



A review of the tolerability of the candidate TB vaccine, MVA85A compared with BCG and Yellow Fever vaccines, and correlation between MVA85A vaccine reactogenicity and cellular immunogenicity

Rosalind Rowland^a, Nathaniel Brittain^a, Ian D Poulton^a, Angela M Minassian^a, Clare Sander^a, David W Porter^a, Nicola Williams^b, Iman Satti^a, Ansar A Pathan^{a,1}, Alison M Lawrie^a, Helen McShane^{a,*}

^aThe Jenner Institute, Old Road Campus Research Building, Oxford University, Roosevelt Drive, Oxford OX3 7DQ, UK

^bCentre for Statistics in Medicine, Wolfson College Annexe, University of Oxford, Linton Road, Oxford OX2 6UD, UK

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ABSTRACT

Background: The development of a new, more effective vaccine against tuberculosis (TB) for use in healthy and HIV-infected adults, children and infants, remains a global health priority. MVA85A is a candidate tuberculosis vaccine designed to enhance immunity to the existing vaccine, Bacillus Calmette-Guerin (BCG). MVA85A entered clinical trials in 2002 and has now progressed to Phase IIb proof-of-concept efficacy trials in infants and HIV-infected adults in Africa.

Methods: A detailed analysis was conducted of the cumulative safety data of intradermal delivery of MVA85A in 112 healthy adult subjects in a series of open label, single arm, non-controlled, Phase I safety and immunogenicity clinical trials in the UK. The trials differed with respect to previous mycobacterial exposure, vaccine regime and dose. Objective safety measures (local reaction size and body temperature) were evaluated for correlations with adaptive antigen-specific immune responses.

Results: All subjects in the combined mid-dose group developed a local reaction, of which 92% were mild, 8% were moderate and no reactions were severe. Around 90% of subjects in each group reported at least one systemic adverse event, most commonly headache, myalgia, malaise, feeling feverish, fatigue and arthralgia. Of all systemic adverse events in the combined mid-dose group, 96% were mild, 3% were moderate and 1% were severe (but none of these were judged to be vaccine-related). Pre-vaccination mycobacterial exposure did not affect the adverse event profile. The size of local reaction and frequency of systemic adverse events increased with MVA85A vaccine dose. There were no documented fevers in the low-dose group, whilst 3% of subjects in the combined mid-dose group and 21% in the high-dose group had documented fevers. Peak local reactions were larger after a second poxvirus vaccination, but other local and systemic adverse events were comparable to a single MVA85A vaccination. No severe systemic AEs or serious adverse events in any group were judged to be vaccine-related. Local AEs compared favourably to BCG vaccine-induced local AE and systemic AEs after MVA85A vaccination were comparable to those after the live viral Yellow Fever vaccine in similar populations. There were no correlations found between local reaction size or body temperature and adaptive immune responses (measured by *ex vivo* interferon gamma Enzyme Linked Immunospot).

Conclusions: The candidate TB vaccine, MVA85A has been safely administered to over 100 healthy adults in the UK. Intradermal vaccination with MVA85A induced a transient, superficial reaction local to the injection site and mild short-lived viral symptoms. The local and systemic AE profile of MVA85A vaccination was comparable to published data of other intradermal vaccines and live viral vaccines respectively. Local reaction sizes and body temperature measurements did not correlate with the adaptive cellular immune response to MVA85A.

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* Corresponding author. Tel.: +44 01865 617606; fax: +44 01865 857471.

E-mail address: helen.mcshane@ndm.ox.ac.uk (H. McShane).

¹ Present address: Centre for Infection, Immunity and Disease Mechanisms, Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge, Middlesex UB8 3PH, UK.

1. Introduction

Tuberculosis (TB) is one of the leading global causes of death and disability from a single infectious agent, *Mycobacterium tuberculosis* (*M.tb*), with an estimated 8.8 million new infections and 1.5 million deaths in 2010 [1]. The Stop TB Partnership goals include reducing the global burden of TB (prevalence and death rates) by 50% by 2015 compared to 1990 levels and eliminating TB as a public health problem by 2050. Prophylactic immunization is a key strategy in reducing the incidence of TB. *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG), the only licensed TB vaccine, is given in mass immunisation campaigns to neonates in high-risk populations as part of the WHO Expanded Programme on Immunisation (EPI). BCG consistently protects against TB meningitis and disseminated TB in children but its efficacy wanes with time [2–4]. In addition, BCG affords highly variable protection against pulmonary disease, which accounts for the burden of global TB mortality and morbidity [5]. A new, more effective TB vaccine is a major global health priority. A feasible and promising strategy is for a new prophylactic vaccine to be given in a regime which includes BCG, in order to enhance the immunity afforded by BCG.

We are developing a subunit viral-vectored vaccine, using Modified Vaccinia Virus Ankara (MVA) as a delivery system for the mycobacterial antigen 85A. This candidate vaccine is designated MVA85A and has been evaluated in a series of small Phase I safety and immunogenicity clinical trials in the UK since 2002 [6–9]. The promising safety and immunogenicity of MVA85A led to further clinical trials in target populations in South Africa, The Gambia and Senegal [10–15]. Two proof-of-concept (Phase IIb) efficacy trials are now underway in BCG-vaccinated South African infants and HIV-infected African adults. As the early UK trials had small group sizes (typically 12 subjects), only very common adverse events (AEs) were detected by individual trials. Now that over 100 healthy adult subjects in the UK have received MVA85A vaccination, we have the opportunity to perform an integrated further evaluation of the cumulative safety and tolerability of MVA85A vaccination in a larger cohort.

2. Subjects and methods

2.1. Clinical trials

Safety data from seven open label, single arm, non-controlled safety clinical trials were analysed (Table 1) [7–9,16,17] (Porter, unpublished data). The trial protocols all received full ethical approval from the Oxfordshire Research Ethics Committee (OXREC) or the Gene Therapy Advisory Committee. Regulatory approval for these studies was granted by the Medicines and Healthcare products Regulatory Agency (MHRA), UK.

2.2. Location

The trials were conducted at the Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford and were sponsored by the University of Oxford. Northwick Park Hospital, London was used as a second site for recruitment and follow up of *M.tb*-infected subjects [7].

2.3. Subjects

Healthy adult subjects between the ages of 18 and 55 years were recruited from the Oxford region and, for latently *M.tb*-infected (LTBI) subjects, from TB contact clinics in Oxford and London [7]. Fully informed written consent was obtained from all subjects prior to any study procedures being performed. Before enrolment, all subjects underwent medical screening, which included medical history, physical examination, urinalysis and blood tests. Specific exclusion criteria included significant allergy; immunosuppression; clinically significant past or current medical history; psychiatric disorders; injecting drug use or excess alcohol use; confirmed or planned pregnancy; and any previous MVA or Fowlpox (FP9) vaccinations. Subjects with clinically significant abnormalities in their routine haematology, biochemistry or urinalysis results, or infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV) were also excluded. Subjects were required to consent to refrain from blood donation throughout the trials and females were required to use continuous effective contraception.

2.4. MVA85A

The construction of MVA85A has been described previously [18]. Clinical grade MVA85A (batch number 010402) was manufactured to Good Manufacturing Practice standard by IDT Biologika GmbH (Dessau, Germany). MVA85A was administered by intradermal injection into the deltoid area of the arm on the day of vaccination at doses of 1×10^7 plaque forming units (pfu) (low-dose); 5×10^7 pfu (mid-dose) or 1×10^8 pfu (high-dose) (Table 1). The low and mid-dose vaccinations were administered as a single intradermal injection. The high-dose vaccinations were administered as two injections, each a dose of 5×10^7 pfu, delivered simultaneously one into each arm.

2.5. Enrolment and follow up

Subjects received their first MVA85A vaccination on the day of enrolment and were followed up for 24 or 52 weeks following vaccination, depending on the individual trial protocol.

Table 1
Demographics of subjects vaccinated with MVA85A in the UK according to group.

Group	Vaccine dose (pfu)	N	Males (%)	Median age (range) (years)	Clinicaltrials.gov reference and citation
M ^a	5.0×10^7	14	5 (36)	29 (19–54)	NCT00423566 [9]
MM ^b	5.0×10^7	11 ^a	5 (45)	31 (20–48)	NCT00423566 [9]
BM ^c low-dose	1.0×10^7	12	4 (33)	27 (21–42)	NCT00465465 [17]
BM mid-dose	5.0×10^7	43	17 (40)	26 (23–54)	NCT00427453 [8] NCT00427830 [9] NCT00653770 [16]
BM high-dose	1.0×10^8	24	11 (46)	24 (19–32)	NCT00465465 [17] NCT00548444 (Porter, unpublished data)
LTBI ^d	5.0×10^7	12	10 (83)	31 (20–49)	NCT00456183 [7]
BFM ^e	5.0×10^7	7	3 (43)	30 (24–47)	NCT00653770 [16]

^a M = single vaccination with MVA85A.

^b MM = Two sequential vaccinations with MVA85A (of the 14 subjects vaccinated with MVA85A, 11 subjects received a second MVA85A vaccination after an interval of 4 weeks within the same clinical trial).

^c BM = single vaccination with MVA85A in previously BCG-vaccinated subjects.

^d LTBI = Latent *M.tb* infection (10 of the 12 subjects had evidence of prior BCG vaccination).

^e BFM = Sequential vaccination with FP85A, followed by MVA85A after an interval of 4 weeks in previously BCG-vaccinated subjects.

2.6. Groups

Subjects were grouped according to their previous exposure to mycobacteria, vaccination regime and vaccine dose. The mid-dose vaccination groups are named M, MM, BM, LTBI and BFM and consisted of 76 subjects who received a total of 87 vaccinations with MVA85A. The mycobacterially naïve M group ($n = 14$) had received no prior BCG vaccination and had negative tuberculin skin test pre-vaccination before vaccination with MVA85A. The MM groups were 11 of the 14 mycobacterially naïve subjects who received a second MVA85A vaccination. The BM group ($n = 43$) had no evidence of LTBI but had received prior BCG vaccination. Ten subjects in the LTBI group ($n = 12$) had evidence of prior BCG vaccination. The BFM group ($n = 7$) had been previously BCG-vaccinated and were vaccinated with another candidate TB vaccine (FP85A) 4 weeks prior to MVA85A. The low-dose group ($n = 12$) and high-dose groups ($n = 24$) had all been BCG-vaccinated prior to enrolment and each received one MVA85A vaccination.

2.7. Safety evaluation

The safety profile of the vaccine was evaluated by active and passive AE collection for the duration of follow up. Clinically qualified investigators conducted all screening, vaccination and follow-up visits. Subjects were given a diary card to complete for the first 7 days following immunisation. At regular follow up appointments, the vaccine injection site was reviewed, and solicited and unsolicited AEs were recorded by the investigators ([Supplementary data, Table S1](#)). Blood samples for routine haematology and biochemistry were taken before and after vaccination. At the time of this analysis, all AEs were assigned grades for causality and severity ([Supplementary data, Table S1](#)). For maximum stringency, all solicited AEs reported with a reasonable temporal relationship to the vaccine were recorded as vaccine-related and any AE designated as possibly, probably or definitely related to MVA85A vaccination were evaluated as vaccine-related AEs. Unrelated AEs were also analysed. Documented fever was defined as body temperature greater than 38.0 °C. An AE was defined as serious if it was life threatening, caused persistent or significant disability or incapacity, or resulted in admission to hospital.

2.8. Immunological evaluation

Ex vivo interferon gamma (IFN γ) Enzyme Linked ImmunoSorbent (ELISpot) assays were the main readout of vaccine immunogenicity and were performed on blood taken prior to and 1 week after vaccination and at regular time points during the follow up period as previously described [7–9]. Cryopreserved peripheral blood mononuclear cells (PBMC) and serum were stored and used after trials ended to comprehensively characterise vaccine-induced immunogenicity [19,20].

2.9. Literature reviews

As the early clinical trials of MVA85A did not include control groups, the reactogenicity of two licensed vaccines, BCG and a Yellow Fever vaccine, were summarised in order to provide comparative data for MVA85A. BCG is the only vaccine currently licensed for immunisation against TB and is administered intradermally. Yellow Fever vaccine is a live viral vaccine, which is widely used for immunisation of adults and is administered subcutaneously or intradermally. An electronic database (medline) was searched for systematic reviews, meta-analyses and prospective clinical trials recruiting healthy adult subjects in Europe or North America. The following search terms were entered into medline: “BCG vaccine AND humans AND tuberculosis” (limits: clinical

trial); “BCG” (limits: meta-analysis); “BCG” (limits: review); “Yellow Fever vaccine AND humans” (limits: clinical trial); “Yellow Fever vaccine AND systematic review” and “Yellow Fever vaccine” (limits: humans, meta-analysis).

2.10. Statistical analysis

The analysis of AE frequencies was descriptive. For statistical analysis of objective measures (local reaction diameter and body temperature), non-parametric tests were used as the data were not normally distributed. The diameters of local reactions between groups were compared using the Mann Whitney U test (Stata 9). The Wilcoxon signed rank test for paired data were used to compare diameters of sequential vaccinations within a group (Stata 9). The Spearman test for non-parametric data were used to test for correlations between peak IFN γ ELISpot responses to 85A and both local reaction diameters and peak recorded temperatures (Stata 9).

3. Results

3.1. Subjects

Safety data from all healthy subjects in the UK who had received an MVA85A vaccination were evaluated ([Fig. 1](#)). There were more females than males and the median age of enrolled subjects was similar between genders ([Fig. 1](#)). There were fewer females than males in the LTBI group, but more females than males in all other groups ([Table 1](#)).

3.2. Adverse events

3.2.1. Local reactions

For the mid-dose MVA85A vaccinations, the profiles of AEs local to the injection site were similar for groups M, MM and BM, with

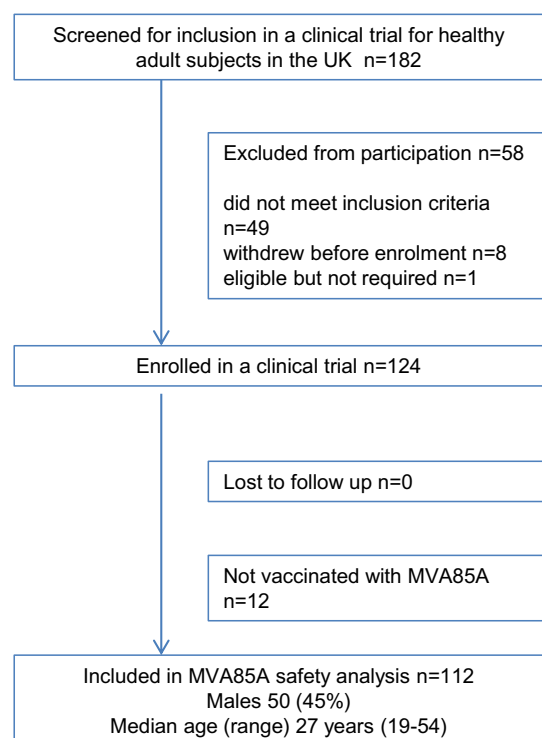


Fig. 1. Subjects shows the flow of subjects from screening for the individual clinical trials to inclusion in this analysis.

respect to local AE frequency and severity (Fig. 2). Based on a combined analysis of local AEs in all 69 subjects in the mid-dose M, BM and LTBI groups, all subjects developed injection site erythema and swelling, and most subjects also reported local scaling, pain and pruritus (Fig. 2, Table 2). Of all 330 local AEs, 92% (62) were mild, 8% (27) were moderate and none were severe. Injection site erythema, swelling and warmth developed on the day of vaccination, followed by pain, pruritus and scaling (Table 3). Pain, pruritus and warmth were present for a few days in most cases; scaling and swelling for a few weeks; and erythema for 3–4 months (Table 3). Unsolicited local AEs reported were injection site ooze (3/330, 1%), rash (2/330, 0.6%), scar (1/330, 0.3%), limitation (1/330, 0.6%) and localised muscular pain (2/330, 0.6%) and all were mild.

Severe erythema or swelling were reported for three subjects in the MM group and five subjects in the BFM group, but no subjects in the other groups had severe local reactions (Fig. 2). Severity classifications of erythema and swelling were based on measurements of the diameters. Peak (days 1 and 2) diameters of local erythema recorded in subject diary cards were larger after MVA85A vaccination in the BFM group compared to the BM group (Fig. 3, Table 4). After a second vaccination with MVA85A (group MM), peak (days 1 and 2) diameters of erythema were larger compared to the peak diameters recorded after the first vaccination in the same subjects (group M) (Fig. 3, Table 4). Diameters of swelling were also larger after the second vaccination (MM) compared to the first vaccination (M) (data not shown).

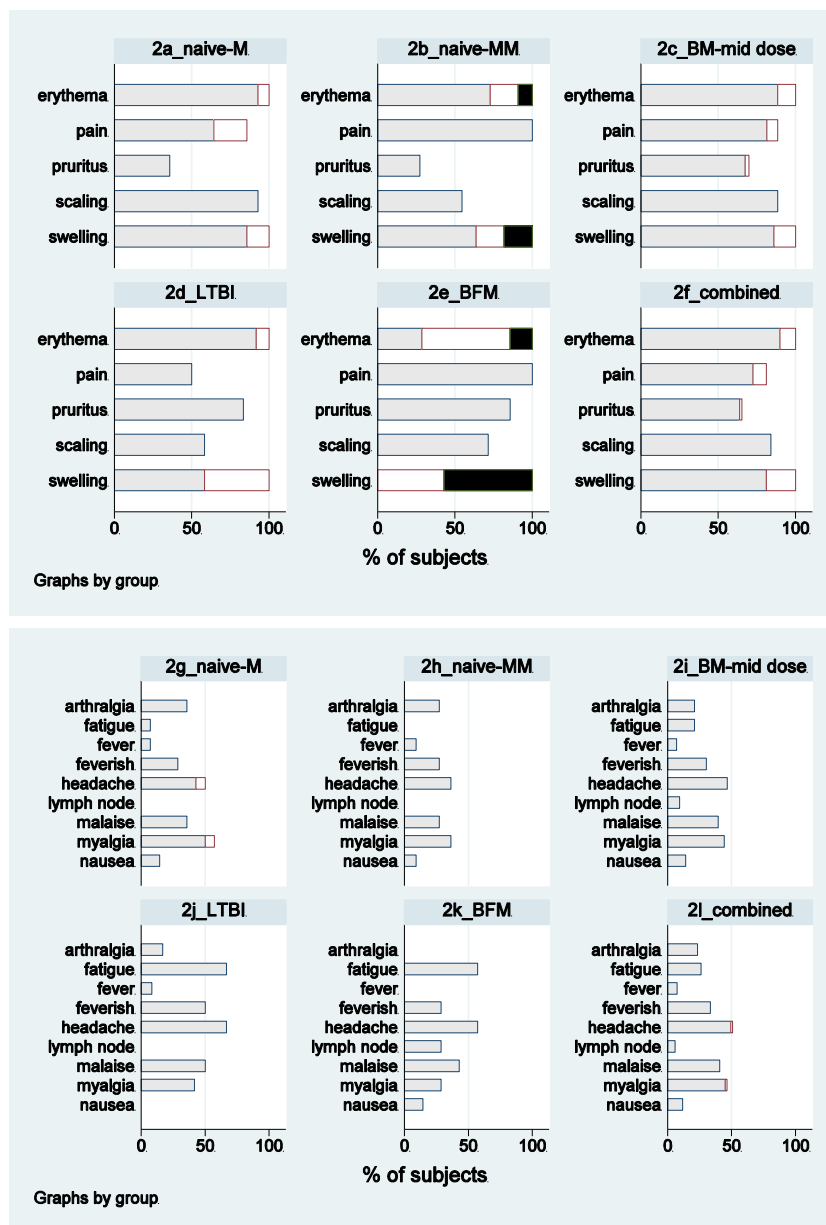


Fig. 2. AE frequency and severity after MVA85A vaccination. Severity and frequency of the most frequently reported local and systemic AEs following immunisation with 5×10^7 pfu (mid-dose) of MVA85A vaccination. Naïve M = first MVA85A vaccination (5×10^7 pfu) in mycobacterially naïve subjects ($n = 14$); naïve MM = second MVA85A vaccination (5×10^7 pfu) in mycobacterially naïve subjects ($n = 11$); BM = single MVA85A vaccination (5×10^7 pfu) in previously BCG-vaccinated subjects ($n = 43$); LTBI = single MVA85A vaccination (5×10^7 pfu) in LTBI subjects ($n = 12$, 10 were previously BCG-vaccinated); BFM = single MVA85A vaccination (5×10^7 pfu) in previously BCG-vaccinated subjects, vaccinated with candidate TB vaccine FP85A 4 weeks prior ($n = 7$); mid-dose (combined) = all subjects receiving single vaccination with MVA85A (5×10^7 pfu; groups M, BM, LTBI; $n = 69$); Bars are subdivided according to severity: grey = mild; white = moderate; black = severe.

Table 2

AE profiles of Yellow Fever and BCG vaccines. Frequency of AEs in the combined mid-dose of MVA85A and from published data of BCG and Yellow Fever vaccines. The displayed data are the percentage of subjects reporting each AE, with the number of subjects in parentheses.

Vaccine Route Study	MVA85A id	BCG id	BCG id dbRCT ^a	BCG id	BCG id RCT	BCG id RCT	YF id RCT	YF sc RCT	YF sc dbRCT	YF sc RCT	YF sc dbRCT
Source		[21]	[22]	[24]	[23]	[25]	[29]	[29]	[27]	[28]	[26]
N	69	14	17	20	48	29	77	78	659	76	106
Local erythema	100% (69)	100% (14)	88% (15)			97% (28)	82% (63)	32% (25)	30% (198)	18% (14)	5% (5)
Local pain	65% (45)	100% (14)	65% (11)			90% (26)	8% (6)	19% (15)	42% (274)	24% (18)	9% (10)
Local pruritus	84% (58)	64% (9)									
Local swelling	100% (69)	100% (14)	88% (15)				68% (52)	12% (9)	21% (139)	16% (12)	0% (0)
Local ulcer			100% (17)	100% (20)	98% (47)	83% (24)					
Axillary lymph nodes	6% (4)	14% (2)			few cases					5% (4)	
Arthralgia	23% (16)	29% (4)								9% (7)	1% (1)
Asthenia/fatigue	26% (18)								30% (197)	20% (15)	8% (8)
Fever ^b	3% (2)	0% (0)			0% (0)		5% (4)	8 (10)	16% (102)	8% (6)	0% (0)
Feverish ^c	33% (23)	14% (2)									
GI event									5% (32)	13% (10)	5% (5)
Headache	51% (35)	36% (5)							32% (210)	39% (30)	12% (13)
Malaise	41% (28)								19% (123)		
Myalgia	46% (32)	36% (5)					16% (12)	22% (27)	26% (171)	10% (8)	8% (9)
Nausea or vomiting	12% (8)	7% (1)							3% (21)		

^a db = Double blind, RCT = randomised controlled trial.

^b Fever = documented fever.

^c Feverish = symptoms in the absence of documented fever.

Table 3

AE onset and duration. The median day of onset and duration of possibly, probably or definitely vaccine-related AE in the combined mid-dose group are displayed. Minimum and maximum results are in parentheses.

Adverse event	Median day of onset (range)	Median duration, days (range)
Systemic	Arthralgia	1 (0–5)
	Documented fever	1 (0–4)
	Fatigue	1 (0–6)
	Feverish	1 (0–5)
	Headache	1 (0–6)
	Malaise	1 (0–6)
	Myalgia	1 (0–6)
Injection site	Erythema	0 (0–7)
	Pain	1 (0–14)
	Pruritus	4 (0–28)
	Scaling	7 (0–28)
	Swelling	0 (0–7)
	Warmth	0 (0–6)

The frequencies of local AEs in the low and high-dose groups were similar to those in the combined mid-dose group, except injection site pain was more frequent in the high-dose group (96%, 23/24) than the low-dose group (50%, 6/12) (data not shown) [17]. There was one report of severe swelling in the low-dose group and one report of severe pain in the high-dose group [17]. Peak (days 1 and 2) erythema diameters from subject diary cards were larger at high-dose compared to mid- and low-doses (Fig. 3, Table 4). Peak diameters of swelling were similar between the three doses (data not shown).

3.3. Systemic AEs

The frequencies and severities of systemic AEs were similar between the mid-dose groups, except a smaller proportion of subjects in the MM group (7/11, 64%) reported any systemic AE, compared to the other groups (M 93%, 13/14; BM 86%, 37/43; LTBI 92% 11/12; BFM 86%, 6/7) (Fig. 2).

In the combined analysis of a single mid-dose vaccination of MVA85A (groups M, BM and LTBI), the most commonly reported systemic AEs which were deemed possibly, probably or definitely

related to vaccination were headache, myalgia, malaise, feeling feverish, fatigue and arthralgia (Fig. 2, Table 2). Vaccine-related systemic AEs reported less frequently were nausea (8/69, 12% subjects), documented fever (5/69, 7%) and axillary lymphadenopathy (4/69, 6%). Vasovagal symptoms, dizziness and diarrhoea were infrequently reported (1% of AEs). Upper respiratory tract infections comprised 8% of all systemic AEs reported (included unrelated AEs) but none were deemed definitely or probably related to MVA85A vaccination (Supplementary data, Table S2). MVA85A vaccine-related systemic AEs developed a median of 1 day after vaccination and resolved after a median of 1 day (Table 3). Of all 293 systemic AEs, 281 (96%) were mild; nine (3%) were moderate and three AEs (1%) were severe (Supplementary data, Table S2). The three AEs classified as severe were all serious adverse events, but none were deemed MVA85A vaccine-related. These were a fractured ankle 9 days after vaccination requiring hospitalisation (BCG-primed group), pregnancy diagnosed 6 weeks after vaccination (mycobacterially naïve group) and a drug overdose 11 months after vaccination (LTBI group). There were three moderate vaccine-related AEs (vasovagal faint, headache and muscle aches), all reported by one subject in the mycobacterially naïve group.

The profile of systemic AEs was similar for the low and high-dose groups, although the frequencies of vaccine-related systemic AE increased with dose. The proportion of subjects with any systemic AE in the low-dose group was 42% (5/12); 88% (61/69) in the combined mid-dose group and 100% (24/24) in the high-dose group. Similarly, there were no documented fevers in the low-dose groups, whilst 3% (2/69) of subjects in the combined mid-dose group and 21% (5/24) subjects in the high-dose group had documented fevers.

3.4. AEs after BCG and Yellow Fever vaccines: Literature review

No systematic reviews or meta-analysis that included an evaluation of the reactogenicity of BCG or Yellow Fever vaccines in healthy adult subjects were identified. The search for clinical trials of intradermal BCG vaccination in healthy adults in Europe and North America yielded 211 manuscripts, of which seven met the eligibility criteria for inclusion [21–25]. BCG vaccination was associated with injection site erythema, swelling, pain and ulceration (Table 2). Systemic AEs (headache, lymphadenopathy,

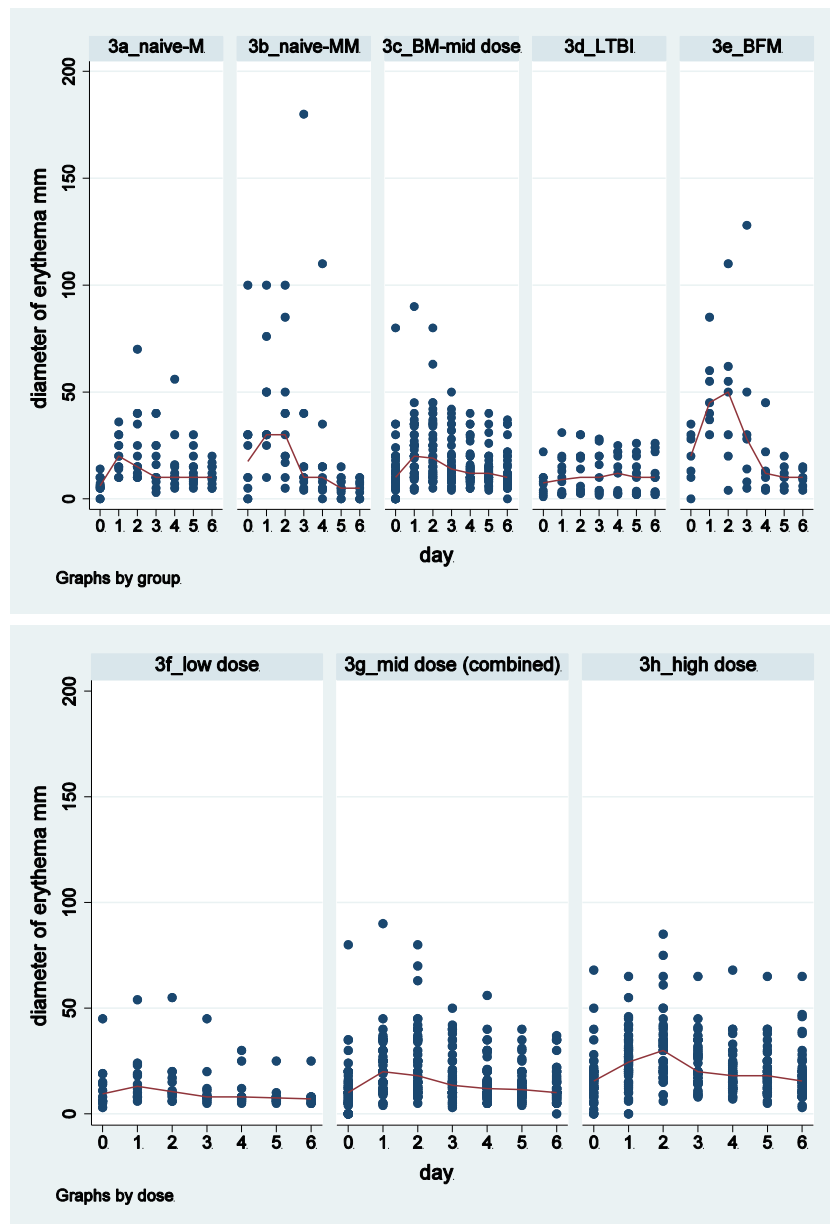


Fig. 3. Diameter of local reactions following vaccination with MVA85A. Individual measurements of diameter of local erythema recorded by subjects in diary cards for 7 days following vaccination (where day zero is the day of vaccination). Median diameters for each day are connected with lines. M = first MVA85A vaccination (5×10^7 pfu) in mycobacterially naïve subjects ($n = 14$); MM = second MVA85A vaccination (5×10^7 pfu) in mycobacterially naïve subjects ($n = 11$); BM = single MVA85A vaccination (5×10^7 pfu) in previously BCG-vaccinated subjects ($n = 43$); LTBI = single MVA85A vaccination (5×10^7 pfu) in LTBI subjects ($n = 12$, 10 were previously BCG-vaccinated); BFM = single MVA85A vaccination (5×10^7 pfu) in previously BCG-vaccinated subjects, vaccinated with candidate TB vaccine FP85A 4 weeks prior ($n = 7$); low-dose = single MVA85A vaccination (1×10^7 pfu) in previously BCG-vaccinated subjects ($n = 12$); mid-dose (combined) = all subjects receiving single vaccination with MVA85A (5×10^7 pfu; groups M, BM, LTBI; $n = 69$); high-dose = MVA85A vaccination (1×10^8 pfu) in previously BCG-vaccinated subjects ($n = 24$).

Table 4

Statistical analysis of diameter of erythema. Peak diameter of erythema (days 1 and 2) from measurements recorded in subject diary cards were compared between groups.

	Median diameter (range)	Difference in medians (95% confidence interval)	Mann Whitney U test
BFM	48 mm (4–110)		
BM	20 mm (0–90)	25 mm (12–37)	$p = 0.0001$
High-dose	26 mm (0–85)		
Mid-dose	19 mm (2–90)	8 mm (5–11)	$p = 0.0000$
Low-dose	12 mm (6–55)	4 mm (1–8)	$p = 0.045$
MM	30 mm (5–100)	Median difference (range):	Wilcoxon sign rank test:
M	18 mm (10–70)	11 mm (–20–85)	$p = 0.004$

and myalgia) were reported in one single arm study in 14 subjects conducted by our research group (Table 2) [21].

The search for Yellow Fever vaccine clinical trials in healthy adults in Europe and North America yielded 54 manuscripts, of

which four met the eligibility criteria for inclusion [26–29]. Yellow Fever vaccine was administered intradermally to healthy adults in one randomised controlled trial (RCT) and subcutaneously in four RCTs. Injection site erythema, swelling and pain were more frequent after intradermal than subcutaneous injections in one RCT comparing the two routes (Table 2) [29]. Systemic AEs after Yellow Fever vaccination were headache, myalgia, fatigue, malaise, GI events, fever and arthralgia (Table 2).

3.5. Correlation of immune responses with adverse events

Peak *ex vivo* IFN γ ELISpot responses to a single pool of 15mer antigen 85A peptides after high-dose vaccination with MVA85A in 12 subjects (clinical trials.gov identifier NCT00465465) were plotted against peak body temperatures for the same subjects. IFN γ responses to antigen 85A were detected in all 12 subjects and peaked 1 week after vaccination (median 6493, range 3030–8409 spot forming cells) [17]. Peak documented body temperature was in the range 40.0–40.4 °C for one subject; 38.5–38.9 °C for one subject; 38.0–38.4 for two subjects; 37.5–37.9 for two subjects and below 37.5 °C for the remaining six subjects. There was no correlation between peak *ex vivo* IFN γ ELISpot responses to antigen 85A and peak documented fever (Fig. 4A).

Peak *ex vivo* IFN γ ELISpot responses to summed pools of antigen 85A peptides after 104 vaccinations with MVA85A in six clinical trials were plotted against peak diameter of erythema for the same subjects (clinical trials.gov identifiers NCT00423566; NCT00653770; NCT00465465; NCT00456183; NCT00427830; NCT00427453); [7–9,16,17]. The median peak diameter of erythema was 22.5 mm (range 0–180 mm). IFN γ ELISpot responses to antigen 85A peaked 1 week after vaccination (median 2309, range 0–11,066 spot forming cells). There was no correlation between peak *ex vivo* IFN γ ELISpot responses to antigen 85A and peak diameter of erythema (Fig. 4B).

4. Discussion

A combined safety evaluation of the candidate TB vaccine, MVA85A from a series of small, non-controlled, clinical trials in adults in the UK has been presented. Vaccination safety and immunogenicity has been evaluated in the context of increasing mycobacterial exposure; different vaccine doses; and homologous or heterologous prime with MVA85A or another poxvirus vaccine. Since biomarkers of protection against *M.tb* have not yet been identified, large clinical trials in TB endemic areas are currently the only means for testing TB vaccine efficacy in humans. The aims of these early studies were to quickly identify any safety concerns and demonstrate immunogenicity, in order to provide a platform for performing efficacy clinical trials. In view of this and the resources available, no vaccination or placebo control groups were included.

A combined mid-dose analysis was performed for subjects receiving a single vaccination of 5×10^7 pfu MVA85A. The AE profile after MVA85A vaccination was similar between mycobacterially naïve, previously BCG-vaccinated and LTBI subjects, in terms of AE nature, frequency, severity and duration. All subjects developed local erythema and swelling, which were associated with tenderness, pruritus and scaling in over two thirds of subjects 1–2 days after vaccination. Warmth and pain, markers of the acute inflammatory process, were present for a median of 3–4 days. Erythema resolved over 3–6 months, but this term simply describes any redness or pinkness around the injection site. 92% of local AEs were mild and none were severe. The majority of subjects also reported brief and mild viral symptoms 1 day after MVA85A vaccination,

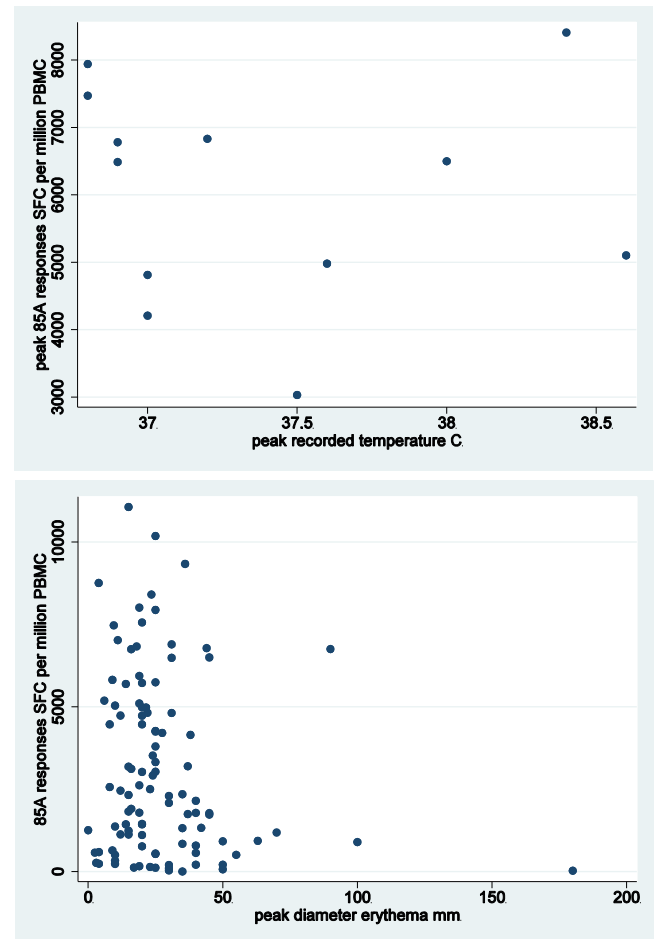


Fig. 4. Correlation between peak immunogenicity and adverse events. (4a) Peak recorded body temperature and the peak IFN γ ELISpot responses (1 week after vaccination) for 12 subjects who had received the highest dose (1×10^8 pfu) of MVA85A vaccine (Spearman rho = -0.21 , $p = 0.51$). (4b) Peak diameter of erythema recorded and peak IFN γ ELISpot responses (1 week after vaccination) for 104 subjects (Spearman rho = -0.14 , $p = 0.17$).

with documented fever in 3% of subjects. There were no severe systemic vaccine-related AEs.

As previously described, there was a lower frequency of pain in the low-dose group compared to the high-dose group and systemic AEs were more frequent in the high-dose groups [17]. A dose effect was also seen with objective measures of AEs. The diameter of local erythema increased with vaccine dose and 21% of subjects reported documented fever in the high-dose group compared to 3% in the combined mid-dose groups and none in the low-dose group. When MVA85A was the second poxvirus vaccination (MM and BFM groups), the peak diameter of erythema (and swelling in the MM group) was larger compared to a single vaccination with MVA85A. This effect was more marked in the BFM group, in which the peak diameter of swelling was classified as moderate or severe in all seven subjects and in five of the seven subjects for erythema. However, group sizes were small, the diameters of reactions were only larger on days 1 and 2, and frequency and severity of other local AEs and systemic AEs were similar to those in the other mid-dose groups.

Since these trials did not include a placebo or vaccination control group, tolerability was compared to published data of two licensed vaccines, the existing TB vaccine, live attenuated *M. bovis* BCG and a live viral vaccine against Yellow Fever. These vaccines were selected since they have similarities to MVA85A in terms of

mycobacterial antigen (BCG) or live viral vector (Yellow Fever) and are widely administered, including to healthy adults in temperate countries. The transient local inflammation after MVA85A vaccination compares favourably to the injection site ulceration associated with BCG vaccination. Although most trials did not report any systemic AEs after BCG vaccination, it is a live mycobacterial vaccine with a risk of more serious systemic (disseminated) BCG infection. Disseminated BCG infection is unusual in immunocompetent individuals, but the risk is much higher in the immunocompromised, and HIV infection in infants is now a contraindication to vaccination with BCG [30]. Yellow Fever vaccination induced erythema, swelling and pain in most subjects when administered intradermally and less frequently when subcutaneous (the usual route). Comparable to MVA85A, Yellow Fever vaccination induces a transient mild viral syndrome, with documented fever reported in some subjects. Without a placebo control group, the frequencies of non-specific systemic AEs that would be reported by the same population in the same conditions in the absence of MVA85A vaccination are not known. Randomised controlled trials (RCT) of other vaccines have reported high rates of headache (19%), tiredness (18%) and any systemic AE (50%) in the placebo groups [31,32]. Interestingly, the only clinical trial reporting any systemic AEs after BCG vaccination was performed by our research group, using the same methods as the MVA85A clinical trials, with a 7 days subject diary card and soliciting the same AEs [21]. In this trial, headache, arthralgia, myalgia and feverishness were reported by up to one third of subjects.

The frequency of local reactions seen after immunisation with MVA85A, particularly erythema and swelling, reflects the intradermal route of administration as well as the vaccine itself. Local reactions are more frequent after intradermal compared to intramuscular or subcutaneous injections [29,31–35]. Safety and immunogenicity of different routes of vaccination with MVA85A are currently being evaluated. The systemic AEs reported after MVA85A vaccination are more comparable to those reported after other live viral-vectored vaccines than the existing TB vaccine, BCG, suggesting the vector determines AEs to a greater extent than the insert [33,36–41]. This is intuitive, since the viral vector comprises the vast majority of the antigenic stimulus, and MVA is chosen as a vector for antigen delivery precisely because of the powerful innate immune response induced by the vector.

Protein-adjuvant vaccines are an alternative approach being developed for new vaccines for TB and other infectious diseases. These include the leading malaria vaccine RTS,S adjuvanted with AS01B or AS02A; TB vaccine M72 in AS02; malaria vaccine AMA1-CA/ISA 720; and TB vaccine Hybrid 1 (Antigens 85B and ESAT-6 in adjuvant IC31) [42–45]. Current data indicate that using adjuvants will, like live viral vectors, induce local and systemic reactions and that MVA85A is similar in tolerability to candidate protein-adjuvant vaccines.

No correlations were found between peak immune responses (1 week after vaccination) as measured by *ex vivo* IFN γ ELISpot and objective measures of systemic AEs (documented fever) or local reactions (diameter of erythema). The immune assays were evaluating the adaptive cellular immune response 7 days post-vaccination. As both the onset of systemic AEs and the peak diameter of erythema and swelling occur within the first 24–48 h after vaccination, we speculate that the innate immune response is largely responsible for the AE profile of MVA85A. If the effect of sequential poxvirus vaccination on increasing local reaction size is real, this may indicate that the adaptive immune response is also involved. Pathways that have been shown to play important roles in sensing MVA and coordinating the innate response include TLR2–TLR6–MyD88, MDA-5–IPS-1 and NALP3 [46,47]. Work analysing the innate immune response after MVA85A vaccination will

enable analysis of the relationship between vaccine-induced innate immunity and AE profile.

In summary, MVA85A is a well-tolerated TB vaccine candidate that elicits a strong cellular immune response. Comparison to published safety data for other vaccines suggests it has comparable reactivity to other live viral vaccines, protein-adjuvant vaccines and intradermal vaccines. Local and systemic AEs reported for MVA85A are not affected by increasing mycobacterial exposure and do not correlate with the adaptive cellular immune response to MVA85A. Further work to characterise the innate immune response and evaluate its relationship with both AE profile and adaptive immune response is underway. It is essential that any new TB vaccine being developed can be safely given to high-risk groups and the safety evaluation of immunisation of HIV-infected subjects is an important component of the ongoing clinical development of MVA85A[6]. Current evaluation of the safety and immunogenicity of MVA85A is also focusing on alternative routes of vaccination and vaccine efficacy in target populations.

Clinicaltrials.gov identification numbers for the trials included in this analysis: NCT00423566; NCT00465465; NCT00427453; NCT00427830; NCT00653770; NCT00548444; NCT00456183; NCT00653770.

Financial disclosure

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Conflict of interest statement

AAP and HMCS are named inventors on a composition of matter patent for MVA85A and are shareholders in a Joint Venture formed for the further development of this vaccine.

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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.trivac.2012.07.001>.

References

- [1] Global Tuberculosis Control, WHO report 2011, World Health Organisation, 2011. Report No.: 978 92 4 1564380.
- [2] B.B. Trunz, P. Fine, C. Dye, Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: A meta-analysis and assessment of cost-effectiveness, *Lancet* 367 (2006) 1173–1180.
- [3] J.A. Sterne, L.C. Rodrigues, I.N. Guedes, Does the efficacy of BCG decline with time since vaccination?, *Int J. Tuberc. Lung. Dis.* 2 (1998) 200–207.
- [4] L.C. Rodrigues, V.K. Diwan, J.G. Wheeler, Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: A meta-analysis, *Int. J. Epidemiol.* 22 (1993) 1154–1158.
- [5] G.A. Colditz, T.F. Brewer, C.S. Berkey, M.E. Wilson, E. Burdick, H.V. Fineberg, et al., Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature, *JAMA* 271 (1994) 698–702.
- [6] A.M. Minassian, R. Rowland, N.E. Beveridge, I.D. Poulton, I. Satti, S. Harris, et al., A Phase I study evaluating the safety and immunogenicity of MVA85A, a candidate TB vaccine, in HIV-infected adults, *BMJ Open* 1 (2011) e000223.
- [7] C.R. Sander, A.A. Pathan, N.E. Beveridge, I. Poulton, A. Minassian, N. Alder, et al., Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in *Mycobacterium tuberculosis*-infected individuals, *Am. J. Respir. Crit. Care Med.* 179 (2009) 724–733.

- [8] A.A. Pathan, C.R. Sander, H.A. Fletcher, I. Poulton, N.C. Alder, N.E. Beveridge, et al., Boosting BCG with recombinant modified vaccinia Ankara expressing antigen 85A: Different boosting intervals and implications for efficacy trials, *PLoS ONE* 2 (2007) e1052.
- [9] H. McShane, A.A. Pathan, C.R. Sander, S.M. Keating, S.C. Gilbert, K. Huygen, et al., Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans, *Nat. Med.* 10 (2004) 1240–1244.
- [10] T.J. Scriba, M. Tameris, E. Smit, L. van der Merwe, E.J. Hughes, B. Kadira, et al., A phase IIa trial of the new TB vaccine, MVA85A, in HIV and/or *M. tuberculosis* infected adults, *Am. J. Respir. Crit. Care Med.* (2012).
- [11] M.O. Ota, A.A. Odutola, P.K. Owiafe, S. Donkor, O.A. Owolabi, N.J. Brittain, et al., Immunogenicity of the tuberculosis vaccine MVA85A is reduced by coadministration with EPI vaccines in a randomized controlled trial in Gambian infants, *Sci. Transl. Med.* 3 (2011) 88ra56.
- [12] T.J. Scriba, M. Tameris, N. Mansoor, E. Smit, L. van der Merwe, K. Mauff, et al., Dose-finding study of the novel tuberculosis vaccine, MVA85A, in healthy BCG-vaccinated infants, *J. Infect. Dis.* 203 (2011) 1832–1843.
- [13] R.H. Brookes, P.C. Hill, P.K. Owiafe, H.B. Ibinga, D.J. Jeffries, S.A. Donkor, et al., Safety and immunogenicity of the candidate tuberculosis vaccine MVA85A in West Africa, *PLoS ONE* 3 (2008) e2921.
- [14] T. Hawkrige, T.J. Scriba, S. Gelderbloem, E. Smit, M. Tameris, S. Moyo, et al., Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in healthy adults in South Africa, *J. Infect. Dis.* 198 (2008) 544–552.
- [15] H.B. Ibinga, R.H. Brookes, P.C. Hill, P.K. Owiafe, H.A. Fletcher, C. Lienhardt, et al., Early clinical trials with a new tuberculosis vaccine, MVA85A, in tuberculosis-endemic countries: Issues in study design, *Lancet Infect. Dis.* 6 (2006) 522–528.
- [16] R. Rowland, A.A. Pathan, I. Satti, I.D. Poulton, M.M.L. Matsumiya, M. Whittaker, et al., Safety and Immunogenicity of an FP9-Vectored Candidate Tuberculosis Vaccine, Alone and With Candidate Vaccine, MVA85A: A Phase I Clinical Trial, 2012. Submitted for publication.
- [17] A.A. Pathan, A.M. Minassian, C.R. Sander, R. Rowland, D.W. Porter, I.D. Poulton, et al., Effect of vaccine dose on the safety and immunogenicity of a candidate TB vaccine, MVA85A, in BCG vaccinated UK adults, *Vaccine* (2012).
- [18] H. McShane, S. Behboudi, N. Goonetilleke, R. Brookes, A.V. Hill, Protective immunity against *Mycobacterium tuberculosis* induced by dendritic cells pulsed with both CD8(+) and CD4(+) T-cell epitopes from antigen 85A, *Infect. Immun.* 70 (2002) 1623–1626.
- [19] N.E. Beveridge, H.A. Fletcher, J. Hughes, A.A. Pathan, T.J. Scriba, A. Minassian, et al., A comparison of IFN γ detection methods used in tuberculosis vaccine trials, *Tuberculosis (Edinb.)* 88 (2008) 631–640.
- [20] N.E. Beveridge, D.A. Price, J.P. Casazza, A.A. Pathan, C.R. Sander, T.E. Asher, et al., Immunisation with BCG and recombinant MVA85A induces long-lasting, polyfunctional *Mycobacterium tuberculosis*-specific CD4+ memory T lymphocyte populations, *Eur. J. Immunol.* 37 (2007) 3089–3100.
- [21] K.T. Whelan, A.A. Pathan, C.R. Sander, H.A. Fletcher, I. Poulton, N.C. Alder, et al., Safety and immunogenicity of boosting BCG vaccinated subjects with BCG: Comparison with boosting with a new TB vaccine, MVA85A, *PLoS ONE* 4 (2009) e5934.
- [22] D.F. Hoft, A. Blazevic, G. Abate, W.A. Hanekom, G. Kaplan, J.H. Soler, et al., A new recombinant Bacille Calmette-Guerin vaccine safely induces significantly enhanced tuberculosis-specific immunity in human volunteers, *J. Infect. Dis.* 198 (2008) 1491–1501.
- [23] D.F. Hoft, C. Leonardi, T. Milligan, G.T. Nahass, B. Kemp, S. Cook, et al., Clinical reactivity of intradermal Bacille Calmette-Guerin vaccination, *Clin. Infect. Dis.* 28 (1999) 785–790.
- [24] P. Ravn, H. Boesen, B.K. Pedersen, P. Andersen, Human T cell responses induced by vaccination with *Mycobacterium bovis* Bacillus Calmette-Guerin, *J. Immunol.* 158 (1997) 1949–1955.
- [25] E.B. Kemp, R.B. Belshe, D.F. Hoft, Immune responses stimulated by percutaneous and intradermal Bacille Calmette-Guerin, *J. Infect. Dis.* 174 (1996) 113–119.
- [26] J. Lang, J. Zuckerman, P. Clarke, P. Barrett, C. Kirkpatrick, C. Blondeau, Comparison of the immunogenicity and safety of two 17D yellow fever vaccines, *Am. J. Trop. Med. Hyg.* 60 (1999) 1045–1050.
- [27] T.P. Monath, R. Nichols, W.T. Archambault, L. Moore, R. Marchesani, J. Tian, et al., Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase III multicenter, double-blind clinical trial, *Am. J. Trop. Med. Hyg.* 66 (2002) 533–541.
- [28] M. Pfister, O. Kursteiner, H. Hilfiker, D. Favre, P. Durrer, A. Ennaji, et al., Immunogenicity and safety of BERNA-YF compared with two other 17D yellow fever vaccines in a phase 3 clinical trial, *Am. J. Trop. Med. Hyg.* 72 (2005) 339–346.
- [29] A.H. Roukens, A.C. Vossen, P.J. Bredenbeek, J.T. van Dissel, L.G. Visser, Intradermally administered yellow fever vaccine at reduced dose induces a protective immune response: A randomized controlled non-inferiority trial, *PLoS ONE* 3 (2008) e1993.
- [30] WHO. Revised BCG vaccination guidelines for infants at risk for HIV infection, *Wkly Epidemiol. Rec.* 82 (2007) 193–196.
- [31] L.A. Jackson, M.J. Gaglani, H.L. Keyserling, J. Balsler, N. Bouveret, L. Fries, et al., Safety, efficacy, and immunogenicity of an inactivated influenza vaccine in healthy adults: A randomized, placebo-controlled trial over two influenza seasons, *BMC Infect. Dis.* 10 (2010) 71.
- [32] S. Vasan, S.J. Schlesinger, Z. Chen, A. Hurley, A. Lombardo, S. Than, et al., Phase 1 safety and immunogenicity evaluation of ADMVA, a multigenic, modified vaccinia Ankara-HIV-1 B/C candidate vaccine, *PLoS ONE* 5 (2010) e8816.
- [33] M.B. Wilck, M.S. Seaman, L.R. Baden, S.R. Walsh, L.E. Grandpre, C. Devoy, et al., Safety and immunogenicity of modified vaccinia Ankara (ACAM3000): Effect of dose and route of administration, *J. Infect. Dis.* 201 (2010) 1361–1370.
- [34] M. Walther, F.M. Thompson, S. Dunachie, S. Keating, S. Todryk, T. Berthoud, et al., Safety, immunogenicity, and efficacy of prime-boost immunization with recombinant poxvirus FP9 and modified vaccinia virus Ankara encoding the full-length *Plasmodium falciparum* circumsporozoite protein, *Infect. Immun.* 74 (2006) 2706–2716.
- [35] M.J. Warrell, A. Riddell, L.M. Yu, J. Phipps, L. Diggie, H. Bourhy, et al., A simplified 4-site economical intradermal post-exposure rabies vaccine regimen: A randomised controlled comparison with standard methods, *PLoS Negl. Trop. Dis.* 2 (2008) e224.
- [36] A. von Krempelhuber, J. Vollmar, R. Pokorny, P. Rapp, N. Wulff, B. Petzold, et al., A randomized, double-blind, dose-finding Phase II study to evaluate immunogenicity and safety of the third generation smallpox vaccine candidate IMVAMUNE, *Vaccine* 28 (2010) 1209–1216.
- [37] J. Vollmar, N. Arndtz, K.M. Eckl, T. Thomsen, B. Petzold, L. Mateo, et al., Safety and immunogenicity of IMVAMUNE, a promising candidate as a third generation smallpox vaccine, *Vaccine* 24 (2006) 2065–2070.
- [38] V.S. Moorthy, M. Pinder, W.H. Reece, K. Watkins, S. Atabani, C. Hannan, et al., Safety and immunogenicity of DNA/modified vaccinia virus Ankara malaria vaccination in African adults, *J. Infect. Dis.* 188 (2003) 1239–1244.
- [39] I. Cebere, L. Dorrell, H. McShane, A. Simmons, S. McCormack, C. Schmidt, et al., Phase I clinical trial safety of DNA- and modified virus Ankara-vectored human immunodeficiency virus type 1 (HIV-1) vaccines administered alone and in a prime-boost regime to healthy HIV-1-uninfected volunteers, *Vaccine* 24 (2006) 417–425.
- [40] D.P. Webster, S. Dunachie, S. McConkey, I. Poulton, A.C. Moore, M. Walther, et al., Safety of recombinant fowlpox strain FP9 and modified vaccinia virus Ankara vaccines against liver-stage *P. falciparum* malaria in non-immune volunteers, *Vaccine* 24 (2006) 3026–3034.
- [41] B. Abel, M. Tameris, N. Mansoor, S. Gelderbloem, J. Hughes, D. Abrahams, et al., The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults, *Am. J. Respir. Crit. Care Med.* 181 (2010) 1407–1417.
- [42] M.A. Pierce, R.D. Ellis, L.B. Martin, E. Malkin, E. Tierney, K. Miura, et al., Phase 1 safety and immunogenicity trial of the *Plasmodium falciparum* blood-stage malaria vaccine AMA1-C1/ISA 720 in Australian adults, *Vaccine* 28 (2010) 2236–2242.
- [43] J.T. van Dissel, S.M. Arend, C. Prins, P. Bang, P.N. Tingskov, K. Lingnau, et al., Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in naive human volunteers, *Vaccine* 28 (2010) 3571–3581.
- [44] K.E. Kester, J.F. Cummings, O. Ofori-Anyinam, C.F. Ockenhouse, U. Krzych, P. Moris, et al., Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naïve adults: Safety, efficacy, and immunologic associates of protection, *J. Infect. Dis.* 200 (2009) 337–346.
- [45] K. Von Eschen, R. Morrison, M. Braun, O. Ofori-Anyinam, E. De Kock, P. Pavithran, et al., The candidate tuberculosis vaccine Mtb72F/AS02A: Tolerability and immunogenicity in humans, *Hum. Vaccine* 5 (2009) 475–482.
- [46] J. Delaloye, T. Roger, Q.G. Steiner-Tardivel, D. Le Roy, M. Knaup Reymond, S. Akira, et al., Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome, *PLoS Pathog.* 5 (2009) e1000480.
- [47] J. Zhu, J. Martinez, X. Huang, Y. Yang, Innate immunity against vaccinia virus is mediated by TLR2 and requires TLR-independent production of IFN- β , *Blood* 109 (2007) 619–625.