18 days-post vaccination; the three SAEs for gastroenteritis was at 33, 157 190 days post-vaccination. On evaluations, it was decided that these episodes were due to intercurrent illness experienced by infants of this age in this community and were not unexpected. Three of these SAEs (two cases of gastroenteritis and one case of urinary tract infection) were also graded as severe. Two additional non-serious SAEs were also reported, both in group 4 (increased white blood cell counts considered due to infected insect bites and decreased neutrophil count seven days post second dose, possibly related to study vaccine).

3.3. CD4 and CD8T cell responses: WB ICS

We measured frequencies of T cells expressing IFN- γ , TNF- α , IL-2 and IL-17 alone or in combination after incubation of whole blood with vaccine antigens. In groups 2, 3 and 4, increases in Ag85-specific (Fig. 2A and C) and TB10.4-specific (Fig. 2B and D) CD4T cells were shown, peaking 28 days post-vaccination in vaccine recipients but not in placebo recipients (Fig. 2A and B). AERAS-402 induced both CD4 (Fig. 2A and B) and CD8T cells (Fig. 2C and D). Overall, induction of CD4T cells was greater than that of CD8T cells (Supplementary Fig. 2A–F).

AERAS-402-induced CD4T cells that expressed IFN- γ and/or TNF- α and/or IL-2, but little or no IL-17, a cytokine which may also have a role in immunity against TB [17] (Fig. 3A–D). The majority of

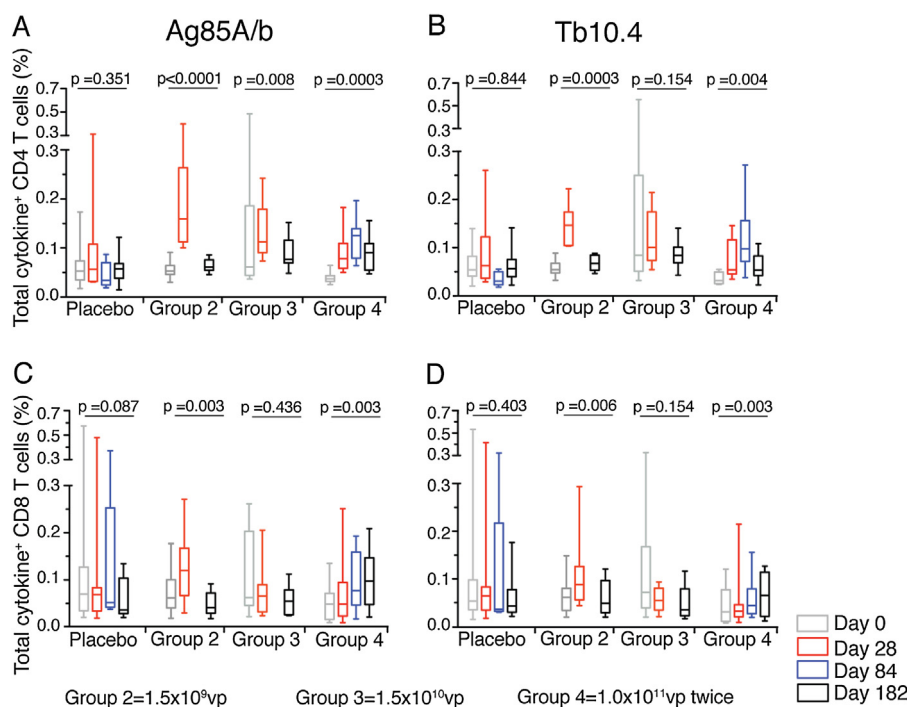


Fig. 2. Frequencies of specific T cells induced by AERAS-402. Specific CD4T cells ((A) and (B)) and CD8T cells ((C) and (D)) were defined by expression of any cytokine following incubation with Ag85A/b ((A) and (C)) or TB10.4 ((B) and (D)) in a whole blood intracellular cytokine staining assay. Frequencies of cytokine-expressing cells in the unstimulated control were subtracted to calculate the shown results. Frequencies are shown for pre-vaccination (day 0) and for post-vaccination time points; participants received either placebo or one of three doses of AERAS-402 (see Section 2, also for number of participants). Medians, interquartile ranges and ranges are shown with the boxplots. The Kruskal–Wallis test was used to assess differences within groups over for each immunogenicity time.

specific CD8T cells expressed IFN- γ , Fig. 3E); very little TNF- α , IL-2 or IL-17 induction was observed (data not shown). The patterns of the specific cytokine expressing T cells were similar regardless of the stimulation with Ag85A/b or TB10.4 peptide pools (data not shown).

3.4. CD4 and CD8 T cell responses: PBMC ICS

We also measured the AERAS-402-induced CD4 and CD8T cell responses by ICS following stimulation of PBMCs with peptide pools of Ag85A, Ag85B and TB10.4. Within each group and time point, we used Wilcoxon matched paired test to compare CD4 and CD8T cell responses. At the peak of the response, the vaccine-induced CD8T cell response appeared more dominant than CD4T cell response, but only significant in group 4 and after the second vaccination (Fig. 4A and B). A second dose in the highest dose group resulted in an increased T cell response (Fig. 4A–C). Overall, irrespective of the vaccine dose, single AERAS-402 vaccination generally induced very low antigen-specific T cell responses detected by PBMC ICS assay.

3.5. Effects of vaccine dose and repeated vaccination on specific T cell responses

Single vaccination at lower AERAS-402 doses induced Ag85-specific (Fig. 5A and C) and TB10.4-specific (Fig. 5B and D) CD4T cells that predominantly expressed IFN- γ alone, Fig. 5A–D). Repeat vaccination at the highest dose induced a predominantly polyfunctional CD4T cell population, i.e., cells expressing all 3 type 1 cytokines together (Fig. 5E and F). In both groups 2 and 3, frequencies of polyfunctional CD4T cells were not significantly greater at 182 days post-vaccination, than at day 0 (pre-vaccination). In contrast, participants in group 4 showed significantly greater frequencies of polyfunctional CD4T cells at 182 days post-vaccination than at day 0.

3.6. Effect of pre-existing mycobacteria-specific T cell immunity on vaccination-induced T cell responses

Mycobacteria-specific immunity present before boost vaccination may influence vaccine-induced T cell response. We observed a positive correlation between baseline mycobacteria-specific CD4 and/or CD8T cell frequencies and AERAS-402-induced response magnitude at day 28 (Fig. 6A–D).

4. Discussion

We showed AERAS-402 is safe and well tolerated, regardless of escalating dose or number of administrations. With WB ICS assay, the vaccine-induced CD4T cells producing type 1 cytokines were most prominent, although CD8T cells were also induced. In contrast, with PBMC ICS assay, CD8T cells dominated the specific response, but only with the highest dose and after a second vaccination. We also showed a positive correlation between pre-existing mycobacteria-specific T cell frequency and the vaccine-induced T cell response.

In our study, AERAS-402 safety profile was similar to that shown in two previous adult trials of the same vaccine [6,7]. Administered intradermally, MVA85A, a pox viral-vectored TB vaccine results in local reactions to nearly 90% of infant recipients [15]. By contrast, only 12 of 40 (30%) of our infant recipients of AERAS-402 experienced a local reaction. The dose association with local pain, tenderness and erythema has also been described in other clinical trials of adenovirus-35-vectored vaccines [6,7,18]. In these trials, it appeared systemic symptoms such as headache, fever, malaise, myalgia and chills were dose-related; in our infant cohort, fever appeared more common in the highest dose group.

We demonstrated a possible association between AERAS-402 administration and rhinitis or upper respiratory tract infections;

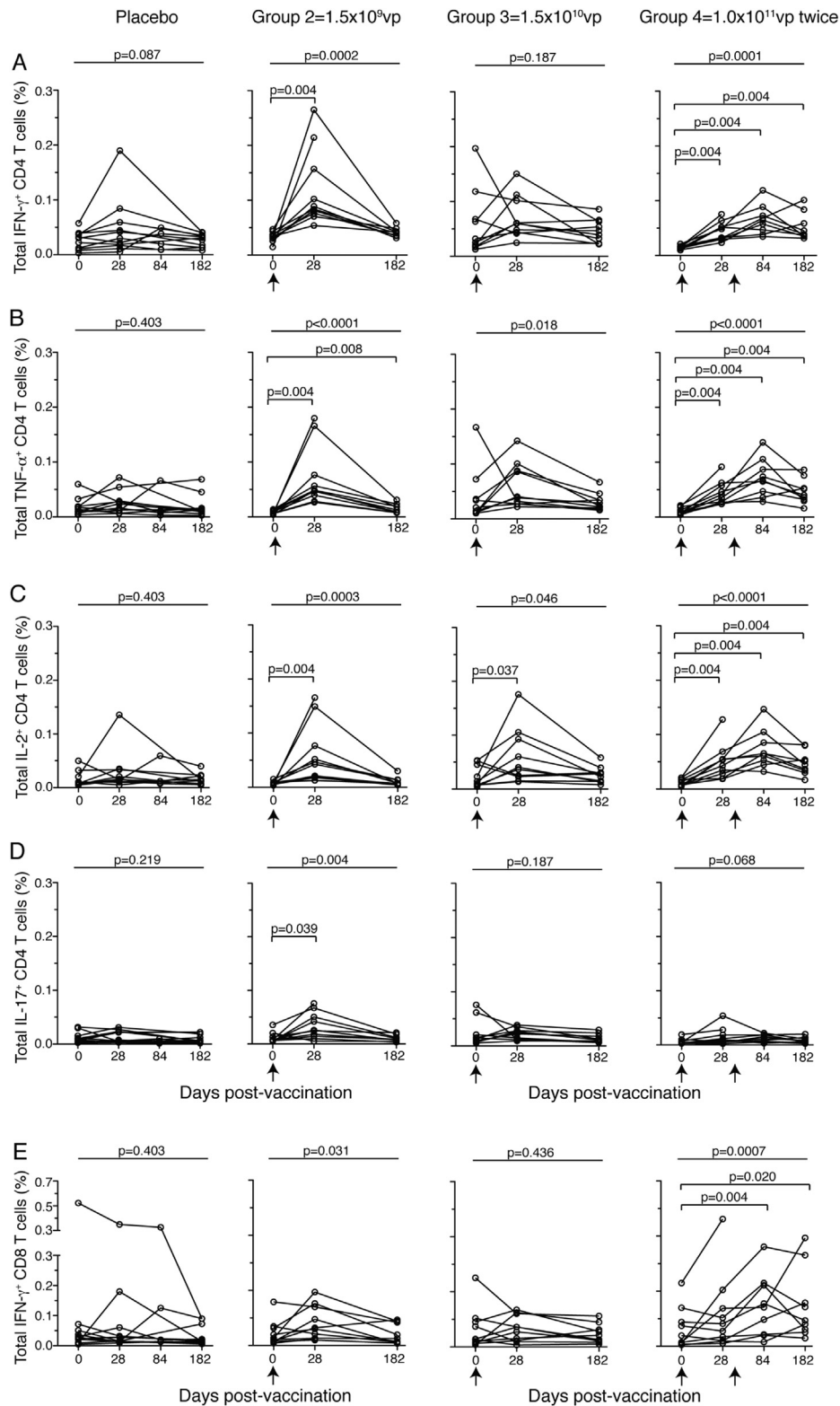


Fig. 3. Frequencies of T cells expressing different cytokines after AERAS-402 vaccination. Whole blood was incubated with peptide pools of Ag85A/b and the frequencies of CD4T cells producing IFN- γ (A), TNF- α (B), IL-2 (C), IL-17 (D), and of CD8T cells producing IFN- γ (E) measured on days 0, 28, 84 (group 4 only) and 182 post-vaccination with AERAS-402 or placebo. The Kruskal–Wallis test was used to assess differences within groups over time. Differences between individual time points were assessed using the Wilcoxon matched pairs test. Only significant differences are shown.

interestingly, this was also shown in previous adult trials of AERAS-402 [6,7]. Results from adenovirus-5-vectored vaccine trials have also shown similar associations although causal relationships have been questioned, due to seasonal associations with outcomes

[19–22]. Although the SAEs were not unexpected, and thought not to be due to vaccination, larger clinical trials would have to address whether there is a specific association between this vaccine and respiratory or gastrointestinal tract infections. When adenovirus

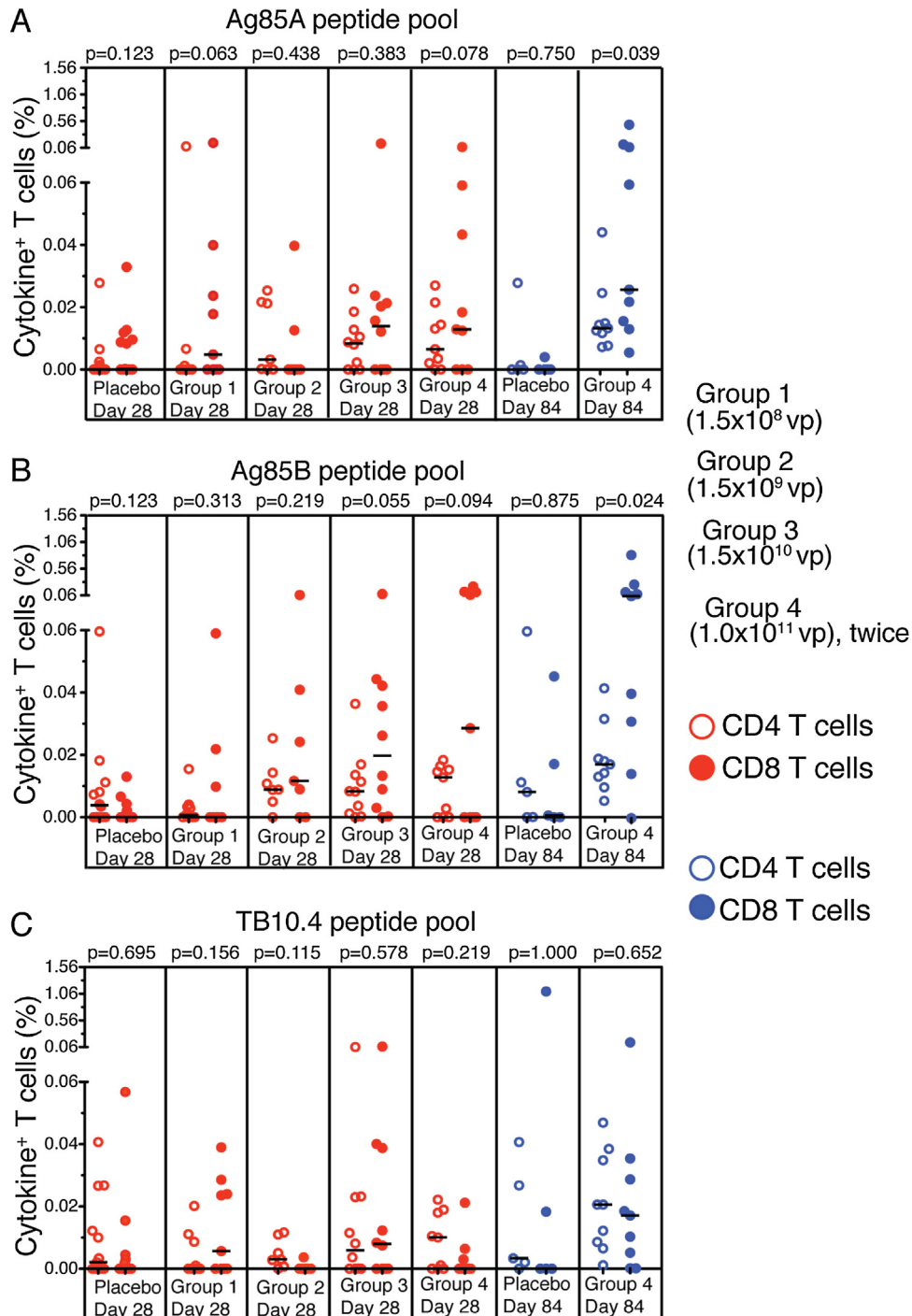


Fig. 4. Comparison of the frequencies of specific CD4 and CD8 T cells measured by a PBMC intracellular cytokine staining assay at the peak of the vaccine-induced response. PBMCs were stimulated for six hours with peptide pools of Ag85A (A), Ag85B (B) and TB10.4 (C) and the frequencies of specific CD4 or CD8 T cells producing either IFN- γ or TNF- α or IL-2 were measured on days 28 and 84 (only group 4) post-vaccination by intracellular cytokine staining in AERAS-402 or placebo vaccinated infants. Within each group and time point, we used Wilcoxon matched paired test to compare CD4 and CD8 T cell responses.

vaccines have been used in the past, the vaccine virus could not be isolated from cultures from these sites when similar adverse events occurred.

The safety profile of AERAS-402 in this infant study is encouraging. Further development of AERAS-402 vaccine now focus on aerosol vaccination [23]. This route of AERAS-402 vaccine delivery may result in an induction of mycobacteria-specific T cell immunity at the site of the disease, as demonstrated in a non-human primate model [23].

Turning to the immunology, some similarities and differences could be observed when AERAS-402-induced immunity in infants was compared with that in adults [6].

One difference was the CD4 T cell response in infants appeared to be of lower magnitude. Similar age effect for Ag85A-specific CD4 T cells was observed in clinical trials of the MVA85A vaccine [12]. We observed differences in specific CD4 and CD8 T cell responses detected by WB and PBMC ICS assays in infants vaccinated with the highest dose and after a second vaccination. This contrasting

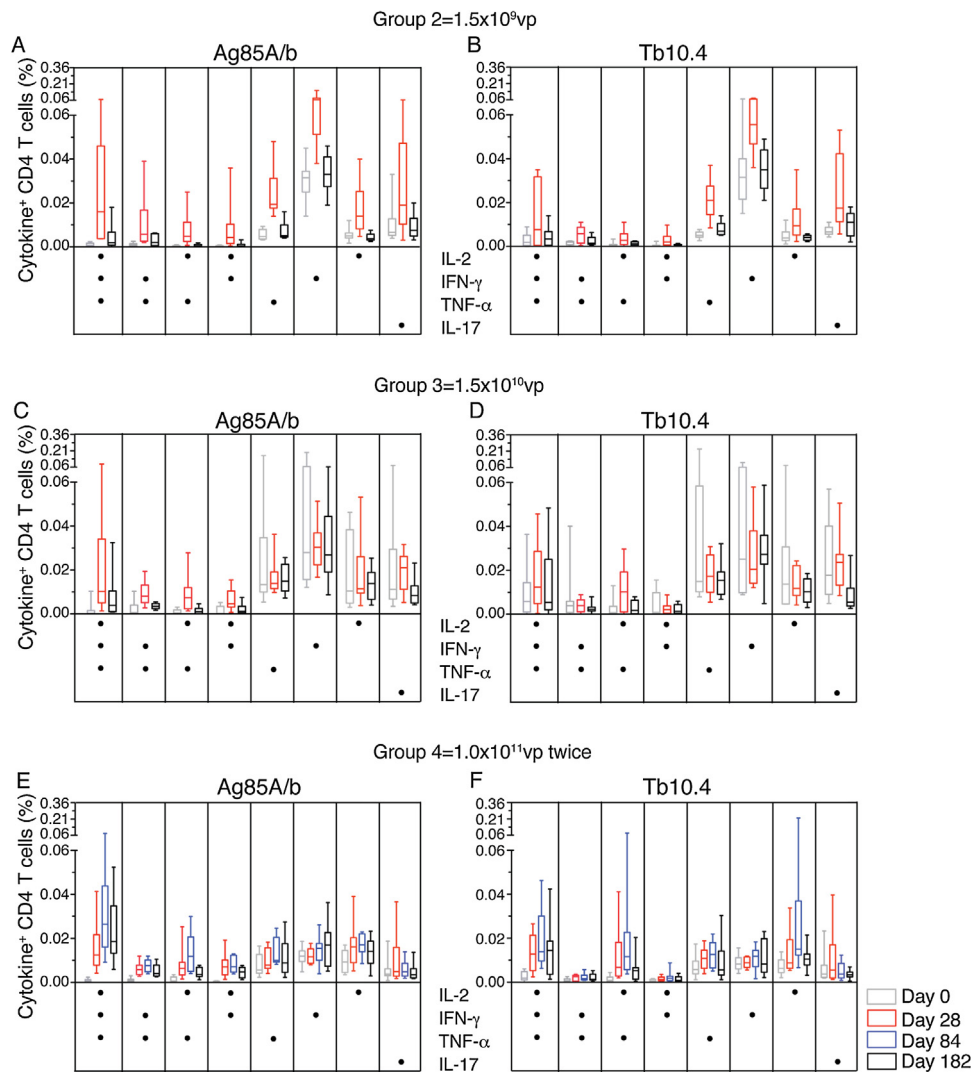


Fig. 5. Patterns of cytokine production in vaccination-induced CD4T cells. Frequencies of monofunctional, bifunctional and polyfunctional CD4T cells, based on combinations of cytokine expression, following incubation of whole blood with peptide pools of either Ag85A/b ((A), (C) and (E)) or Tb10.4 ((B), (D) and (F)). The different combinations of cytokines are shown below the boxplots of the frequencies at various study time points.

observation between the two assays could be, in part, due to differences in stimulation time resulting in the activation of low affinity T cells as well as the effects of cryopreservation. Costantini et al. reported changes in phenotypic and functional characteristics of cryopreserved T cells, among them, detection of increased proportions of effector CD8T cells [24]. Our PBMC ICS showed 2 doses of AERAS-402 induced antigen-specific bi-functional CD8T cells expressing IFN- γ and TNF- α , as well as a smaller proportion of single IFN- γ , typical of effector CD8T cells.

One similarity was that polyfunctional and dual-functional vaccine-specific CD4T cells were detected with WB ICS in adults and infants, without co-expression of IL-17. However, we showed in 10-week old infants that the magnitude and patterns of cytokine expression of BCG-induced CD4T cells do not correlate with the risk of TB disease [25]. Similarly, despite induction of readily detectable polyfunctional Ag85A-specific Th1 responses, MVA85A vaccination of infants did not afford additional protection above BCG vaccination [26]. However, we recently showed that the proportion of mycobacteria-specific polyfunctional CD4T cells increased during TB-treatment of adults [27], supporting a possible role for polyfunctional CD4T cells in immunity against *M.tb* or active suppression of function during infection. Conversely, Sutherland et al.

reported higher frequencies of mycobacteria-specific polyfunctional T cells in patients with TB disease, compared with persons with latent *M.tb* infection [28]. These findings highlight the urgency with which host correlates of protection against TB disease are needed, for application to rational vaccine design. In addition, differences in T cell immunity following administration of AERAS-402 were more apparent when we evaluated “qualitative” as opposed to “quantitative” outcomes in both infants and adults studies. This observation highlights the importance of assessing different aspects of vaccine-induced T cell immunity in TB vaccines clinical trials.

Another difference was a greater induction of CD8T cells observed in adults, compared with infants. This observation may relate to relative immaturity of the infant immune response [29]; alternatively, pre-vaccination mycobacterial exposure might have been greater in adults, resulting in a greater CD8 response. In fact, infants who had a higher baseline T cell response to Ag85A/b and/or Tb10.4 had a greater magnitude of AERAS-402 vaccine-induced T cell responses. We have recently shown in adults that pre-existing mycobacteria-specific immunity, driven by either environmental NTM or *M.tb* infection, resulted in a quantitatively greater T cell response to M72/AS01, another new subunit TB vaccine [30]. Based on current and previous observations, and on the

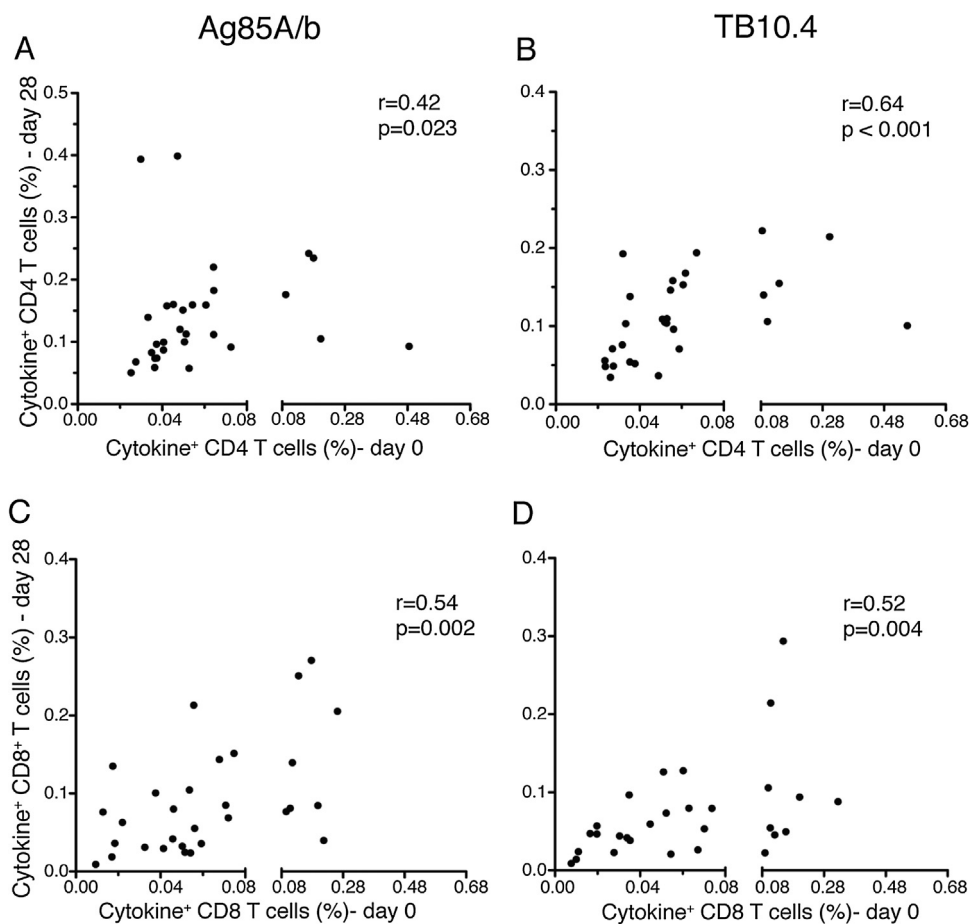


Fig. 6. Association between pre-existing mycobacteria-specific T cell immunity and the vaccination-induced response. Associations between frequencies of total cytokine-expressing CD4 and CD8T cells following incubation of whole blood with peptide pools of either Ag85A/b ((A) and (C)) or peptide pools of TB10.4 ((B) and (D)), measured before vaccination (day 0, X axis) and on study day 28 (Y axis). Results were analysed by Spearman correlation test.

demonstrated effect of age, we suggest that the most significant priming event for novel boost vaccination might not be BCG, but rather recent mycobacterial exposure. We base this statement on a previous observation [31] that immune responses induced by BCG vaccination of newborns wane rapidly over the first year of life. This is an important finding because majority of TB vaccines currently in clinical trials are designed to boost BCG-primed immunity; however, there are now vaccines such as recombinant BCG [32] or attenuated *M.tb* [33] designed to replace BCG as a prime vaccine. These vaccines are already in clinical trials [32] and may provide more optimal priming, and possibly, enhanced boosting.

Finally, in our study setting with high mycobacterial exposure, homologous boosting with AERAS-402 resulted in higher T cell responses in infants, whereas this was not observed in adults [6]. The population-level prevalence of neutralizing antibodies to Ad35 is relatively low, compared with that of several other adenovirus serotypes [34], and is expected to be even lower in infants: this may be the reason why we did see a boost response in infants.

In summary, our results show AERAS-402 is safe and immunogenic when given to infants. In our study setting with high mycobacterial exposure, homologous boost using the highest dose of AERAS-402 did not show an improved vaccine-induced T cell response among adults [6]. In contrast, the same vaccination strategy with the highest dose showed an enhanced vaccine-induced T cell response in infants. However, the study

design in both adults' and infants' trials did not evaluate the effect of a repeat vaccination with low doses. Therefore, we cannot make conclusions on the differences in immune responses between low and high AERAS-402 dose following homologous vaccination.

Funding

Aeras.

Conflict of interest statement

None declared.

Author's contributions

WAH, JCS, HM and GDH designed the study. MT, HG, MH, MK, AV, JH, CK, ALKK clinical and fieldwork. BMNK, BA, NM TJS, WW, HA, MD, MGP, JH, BMcC, JB and DAH performed or supervised the immunology experiments. MAS, BMNK, BA and MT performed statistical analysis. WAH, JCS, HM, TJS, DAH, GDH interpreted the results. BMNK and MT wrote the first draft of the manuscript. All authors reviewed and gave input to the subsequent manuscript drafts.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2014.09.001>.

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