CLINICAL TRIAL PROTOCOL

A phase I trial to evaluate the safety and immunogenicity of candidate TB vaccine MVA85A administered by the aerosol inhaled route in healthy adult volunteers who are latently infected with *Mycobacterium tuberculosis*

Short title: MVA85A aerosol vaccination in adults with latent *M.tb* infection

Trial Reference:	TB040
EudraCT number:	2015-001826-41
REC Reference:	15/SC/0370
IRAS Reference:	181214

Date and Version Number	19 December 2017, V7.0
Chief Investigator:	Professor Helen McShane
Sponsor:	University of Oxford
Local Safety Committee Chair:	Professor Brian Angus
Funding body:	TBVI, Wellcome Trust
Author:	Dr Julia Marshall and Dr Rebecca Powell Doherty

Confidentiality Statement

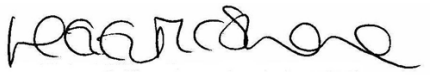
THE JENNER INSTITUTE
DEVELOPING INNOVATIVE VACCINES

TABLE OF CONTENTS

1 STATEMENT OF COMPLIANCE

Investigator Agreement

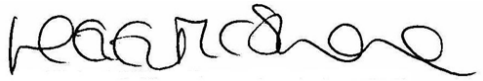
"I have read this protocol and agree to abide by all provisions set forth therein. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Professor Helen McShane		16.01.18
Chief Investigator	Investigator Signature	Date

Professor Helen McShane

Conflict of Interest

"According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no conflict of interest with any Investigators"

Professor Helen McShane		16.01.18
Chief Investigator	Investigator Signature	Date

Professor Helen McShane

2 AMENDMENT HISTORY

Protocol v7.0 Substantial amendment

Section	From	Changed To
Title	A phase I trial to compare the safety and immunogenicity of candidate TB vaccine MVA85A administered by the aerosol inhaled route and the intramuscular route in healthy adult volunteers who are latently infected with *Mycobacterium tuberculosis* **Short title:** MVA85A aerosol vs intramuscular in adults with latent *M.tb* infection	A phase I trial to evaluate the safety and immunogenicity of candidate TB vaccine MVA85A administered by the aerosol inhaled route in healthy adult volunteers who are latently infected with *Mycobacterium tuberculosis* Short title: MVA85A aerosol vaccination in adults with latent *M.tb* infection
3.0		PIC Addition: St George's University Hospitals NHS Foundation Trust Queen Mary's Hospital Tuberculosis Chest Clinic Roehampton Ln, London SW15 5PN Collaborator Addition: Dr Catherine Cosgrove Consultant Infectious Diseases Physician Adult Lead at the Vaccine Institute SGUL St George's, University Hospitals NHS Foundation Trust Tel: +44 (0)208 725 5827 Email: CCosgrov@sgul.ac.uk
3.0	Investigator Dr Morven Wilke	Investigator Dr Julia Marshall
3.0	Project Manager Samantha Vermaak	Project Manager Dr Rebecca Powell Doherty
3.0	Statistician Sharon Love	Statistician Nicola Williams
4.1	15 subjects: Starter Group: the first 3 volunteers will receive 1 x 10^7 pfu aerosol inhaled MVA85A The next 12 subjects will be randomised to two trial arms: Group A: 6 volunteers receiving 5 x 10^7 pfu aerosol inhaled MVA85A and IM saline placebo Group B: 6 volunteers receiving 5 x 10^7 pfu IM injection of MVA85A and aerosol inhaled saline placebo	6-9 subjects: Starter Group: the first 3 volunteers will receive 1 x 10^7 pfu aerosol inhaled MVA85A Group A: A minimum of 3 and a maximum of 6 volunteers will receive the standard dose of 5 x 10^7 pfu aerosol inhaled MVA85A
4.1		Trial interventions: Removal of IM route

4.1	To evaluate the systemic and mucosal cellular and humoral immunogenicity induced by MVA85A vaccination by the aerosol inhaled and IM route, in healthy volunteers who are latently infected with *M.tb* To evaluate other exploratory markers of cell-mediated and humoral immunogenicity induced by aerosol and IM MVA85A, including transcriptomic analysis	To evaluate the systemic and mucosal cellular and humoral immunogenicity induced by MVA85A vaccination by the aerosol inhaled in healthy volunteers who are latently infected with *M.tb* To evaluate other exploratory markers of cell-mediated and humoral immunogenicity induced by aerosol MVA85A, including transcriptomic analysis
4.1	Route of IMP: Aerosol inhalation by nebuliser or IM needle injection in the deltoid region of the arm Allocation method: Variable block randomisation by sequentially numbered sealed envelopes	Route of IMP: Aerosol inhalation by nebuliser Allocation method: Sequential allocation
4.1	15 subjects: Starter Group: the first 3 volunteers will receive 1 x 10^7 pfu aerosol inhaled MVA85A The next 12 subjects will be randomised to two trial arms: Group A: 6 volunteers receiving 5 x 10^7 pfu aerosol inhaled MVA85A and IM saline placebo Group B: 6 volunteers receiving 5 x 10^7 pfu IM injection of MVA85A and aerosol inhaled saline placebo Follow up duration: 24 weeks from vaccination day	6-9 subjects: Starter Group: the first 3 volunteers will receive 1 x 10^7 pfu aerosol inhaled MVA85A Group A (Standard Dose) : 3-6 volunteers will receive 5 x 10^7 pfu aerosol inhaled MVA85A Follow up duration: Final visit 12 weeks from vaccination day with telephone call at 24 weeks.
4.2 Schedule of visits and procedures	Bronchoscopy at 1 week +/- 2 days	Bronchoscopy at 1 week +4 days,- 2 days
4.2	D168 scheduled visit with vital signs and phlebotomy with immunology	D168 telephone call. No blood tests and no vital signs. Volunteers will be asked to come in for physical examination if deemed medically necessary.
4.2	"U&Es, LFTs (except day 2 when CRP only; if raised, to be repeated on day 7)"	Clarification of wording around when CRP is taken "U&Es, LFTs, CRP (except day 2 when CRP only)"
4.2	"Cumulative blood volume 416ml"	"Cumulative blood volume 356ml" Clarification of blood volume: "Blood volumes stated for Oxford. The exact volumes of blood taken will depend on the local site – cumulative blood volumes for volunteers outside Oxford may be slightly lower or higher than those of Oxford volunteers, due to the use of different volume vacutainers for biochemistry, haematology and serology samples as per local Trust standard procedures"

5.0 Abbreviations		Addition of CRP: C-reactive protein GSTT: Guys and St. Thomas' NHS Foundation Trust PFU: Particle forming unit SGUL: St George's University of London Deletion of: ART: Anti-retroviral therapy CFU: Colony forming unit
Throughout		Minor updates to reflect the fact that TB035 trial has now completed
6.1 Context	A second Phase I safety trial assessing anti-vector immunity with nebulised MVA85A was completed in 2016 (TB035, Clinical Trials.gov reference number NCT01954563). An intermediate dose of 5 x 10^7 pfu was used in TB035. To our knowledge there have been no other clinical studies of viral vectored vaccines delivered by aerosol.	A second Phase I safety trial assessing anti-vector immunity with nebulised MVA85A was completed in 2016 (TB035, Clinical Trials.gov reference number NCT01954563). An intermediate dose of 5 x 10^7 pfu was used in TB035. No vaccine related SAEs were reported (unpublished data). To our knowledge there have been no other clinical studies of viral vectored vaccines delivered by aerosol.
6.2 Hypothesis	We postulate that the aerosol inhaled route is practical and feasible and has an acceptable safety profile, comparable to the systemic safety profile of the IM route of administration of MVA85A in volunteers latently infected with *M.tb*. We hypothesise that the aerosol inhaled route of administration will induce greater mucosal immunity and comparable systemic immunity when compared to the IM (systemic) route of administration in these volunteers.	We postulate that the aerosol inhaled route is practical and feasible and has an acceptable safety profile, in volunteers latently infected with *M.tb*. We hypothesise that the aerosol inhaled route of administration will induce greater mucosal and comparable systemic immunity when compared to historical data of IM (systemic) route of administration in healthy volunteers.
6.2 vaccine dosage	Once the Starter Group is fully enrolled and the second safety review is completed, we will start randomisation to Groups A and B, with dose escalation to 5 x 10^7 pfu (the same dose as currently used in TB035). For safety reasons, the first volunteer will be assigned to Group A (aerosol MVA85A plus saline placebo) and there will be no further vaccinations until 48 hours has elapsed and the safety data reviewed. If the CI decides it is safe to proceed, the remaining volunteers will then be randomised.	Once the Starter Group is fully enrolled and the second safety review is completed, we will start enrolment into Group A (Standard Dose), with dose escalation to 5 x 10^7 pfu (the same dose used in TB035). For safety reasons, the first volunteer in this group will be vaccinated ahead of other volunteers and no other volunteers will be vaccinated until at least 48 hours has elapsed. If the CI decides it is safe to proceed, the remaining volunteers will then be enrolled. The target enrolment for this group is 6 volunteers. However, given the often reduced pace of recruitment from the latently infected population, we will accept a minimum of 3 volunteers if the target has not been reached by October 2018.
6.3 Risks and benefits		Local reactions to IM injection deleted

Table 2	TB034: Study Status: Enrolling TB035: N[b] : 12, 2, 12 Study Status: Enrolling e. Subjects blinded as to whether they received MVA85A via the ID or aerosol inhaled route.	TB034: Study Status: Completed N[b] : 12, 13, 12 Study Status: Completed e. Subjects blinded as to whether they received MVA85A via the ID or aerosol inhaled route; Volunteers in group 2 who were due to receive their day 28 vaccination on or after 2nd September 2015 did not receive this boost vaccination due to a higher frequency of mild respiratory adverse events in this group. Volunteers received placebo by both intradermal and aerosol inhaled group to maintain blinding.
7.0 Objectives and Outcome Measures	At each visit, via diary card for 14 days after vaccination To evaluate the systemic and mucosal cellular and humoral immunogenicity induced by MVA85A vaccination by the aerosol inhaled and IM route, in healthy volunteers who are latently infected with *M.tb*. To evaluate other exploratory markers of cell-mediated and humoral immunogenicity induced by aerosol and IM MVA85A, including transcriptomic analysis	At each visit, via diary card for 14 days after vaccination and at the 6 month phone call To evaluate the systemic and mucosal cellular and humoral immunogenicity induced by MVA85A vaccination by the aerosol inhaled route in healthy volunteers who are latently infected with *M.tb.* To evaluate other exploratory markers of cell-mediated and humoral immunogenicity induced by aerosol MVA85A, including transcriptomic analysis
8.0 Inclusion Exclusion criteria	• Ineligible for chemoprophylaxis for latent *M.tb* infection, declined prophylaxis or considered low risk due to distant contact history	• Considered low risk of reactivation of their latent infection due to distant contact history
9.1 Trial design and procedures	15 volunteers will be enrolled; the first 3 to the starter group, then 12 randomly allocated to either group A or group B (1st volunteer in Group A is not randomised).	9 volunteers will be enrolled; the first 3 to the starter group, then 6 volunteers into Group A.
Table 4	Starter group: 3 Aerosol inhaled MVA85A 1 x 10^7 pfu Group A: 6 Aerosol inhaled MVA85A 5 x 10^7 pfu and IM saline placebo Group B: 6 IM MVA85A 5 x 10^7 pfu and inhaled aerosol saline placebo	Starter group: 3 Aerosol inhaled MVA85A 1 x 10^7 pfu Group A (Standard dose): 3-6 Aerosol inhaled MVA85A 5 x 10^7 pfu

9.2	Volunteers, either ineligible for or declining anti-tuberculous chemoprophylaxis, or considered to be at low risk of reactivation of their latent infection due to distant contact history, will be recruited from TB contact clinics at Oxford University Hospitals, Birmingham Heartlands Hospital, Birmingham Chest Clinic, Royal Free Hospital, King's College Hospital, and St Thomas' Hospital.	Volunteers considered to be at low risk of reactivation of their latent infection due to distant contact history, will be recruited from TB contact clinics at Oxford University Hospitals, Birmingham Chest Clinic Heart of England, Royal Free Hospital, King's College Hospital, Guy's and St Thomas' Hospital and St George's University Hospital
9.2	Prospective anti-TB chemoprophylaxis, provided through the usual NHS routes and TB/chest clinics, would be delayed until after the trial D28 follow-up visit and CT, decision not to enrol, or early withdrawal; whichever occurs first.	Ideally, the volunteer will delay their prospective anti-tuberculous chemoprophylaxis, provided through the usual NHS routes and TB/chest clinics, until after the trial D28 follow-up visit and CT, decision not to enrol, or early withdrawal; whichever occurs first. The study team will keep each volunteer's NHS TB/chest clinic team aware of when these timelines are reached. However, considering the challenges inherent in recruiting latently infected volunteers, if the volunteer does not want their prospective anti-TB chemoprophylaxis delayed they will still be eligible to be enrolled onto the study.
9.5 Randomisation and blinding		Deletion of this section
9.6 Follow up visit	MVA85A vaccination will be performed by the aerosol or intramuscular route according to the site specific SOP. The bronchoscopy visit lasts several hours and takes place at the bronchoscopy suite in the OUH NHS Foundation Trust Hospitals by the specialist team there. Details of the procedure are outlined in section 6.1	Now 9.5 Follow up visit MVA85A vaccination will be performed by the aerosol route according to the trial SOP. The bronchoscopy visit lasts several hours and takes place at the bronchoscopy suite in the OUH NHS Foundation Trust Hospitals by the specialist team there. Details of the procedure are outlined in section 6.1 In exceptional circumstances where volunteers are absolutely unable to travel to Oxford, the bronchoscopy may be performed at the other NHS trust study sites (King's College Hospital NHS Trust, University of Birmingham NHS Trust, Royal Free Hospital NHS Trust). The bronchoscopy will always be performed by a respiratory consultant trained in the procedure and following the trial specific SOP.
10.3	The vaccine will be administered IM over the deltoid region of the non-dominant	The vaccine will be administered by aerosol inhalation, according to the trial-specific SOP.

	upper arm, or by aerosol inhalation, according to the site-specific SOP.	
10.4 Saline	Sterile saline will be used for dilution and placebo doses. The volume of saline used as placebo will be identical to the vaccine volume by the same route. For volunteers receiving the aerosol vaccination (Starter Group, Group A) we will administer the vaccine dose made up to 1ml with saline and at the same time a paired placebo (Group A only) of 60µl of normal saline for IM injection. For volunteers receiving the vaccine IM (Group B) we will administer 60µl and at the same time a paired placebo of 1ml of saline inhaled.	Sterile saline will be used for dilution of MVA85A as needed, to achieve the correct dose. Once prepared, the vaccine will then be made up to 1ml with saline for administration via the MicroAIR NE-U22 nebuliser.
11.7 Assessment of severity		Local injection site reactions table deleted
12.0 Statistics	Following the first three volunteers enrolled into the starter group, six volunteers will be recruited into each experimental arm of the trial (groups A and B). Our previous experience with clinical trials using MVA85A suggests that this sample size is a feasible number to recruit, screen, enroll, and follow up in practical terms, whilst also allowing the determination of any substantial differences in the outcome measures between the two groups. A twofold increase in the magnitude of the cellular immune response (as measured by ELISpot response to 85A peptide stimulation) is considered immunologically meaningful. This sample size is appropriate for a proof-of-concept Phase I safety study and takes into account the low number of potentially eligible healthy adults with latent *M.tb* infection in the UK and the impact of this on recruitment. The sample size has not been determined with the aim of achieving statistical significance. The one-week and six-month responses will be compared between the two groups using a t-test. If the resulting data are not normally distributed then medians and interquartile ranges will be used to summarise the data and the Mann Whitney U test will be used for statistical comparisons. An area-under-curve analysis will be used to compare	This is primarily a safety trial with descriptive endpoints. Following the first three volunteers enrolled into the starter group, 3-6 volunteers will be recruited into Group A, to receive the target dose. Due to existing difficulties recruiting to this clinical trial we have decided to take out Group B (the comparator arm) to ensure that the numbers recruited will still be sufficient to allow for the primary objective, an assessment of safety. The one-week and three-month responses will be compared. Medians and interquartile ranges will be used to summarise the data and the Mann Whitney U test will be used for statistical comparisons. An area-under-curve analysis will be used to compare the overall responses over time comparing these aerosol data with historical data from previous trials. This analysis will make use of data collected at all time points.

	the overall responses over time between the two groups. This analysis will make use of data collected at all time points.	
16.6 Reimbursements	Volunteers will be compensated *pro rata* for their time, travel and for trial procedures while participating in the trial, amounting to a total of approximately £515-£635, depending on the exact number of visits, site of recruitment and whether any repeat or additional visits are necessary.	Volunteers will be compensated *pro rata* for their time, travel and for trial procedures while participating in the trial, amounting to a total of approximately £505-£625, depending on the exact number of visits, site of recruitment and whether any repeat or additional visits are necessary.

Protocol v6.0 Substantial amendment

Section	**From**	**Changed To**
9.2	Patients who fulfil the inclusion criteria will be given a Volunteer Information Sheet at their clinic attendance and asked if they would like to participate. If interested, their details will be passed to the trial team to arrange a screening appointment.	Patients who fulfil the inclusion criteria will be given a Volunteer Information Sheet Summary at their clinic attendance and asked if they would be interested in finding out more about the trial. If so, they will be given the full Volunteer Information Sheet to take with them to read in their own time. If still interested, and with their permission, the trial team will contact the patient to arrange a screening appointment.

Protocol v5.0 Substantial amendment

Section	**From**	**Changed To**
Throughout		Reduction of sample size for Starter Group, Group A and Group B
Throughout		Addition of three new sites
Throughout		Minor typographical corrections
Synopsis	Trial Duration estimated at 24 months	Trial Duration estimated at 30 months

Protocol v4.0 Substantial amendment

Section	**From**	**Changed To**
3.0		Removal of Mike Riste as trial Investigator
8.2	• Healthy adult aged 18-50 years	• Healthy adult aged 18-55 years
9.2	Prospective anti-tuberculous chemoprophylaxis would be delayed until the end of the trial, or early withdrawal; whichever occurs first.	Prospective anti-tuberculous chemoprophylaxis, provided through the usual NHS routes and TB/chest clinics, would be delayed until after the trial D28 follow-up visit and CT, decision not to enroll, or early withdrawal; whichever occurs first. The study team will keep each volunteer's NHS TB/chest

		clinic team aware of when these timelines are reached.
11.9	The LSM will perform independent external safety reviews prior to dose escalations.	[deleted to match other safety sections (11.1) as dose escalation safety review will be performed by PI and team]

Protocol v3.0 Substantial amendment

Section	From	Changed To
9.2		• We will also display posters in GP practices in the hope of attracting interest from latently infected individuals in the community who are no longer attending TB contact clinics.

Protocol v2.2 Non-substantial amendment

Section	From	Changed To
Throughout	• Oxford University Hospitals NHS Trust	• Oxford University Hospitals NHS Foundation Trust

Protocol v2.1 Non-substantial amendment

Section	From	Changed To
8.2	• Use effective contraception for the duration of the trial period (females only)	• Use effective contraception (see below) for the duration of the trial period (females only)
8.3		Explanation added of "Effective contraception for female volunteers".
11.2	• Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation.	• Hospitalisation or prolongation of existing hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation.

Protocol v2.0 Substantial amendment

Section	From	Changed To
Throughout		Incorrect references to placebo for Starter Group removed
Throughout		Clarification that first volunteer in Group A will be assigned, not randomised
3.0	**Principal Investigator:**	**Principal Investigator:**

	Dr Martin Dedicoat Consultant Infectious Diseases Physician Department of Infection & Tropical Medicine Heart of England NHS Trust Birmingham, B9 5SS Tel: +44 (0)121 4242357 Fax: +44 (0)121 4241309 Email: martin.dedicoat@heartofengland.nhs.uk	Prof Paul Moss NIHR-WTCRF Tel: +44 (0)121 414 2824 Email: p.moss@bham.ac.uk
3.0	**Collaborator:** Dr Henry Bettinson Consultant Respiratory and Intensive Care Physician Oxford Centre for Respiratory Medicine Oxford University Hospitals NHS Trust Oxford, OX3 7LE Tel: +44 (0)1865 225234 Fax: +44 (0)1865 225221 Email: henry.bettinson@ouh.nhs.uk	**Collaborators:** Dr Henry Bettinson Consultant Respiratory and Intensive Care Physician Oxford Centre for Respiratory Medicine Oxford University Hospitals NHS Trust Oxford, OX3 7LE Tel: +44 (0)1865 225234 Fax: +44 (0)1865 225221 Email: henry.bettinson@ouh.nhs.uk Dr Martin Dedicoat Consultant Infectious Diseases Physician Department of Infection & Tropical Medicine Heart of England NHS Trust Birmingham, B9 5SS Tel: +44 (0)121 4242357 Fax: +44 (0)121 4241309 Email: martin.dedicoat@heartofengland.nhs.uk
4.1		Trial interventions corrected to match Table 1
Table 1	Diary cards provided / collected	E-diary setup / final review Addition of pulmonary function test at D14 Addition of CRP at D2 Details of biochemistry tests added
Table 2		Missing footnotes added, plus minor changes to match updated Investigator Brochure
9.4	Urinalysis and a pregnancy test will be performed	Addition of clotting profile Deleted (duplication)
9.8	All samples will be in anonymised form	All samples will be in anonymised form at the CCVTM; samples at the NIHR-WTCRF may contain patient numbers and details as per NHS procedures.
11.6		Ediary. Addition: All AEs starting after the diary card period, or persisting after this period, will be recorded in the AE line listing of the CRF.
13.1 and 13.3		Paper CRFs also at CCVTM

	Secure encrypted University of Oxford server	Secure encrypted servers that are outsourced by OpenClinica™.
Throughout		Minor typographical corrections

3 KEY TRIAL CONTACTS AND ROLES

Clinical Trial Units:

Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital,
Old Road
Headington
Oxford, OX3 7LE

Oxford Centre for Respiratory Medicine, and John Warin Ward,
Oxford University Hospital NHS Foundation Trust
Headington
Oxford, OX3 7LE

National Institute for Health Research-Wellcome Trust Clinical Research Facility (NIHR-WTCRF)
Queen Elizabeth Hospital, Edgbaston
Birmingham, B15 2TH

Royal Free London NHS Foundation Trust
Royal Free Hospital
Pond Street
London, NW3 2QG

King's College Hospital NHS Foundation Trust
Denmark Hill
London, SE5 9PJ

PICs:

Heartlands Hospital
Heart of England NHS Foundation Trust
Bordesley Green East
Birmingham, B9 5SS

Guy's and St Thomas' NHS Foundation Trust
St Thomas' Hospital
Westminster Bridge Road
London, SE1 7EH

St George's University Hospitals NHS Foundation Trust
Queen Mary's Hospital
Tuberculosis Chest Clinic
Roehampton Ln,
London SW15 5PN

Chief Investigator:

Professor Helen McShane
CCVTM
Tel: +44 (0)1865 617606
Fax: +44 (0)1865 857471
Email: helen.mcshane@ndm.ox.ac.uk

Principal Investigators:

Prof Paul Moss
NIHR-WTCRF Birmingham
Tel: +44 (0)121 414 2824
Email: p.moss@bham.ac.uk

Dr Marc Lipman
Royal Free Hospital
Tel: +44 (0)20 7317 7560
Email: marclipman@nhs.net

Dr Felicity Perrin
King's College Hospital NHS Foundation Trust
Tel: +44 (0)20 3299 1729
Email: felicity.perrin@nhs.net

Investigators:

Dr Julia Marshall
TB Vaccine Trial Physician
CCVTM

Collaborators:

Dr Henry Bettinson
Consultant Respiratory and Intensive Care Physician
Oxford Centre for Respiratory Medicine
Oxford University Hospitals NHS Foundation Trust
Oxford, OX3 7LE
Tel: +44 (0)1865 225234
Fax: +44 (0)1865 225221
Email: henry.bettinson@ouh.nhs.uk

Dr Martin Dedicoat
Consultant Infectious Diseases Physician
Department of Infection & Tropical Medicine
Heart of England NHS Trust
Birmingham, B9 5SS
Tel: +44 (0)121 4242357
Fax: +44 (0)121 4241309
Email: martin.dedicoat@heartofengland.nhs.uk

Dr Ronan Breen
Consultant Respiratory Physician
Guy's and St Thomas' NHS Foundation Trust
Tel: +44 (0)7788 1714 966
Email: Ronan.Breen@gstt.nhs.uk

Dr Catherine Cosgrove
Consultant Infectious Diseases Physician
Adult Lead at the Vaccine Institute SGUL
St George's, University Hospitals NHS Foundation Trust
Tel: +44 (0)208 725 5827
Email: CCosgrov@sgul.ac.uk

Project Managers:	Dr Rebecca Powell Doherty & Dr Alison Lawrie CCVTM
Local Safety Monitor:	Professor Brian Angus Nuffield Department of Medicine, University of Oxford Old Road, Headington Oxford, OX3 7BN Tel: +44 (0)1865 220154 Fax: +44 (0)1865 222962 Email: brian.angus@ndm.ox.ac.uk
Senior Immunologist:	Dr Iman Satti The Jenner Institute Old Road Campus Research Building Roosevelt Drive, Headington Oxford, OX3 7DQ Tel: +44 (0)1865 617623 Fax: +44 (0)1865 617608 Email: iman.satti@ndm.ox.ac.uk
Statistician:	Nicola Williams Senior Trial Statistician Nuffield Department of Primary Care Health Sciences Radcliffe Observatory Quarter, University of Oxford Woodstock Road, Oxford. OX2 6GG. Tel: +44 (0) 1865 289300.
Sponsor:	Clinical Trials & Research Governance Joint Research Office, University of Oxford Block 60, Churchill Hospital Old Road, Headington Oxford, OX3 7LE
Monitor:	Clinical Trials & Research Governance Joint Research Office, University of Oxford Block 60, Churchill Hospital Old Road, Headington Oxford, OX3 7LE

4 SYNOPSIS

4.1 Synopsis

Trial Title	A phase I trial to evaluate the safety and immunogenicity of candidate TB vaccine MVA85A administered by the aerosol inhaled route in healthy adult volunteers who are latently infected with *Mycobacterium tuberculosis*	
Trial Identifier	TB040	
Clinical Phase	I	
Chief Investigator	Professor Helen McShane	
Trial Centres	Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE John Warin Ward, Oxford University Hospital NHS Foundation Trust, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE NIHR-Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH Royal Free London NHS Foundation Trust, Royal Free Hospital, Pond Street, London, NW3 2QG King's College Hospital NHS Foundation Trust, Denmark Hill, London, SE5 9PJ	
Trial participants	Healthy adult volunteers who are latently infected with *M.tb*	
Planned Sample Size	6-9 subjects: Starter Group: the first 3 volunteers will receive 1 x 10^7 pfu aerosol inhaled MVA85A Group A (Standard Dose) : 3-6 volunteers will receive 5 x 10^7 pfu aerosol inhaled MVA85A	
Vaccination Schedule	Single vaccination at day 0	
Follow-up Duration	Final visit 12 weeks from vaccination day with telephone call at 24 weeks.	
Blood Sampling	See visit schedule	
Trial Interventions	CT thorax at screening and day 28 Pulmonary function tests (as per table 1) Vaccination by aerosol inhaled route Venepuncture Bronchoscopy and BAL	
Trial Duration	Estimated at 42 months	
Planned Trial Period	Planned start date is July 2015	
	Objectives	Outcome Measures
Primary	To evaluate the safety of MVA85A vaccination by the aerosol inhaled route in healthy volunteers who are latently infected with *M.tb*	Actively and passively collected data on adverse events
Secondary	To evaluate the systemic and mucosal cellular and humoral immunogenicity induced by MVA85A vaccination by the aerosol inhaled in healthy volunteers who are latently infected with *M.tb*	Laboratory markers of cell mediated immunity, including *ex-vivo* ELISpot in blood and intracellular cytokine staining in blood and BAL samples
Tertiary	To evaluate other exploratory markers of cell-mediated and humoral immunogenicity induced by aerosol MVA85A, including transcriptomic analysis	Laboratory markers of cell mediated and humoral immunity in blood and BAL samples
IMP	MVA85A	

Dose(s)	1 x 10^7 pfu (Starter group). 5 x 10^7 pfu (Group A)
Route of IMP	Aerosol inhalation by nebuliser
Allocation Method	Sequential allocation

4.2 Schedule of visits and procedures

Table 1. Schedule of trial procedures

Attendance number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Timeline (days)*	S	0	2	7	14	28	84	168[T]
Timeline (weeks)*	S	0		1	2	4	12	24
Time windows (days)			±1	+4, -2	±4	±7	±14	±21
Inclusion/exclusion criteria	X							
Review contra-indications	X	X		X				
Informed consent	X							
Medical history	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Physical examination	X			X				
Vital signs	X	X	X	X	X	X	X	
Urinalysis	X							
CT chest	X					X		
PFTs	X	X	X	X	X			
β-HCG urine test	X	X		X				
Vaccinations		X						
Bronchoscopy				X				
Local & systemic events/reactions		X	X	X	X	X	X	X
Ediary setup		X						
Ediary final review					X			
Biochemistry (4mL)**	X		X	X		X		
Haematology (2mL)	X			X		X		
Coagulation (4mL)	X							
HBV, HCV, HIV (10mL)	X							
HLA typing (4mL)		X						
Exploratory immunology incl ELISpot (16-60mL***)	X	X	X	X	X	X	X	
Blood vol (mL)	30	64	20	56	60	66	60	
Cumulative blood vol (mL)****	30	94	114	170	230	296	356	

S Screening
X Event scheduled to occur
(X) If considered necessary, emphasising any complaint or change in medications
T Telephone call. Final visit is deemed to be at D84 with follow up phone call at D168
* Timeline is approximate only, as exact timings (± windows periods) of visits relate to the actual (not intended) date of the previous visit
** U&Es, LFTs (except day 2 when CRP only;)
*** 60ml at all visits except screening (10ml, T spot), visit 3 (16ml, no ELISpot) and visit 4 (50ml)
**** Blood volumes stated for Oxford. The exact volumes of blood taken will depend on the local site – cumulative blood volumes for volunteers outside Oxford may be slightly lower or higher than those of Oxford volunteers, due to the use of different volume vacutainers for biochemistry, haematology and serology samples as per local Trust standard procedures
Grey Highlights vaccination day Blue Highlights bronchoscopy day

5 ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
BAL	Bronchoalveolar Lavage
BCC	Basal cell carcinoma
BCG	Bacille Calmette-Guérin
β-HCG	Beta - Human Chorionic Gonadotrophin
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CIS	Carcinoma *in situ*
CRF	Case Report Form
CRP	C-reactive protein
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
ELISpot	Enzyme-linked Immunospot
FBC	Full Blood Count
GCP	Good Clinical Practice
GMO	Genetically Modified Organism
GLP	Good Laboratory Practice
GMSC	Genetic Modification Safety Committee
GSTT	Guy's and St. Thomas' NHS Foundation Trust
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICH	International Committee on Harmonisation
ID	Intradermal
IDT	IDT Biologika GmbH
IFN-ɣ	Interferon Gamma
IGRA	Interferon-Gamma Release Assay
IM	Intramuscular
IMP	Investigational Medicinal Product
LFT	Liver Function Test
LSC	Local Safety Committee
MHRA	Medicines & Healthcare Regulatory Authority
M.tb	*Mycobacterium tuberculosis*
MVA	Modified vaccinia Virus Ankara
NIHR	National Institute for Health Research
PBMC	Peripheral Blood Mononuclear Cells
PFT	Pulmonary Function Tests
PFU	Particle Forming Unit
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SGUL	St George's University of London
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TMF	Trial Master File
U&Es	Urea & Electrolytes

VP	Viral Particles
WTCRF	Wellcome Trust Clinical Research Facility

6 BACKGROUND AND RATIONALE

6.1 Context

Mycobacterium tuberculosis (*M.tb*) is a pathogen with worldwide preponderance which infects humans causing tuberculosis (TB), a transmissible disease resulting in very high mortality and morbidity. A third of the world's population is latently infected with *M.tb*, and these people carry a 10% lifetime risk of developing active life-threatening disease (1). In 2013, there were 9 million new cases worldwide and 1.5 million people died of TB (2). Co-infection with human immunodeficiency virus (HIV) greatly increases risk of TB reactivation and death (3, 4). Diagnosis is challenging and drug treatment can be prolonged, harmful, costly and complex. For these reasons an effective vaccine is a global public health priority.

The Bacille Calmette-Guérin (BCG) vaccine is the only licensed *M.tb* vaccine and it has been administered globally to several billion people over a 90 year period (5). Although it does not protect against pulmonary TB in endemic areas, it is effective in preventing disseminated TB disease including tuberculous meningitis in childhood (4, 6, 7).

Recently, heterologous "prime-boost" vaccination strategies, in which two different candidate vaccines expressing antigens in common are given weeks or months apart, have generated strong and sustained cellular immune responses correlating with an *M.tb* protective effect in preclinical animal models. In such a "prime-boost" strategy, BCG would therefore be an ideal priming vaccine.

MVA85A (Modified Vaccinia virus Ankara expressing *M.tb* antigen 85A) has been shown to be immunogenic as a booster vaccine in BCG-primed subjects (8); however, a phase IIb efficacy trial in 2797 BCG-vaccinated infants in South Africa reported no enhancement of BCG-induced protection (9). In this trial, the immunogenicity was significantly lower than that seen in adults, and more potent routes of administration are of interest in the field. A phase IIb trial in HIV infected adults in South Africa and Senegal has also just reported and no efficacy was demonstrated. Immunogenicity in this trial was also lower than that seen in UK adults and innovative ways of enhancing immunogenicity are urgently needed (10).

Basis for the aerosol inhaled route

The route of *M.tb* infection is by inhalation of aerosolised infectious droplets containing tubercle bacilli, leading to the establishment of primary infection in the lung, which has a distinct mucosal immune system characterized by bronchus associated lymphoid tissue (BALT), which is well adapted to encounter and process such antigens. Immunising via the airway should therefore have the advantage, over other routes, of eliciting protective immune responses in the lung mucosa.

There is data from preclinical animal models with MVA85A and other virally vectored vaccines to suggest that immunising the respiratory mucosa may give superior protection against respiratory diseases (11-15). Feasibility studies of aerosolised MVA delivery in non-human primates have demonstrated safe and consistent mucosal delivery to the respiratory tract, with potent and long-lasting insert specific immune responses (16). We know that BCG, administered systemically, protects most effectively against systemic disease in childhood (6, 7). It may follow that airway vaccination is needed to induce effective protection from pulmonary disease. In addition, the aerosol inhaled route of MVA85A delivery could offer practical, tolerability and safety benefits over and above needle-based methods (see below). There is, therefore, considerable international interest in developing this route of administration of TB vaccines.

The inhaled route is a well-established route of drug delivery. Aerosolised droplets of bronchodilating, anti-inflammatory, and antimicrobial drugs are administered by inhalation to millions of patients each day. Clinical trials of aerosolised measles vaccines have shown that mass immunization by the respiratory mucosal route in a developing country is feasible and can induce strong and long lasting immune responses (17-23). There is also valuable experience with aerosol vaccines given to over 4 million schoolchildren in Mexico, where public acceptance was high, costs were low, and fewer side effects were reported than with subcutaneous vaccination (24). A nasally administered influenza vaccine is licensed in North America (25-27) and others are in clinical trials (28-30). The principle of mucosal surface vaccination is well established, as shown by widely used and effective mucosal vaccines against polio, typhoid, cholera and rotavirus.

There are numerous perceived advantages of aerosol inhaled vaccination: it is safe, needle-free, well-tolerated, popular, less technically demanding, economically beneficial, and allows self-administration of vaccine or administration by less technically trained community health workers (24). Furthermore, use of non-injectable vaccines reduces the likelihood of unsafe disposal and reuse of syringes in immunisation programmes (31).

Another important potential advantage of mucosal immunisation is that aerosol vaccination may minimise or avoid induction of systemic anti-vector immunity and allow homologous boosting with the same viral vector; thus minimising cost of vaccine development (32-34).

The first clinical trial of a candidate TB vaccine being delivered by aerosol to humans was completed at Oxford in 2013. This Phase I trial (TB026) assessed the safety and feasibility of administering MVA85A by nebuliser direct into the airways (35). 12 volunteers received aerosolised MVA85A (10 received the low dose (1×10^7 pfu) and 2 received the higher dose that we would normally give intradermally (1×10^8 pfu)) and saline intradermal placebo, while 12 volunteers received MVA85A as intradermal injection with inhaled saline placebo. No vaccine related SAEs were reported and aerosol immunisation was well tolerated (35). Antigen-specific Th1 immunity was higher in the BAL fluid after aerosol administration than after intradermal administration. Antigen-specific Th1 immunity in the blood was comparable between routes of administration. Importantly, no reduction of systemic immunity was seen after aerosol administration. Interestingly no serum anti-MVA antibodies were detected after aerosol administration, in comparison with intradermal administration where anti-MVA antibodies were induced. A second Phase I safety trial assessing anti-vector immunity with nebulised MVA85A was completed in 2016 (TB035, Clinical Trials.gov reference number NCT01954563). An intermediate dose of 5×10^7 pfu was used in TB035. No vaccine related SAEs were reported (unpublished data). To our knowledge there have been no other clinical studies of viral vectored vaccines delivered by aerosol.

Aerosol delivery of a candidate TB vaccine, therefore, shows promise; however, before this potential route of vaccine administration can be evaluated in TB high burden countries, it is imperative that safety in subjects with latent *M.tb* infection be determined, because of the theoretical risk of immunopathology. This trial aims to assess safety and immunogenicity of aerosol MVA85A vaccination by repeating our previous phase I clinical trial of aerosol MVA85A vaccination in healthy adults (35) in UK adult human volunteers with latent *M.tb* infection. The essential safety and immunogenicity data that will be generated from this trial is a critical go/no-go criteria before this potentially promising route of vaccination can be evaluated in TB high burden countries, where an improved TB vaccine is most needed.

Description and pre-clinical experience of MVA and MVA85A

Modified vaccinia virus Ankara (MVA) is a highly attenuated strain of vaccinia virus which cannot replicate in human cells. It is known to be highly immunogenic and is therefore suitable for use as a viral vector in new vaccine development. It has an excellent safety record as it was administered intradermally to approximately 120,000 people during the smallpox eradication campaign (36-39), and

has since been used in numerous clinical trials of candidate vaccines against viral, mycobacterial and protozoal infections (40, 41). Meanwhile recombinant MVA vaccines administered by respiratory mucosal and gut mucosal routes have demonstrated protective efficacy and elicited strong immune responses in both rodents and non-human primates (42).

Antigen 85A is a highly conserved antigen expressed by *M.tb*, BCG, and all other mycobacterial species sequenced to date. It is a 32-kDa protein, and is an enzyme, mycolyl transferase, which is involved in cell wall biosynthesis (43). Antigen 85A is highly immunodominant in both animal and human studies (44-46), and protects against *M.tb* challenge in mice, guinea pigs and non-human primates (47, 48). The recombinant MVA85A vaccine incorporates the 1176 base pair gene for antigen 85A into the viral DNA allowing expression of this secreted antigen.

Experiments in mice, guinea pigs, cattle, and non-human primates have shown that a prime-boost schedule of vaccination with BCG followed by MVA85A, either intradermally, intramuscularly or mucosally, can improve protective efficacy against subsequent *M.tb* challenge, compared to BCG alone (11, 48-50). Animal toxicity studies using the intradermal route of administration revealed no differences from PBS-injected controls apart from irritation at the site of administration.

Clinical studies with MVA85A

Over 2500 volunteers have now received MVA85A, of which (in completed trials) 48 were by the intramuscular route, 12 by the aerosol route and the remainder intradermally. 22 phase I/II clinical studies of MVA85A have been completed, a further three are ongoing. These are summarised in table 2. We have conducted one previous trial evaluating MVA85A in adults with latent *M.tb* infection (TB007). Volunteers were vaccinated intradermally and the vaccine was safe and immunogenic, with no serious adverse events. In addition, several previous studies (TB010, TB011, TB019 and TB021) have all included volunteers with latent *M.tb* infection.

Protocol	Phase	Population	Treatment groups[a]	N[b]	Study Status
TB002	Phase I open label non-randomised	Healthy BCG-naïve adults, UK	5×10^7 pfu MVA85A (Days 0 & 21)	14	Completed
GM920	Phase I open label non-randomised	Healthy BCG-naïve or vaccinated adults, Gambia	5×10^7 pfu MVA85A (Days 0 & 21) BCG naïve 5×10^7 pfu MVA85A (Day 0) BCG-vaccinated	11 10	Completed
TB004	Phase I open label non-randomised	Healthy BCG-naïve adults, UK	1×10^6 pfu BCG prime (Day 0) 5×10^7 pfu MVA85A boost (after 1 month)	10	Completed
TB005	Phase I open label non-randomised	Healthy BCG-vaccinated adults, UK	5×10^7 pfu MVA85A (Day 0)	21	Completed
TB007	Phase I open label non-randomised	Healthy adults latently infected with *M.tb*, UK	5×10^7 pfu MVA85A (Day 0)	12	Completed
TB008	Phase I open label non-randomised	Healthy BCG-naïve or vaccinated adults & adolescents, South Africa	5×10^7 pfu MVA85A (Day 0) adults 5×10^7 pfu MVA85A (Day 0) adolescents	24 12	Completed
TB009	Phase I open label non-randomised	Healthy BCG-vaccinated adults, UK	1×10^7 pfu MVA85A (Day 0) 1×10^8 pfu MVA85A (Day 0)	12 12	Completed
TB010	Phase I open label non-randomised	HIV-positive adults, UK	5×10^7 pfu MVA85A (Day 0) 1×10^8 pfu MVA85A (Day 0)	10 10	Completed
TB011	Phase I open label non-randomised	Adults infected with *M.tb*, HIV, or both, South Africa	5×10^7 pfu MVA85A (Day 0) TB 5×10^7 pfu MVA85A (Day 0) HIV 5×10^7 pfu MVA85A (Day 0) TB & HIV 5×10^7 pfu MVA85A (Day 0) HIV on ART	12 12 12 12	Completed
TB012	Phase II open label non-randomised	Healthy BCG-vaccinated infants, South Africa	<u>Stage 1</u> EPI alone (Day 0) 2.5×10^7 pfu MVA85A with EPI (Day 0) 2.5×10^7 pfu MVA85A alone (Day 0)[c] EPI alone (Day 0) 5×10^7 pfu MVA85A with EPI (Day 0) 5×10^7 pfu MVA85A alone (Day 0)[c] <u>Stage 2</u> EPI alone 5×10^7 pfu MVA85A with EPI (Day 0) 5×10^7 pfu MVA85A alone (Day 0)[c]	 12 12 12 12 12 12 47 47[f] 48[f]	<u>Stage 1</u> Completed <u>Stage 2</u> Completed
TB014	Phase II open label non-randomised	Healthy BCG-vaccinated children & infants, South Africa	5×10^7 pfu MVA85A (Day 0) children 2.5×10^7 pfu MVA85A (Day 0) infants 5×10^7 pfu MVA85A (Day 0) infants 1×10^8 pfu MVA85A (Day 0) infants	24 36 36 36	Completed

			Prevenar (variable dose) (Day 0) infants	36	
TB017	Phase I open label non-randomised	Healthy BCG-vaccinated adults, UK	5×10^7 pfu FP85A (Day 0) 5×10^7 pfu MVA85A (Day 0) then 5×10^7 pfu FP85A (Day 28) 5×10^7 pfu FP85A (Day 0) then 5×10^7 pfu MVA85A (Day 28)	12 12 7	Completed[d]
TB018	Phase I open label non-randomised	Healthy BCG-vaccinated adults, UK	1×10^8 pfu MVA85A (Day 0) 1×10^8 pfu MVA85A (Day 0), 1g/kg deuterium-labelled glucose (Day 4) 1×10^8 pfu MVA85A (Day 0), 1g/kg deuterium-labelled glucose (Day 10)	4 4 4	Completed
TB019	Phase I open label non-randomised	HIV-infected adults, Senegal	1×10^8 pfu MVA85A (Day 0 & 168) 1×10^8 pfu MVA85A (Day 0 & 168) on ART	12 12	Completed
TB020	Phase II double blinded randomised	Healthy BCG-vaccinated HIV-negative infants, South Africa	1×10^8 pfu MVA85A (Day 0) Candin® (Day 0)	1399 1398[g]	Completed
TB021	Phase II double blinded randomised	Healthy HIV-infected adults, South Africa & Senegal	1×10^8 pfu MVA85A (Days 0, 168-252) Candin® (Days 0, 168-252)	324[i] 326[i]	Completed
TB022	Phase I open-label randomised	Healthy BCG-vaccinated adults, UK	1×10^8 pfu MVA85A (IM) (Day 0) 1×10^8 pfu MVA85A (ID) (Day 0)	12 12	Completed
TB023	Phase I open-label non-randomised	Healthy BCG-naïve or vaccinated adults, UK	$1x10^8$ pfu MVA85A (Day 0), BCG challenge (Day 28) BCG; 100µl ~ 2-8 $x10^5$ cfu	24 24	Completed
TB026	Phase I randomised blinded	Healthy BCG-vaccinated adults, UK	$1x10^8$ pfu MVA85A (aerosol inhaled), saline placebo (ID)[e] $1x10^7$ pfu MVA85A (aerosol inhaled), saline placebo (ID)[e] $1x10^7$ pfu MVA85A (ID), saline placebo (aerosol inhaled)[e]	2 10 12	Completed
TB028	Phase I randomised blinded	Health BCG-vaccinated adults, UK	$1x10^7$ pfu MVA85A-IMX313 (ID) (Day 0) $5x10^7$ pfu MVA85A-IMX313 (ID) (Day 0) $5x10^7$ pfu MVA85A (ID) (Day 0)	6 12 12	Completed
TB029	Phase II randomised controlled trial	Infants of HIV infected mothers, South Africa	$1x10^8$ pfu MVA85A or Candin® control at birth BCG: 0.05ml 1-4 $x\ 10^5$ cfu at 8 weeks of age (HIV uninfected)	124 124	Completed
TB032	Phase I open-label non-randomised	Healthy BCG-vaccinated adults, UK	1×10^{11} vp AERAS-402 (Day 0 & 28) followed by 1×10^8 pfu MVA85A (Day 119) 1×10^{11} vp AERAS-402 (Day 0) followed by 1×10^8 pfu MVA85A (Day 56) 1×10^{11} vp AERAS-402 (Day 0, 28 and 119)	12 16[h] 12	Completed
TB034	Phase I open-label randomised trial	Healthy BCG-vaccinated adults, UK	5×10^9 vp ChAdOx1 85A (Day 0) 2.5×10^{10} vp ChAdOx1 85A (Day 0)	6 12	Completed

			2.5×10^{10} vp ChAdOx1 85, (Day 0), 1×10^{8} pfu MVA85A (Day 56) 2.5×10^{10} vp ChAdOx1 85A (Day 0 and 28), 1×10^{8} pfu MVA85A (Day 119)	12 12	
TB035	Phase I open-label randomised trial	Healthy BCG-vaccinated adults, UK	5×10^{7} pfu MVA85A (aerosol inhaled); placebo (ID) (Day 0), 5×10^{7} pfu MVA85A (ID); placebo (aerosol inhaled) (Day 28)[e] 5×10^{7} pfu MVA85A (ID), placebo (aerosol inhaled) (Day 0), 5×10^{7} pfu MVA85A (aerosol inhaled), placebo (ID), (Day 28)[e] 5×10^{7} pfu MVA85A (ID), placebo (aerosol inhaled) (Day 0), 5×10^{7} pfu MVA85A (ID), placebo (aerosol inhaled) (Day 28)[e]	12 13[e*] 12	Completed
TB036	Phase II open label trial	Healthy BCG-vaccinated adolescents, Uganda	1×10^{8} pfu MVA85A (IM); D0 1×10^{8} pfu MVA85A (IM); D0	18 18	Completed

Table 2. Summary of clinical trials of MVA85A

a. Intradermal route of administration unless otherwise stated
b. Number of subjects is actual number enrolled for studies that have completed enrolment, and planned number of subjects for studies in which enrolment is ongoing or which are still blinded
c. EPI deferred for 1 week
d. Enrolment in TB017 was terminated early, due to issues with the supply of the FP85A vaccine
e. Subjects blinded as to whether they received MVA85A via the ID or aerosol inhaled route. Of 9 subjects who received aerosol boost vaccination 1 month after ID prime, 7 experienced transient mild to moderate respiratory AEs. All were self-limiting. This only occurred in subjects receiving an aerosol boost 1 month after an ID prime. The last 3 subjects in this group did not therefore receive an aerosol MVA85A boost.;.*1 subject withdrawn post first vaccination but prior to boost vaccination so was replaced.
f. 47 subjects were randomised to receive MVA85A with EPI and 48 were randomised to receive MVA85A alone. As a result of an error, 1 subject switched groups, resulting in 48 subjects receiving MVA85A with EIP and 47 receiving MVA85A alone
g. Two of the 1398 subjects randomised to the Candin® group were not vaccinated
h. Two of these 16 subjects were vaccinated with AERAS-402 but discontinued from the study before receiving MVA85A
i. One of the 324 subjects randomised to MVA85A/AERAS-485 was not vaccinated; one subject randomised to Candin® received MVA85A/AERAS-485
j. The subjects, the bronchoscopist and immunologists were blinded to treatment assignment; the investigator administering the vaccinations was not blinded

Safety profile

No signs of any Koch reaction in volunteers infected with *M.tb* have been seen with this or any other candidate vaccine to date. One possible vaccine related SAE – early unmasking of pre-existing tuberculous meningitis - occurred in a patient with HIV in South Africa.

The analysis of local and systemic reactions induced by the aerosol route that were reported in the completed Phase I safety trials are summarised in section 6.3 "Risks and Benefits".

Dosing

Dose studies of MVA85A have been performed in a step-wise fashion to minimise the risk of a Koch reaction and the incidence of adverse events. The main systemic dose-finding trial compared boosting doses of 1×10^7 pfu and 1×10^8 pfu to previous trials using boosting doses of 5×10^7 pfu. An intradermal dose of 1×10^8 pfu was found to be significantly more immunogenic than the lower doses without concomitant worsening of adverse event profile, and has subsequently been adopted as the standard dose in all subsequent trials (51, 52). The aerosol dose administered in trial TB026, a Phase I safety trial assessing the safety and feasibility of administering MVA85A by the aerosol route, was 1×10^7 pfu and the dose used in TB035 was 5×10^7pfu. In TB035, 2 patients with a significant history of atopy experienced transient systemic reactions following their 2nd vaccine dose. These included Grade 3 temperature, SOB, feverishness, fatigue, nausea and malaise, Grade 2 wheeze, cough and headache, and Grade 1 phlegm, myalgia, arthralgia and sore throat. These symptoms peaked at 24-48 hours and resolved fully by one week, excepting cough which resolved by day 14. There was a transient but significant reduction in exercise tolerance and PFTs; one volunteer had a 40% drop in FEV1 and FVC which normalized by 72 hours, the other a 15% drop (within normal parameters) which also resolved in this timeframe. As a result of this, volunteers with a significant history of atopy are now excluded from aerosol trials.

Immunogenicity

When evaluated by various assays of cellular immunology including interferon gamma (IFN-γ) ELISpot and intracellular cytokine staining assays, MVA85A has induced a strong and sustained cell-mediated immune response which is known to be important for protective immunity (53). As the phase IIb efficacy trial of MVA85A in BCG-vaccinated infants in South Africa did not show protection against TB disease or *M.tb* infection above that of BCG alone, work to identify correlates of protection from this trial's samples is now underway.

Aerosol delivery devices

Currently the World Health Organisation (WHO) is making a major investment in developing new aerosol devices for portable, low cost vaccine delivery (55). The WHO Measles Aerosol Project aims to develop and license respiratory delivery for measles vaccines currently given by injectable methods. The Edmonton-Zagreb strain of measles vaccine, a live virus vaccine, has been given by mesh nebuliser with excellent results. The most modern devices are small, lightweight, portable, and aerosolise through a mesh to provide small and consistent particle size which deliver vaccine to the distal airway mucosa more consistently and precisely than conventional jet mechanisms.

One such mesh nebuliser is the MicroAIR NE-U22 (Omron® Healthcare Limited, Japan) which uses ultrasound to push liquid through a fine metal mesh. This generates an aerosol mist with a particle diameter of about 4 µm. It is in current use with licensed drugs such as bronchodilating and antimicrobial agents and achieves good bioavailability. MVA particles are typically around 400 nm in size and can therefore be aerosolised with minimal damage.

We demonstrated adequate delivery of our vaccine, MVA85A, by this device in preliminary plaque assays. Using a nebuliser adapted for rodent administration, we performed GLP standard toxicology studies of aerosolised MVA85A (1×10^8 pfu) in BalbC mice, demonstrating satisfactory safety and systemic immunogenicity. We further administered aerosolised MVA85A (1×10^8 pfu) by the MicroAIR

NE-U22 mesh nebuliser to eight macaques, showing a good safety profile and satisfactory delivery by inhaled route (unpublished data). We have subsequently used this Omron device in TB026 and TB035, with a good safety profile.

Bronchoscopy and bronchoalveolar sampling

Protection from TB induced by intra-pulmonary vaccination correlates with cellular immune responses detected in the BAL samples of immunised mice (56). In macaques receiving aerosolised MVA85A we detected significant levels of antigen-specific cellular immune responses in BAL samples (57). In TB026 we detected significant levels of antigen-specific cellular immune responses in these BAL specimens, with higher frequencies in the aerosol group (35). Both CD4+ and CD8+ antigen specific T cells were detectable in the BAL. Clinical trials of intranasal vaccine candidates for other infectious diseases have analysed humoral immunity within nasal lavage, but a cellular immune response is essential for protective immunity against *M.tb* (58). Nasal mucosal samples are unlikely to provide adequate cellular samples as nasal lavage generally yields insufficient and variable numbers of cells (59). Furthermore as the vaccine is nebulised and inhaled orally we do not expect it to reach the nasal mucosa.

BAL samples are obtained by performing a bronchoscopy, a widely and safely used procedure. The short procedure involves the insertion of a narrow flexible fibre-optic tube into the airway under light intravenous sedation and topical local anaesthesia. Under direct vision saline is delivered to a section of lung mucosa and then recollected by suction. In clinical trial TB026 bronchoscopies were well tolerated by volunteers. Bronchoscopy is an essential component of this clinical trial because BAL samples are the ideal method to analyse the lung mucosal cellular immune responses which we intend to induce by vaccination (63).

6.2 Rationale

Hypothesis

We postulate that the aerosol inhaled route is practical and feasible and has an acceptable safety profile, in volunteers latently infected with *M.tb*. We hypothesise that the aerosol inhaled route of administration will induce greater mucosal immunity and comparable systemic immunity when compared to historical data of the IM (systemic) route of administration from previous studies in healthy volunteers.

Vaccine dosage

Trials of MVA85A to date have established 1×10^8 pfu as the optimal dose for intradermal injection in adults. Although the fractional delivered dose of aerosolised viral vectored vaccines to the mucosal surface may be considerably less than 25%, there is evidence that strong mucosal and systemic immune responses, comparable to the injectable route, are nevertheless achieved (16, 64).

As this trial will be the first time that aerosolised MVA85A is given to individuals with latent *M.tb* infection, we plan to begin with a low dose Starter Group, who will receive 1×10^7 pfu MVA85A by aerosol inhalation. This is the dose used in trial TB026 in healthy adults. The first volunteer in this group will be vaccinated ahead of other volunteers and no other volunteers will be vaccinated until at least 48 hours has elapsed. The CI will perform a review of the 48 hour safety data and decide if it is safe to vaccinate the remaining volunteers. There will be a second review of safety data after vaccination of all volunteers in the Starter Group.

Once the Starter Group is fully enrolled and the second safety review is completed, we will start enrolment into Group A (Standard Dose), with dose escalation to 5×10^7 pfu (the same dose used in TB035). For safety reasons, the first volunteer in this group will be vaccinated ahead of other volunteers and no other volunteers will be vaccinated until at least 48 hours has elapsed. If the CI

decides it is safe to proceed, the remaining volunteers will then be enrolled. The target enrolment for this group is 6 volunteers. However, given the often reduced pace of recruitment from the latently infected population, we will accept a minimum of 3 volunteers if the target has not been reached by October 2018.

6.3 Risks and Benefits

Potential risks

The potential risks to participants in this trial include risks associated with:

1. Venepuncture and intravenous cannulation

Localised bruising and discomfort can occur at the site of venepuncture. Infrequently fainting may occur. The total volume of blood drawn over a six month period will be 356mL, which should not compromise the health of these healthy individuals.

An intravenous cannula is routinely inserted into a peripheral (usually forearm) vein prior to bronchoscopy to administer intravenous sedation and removed once the procedure is completed. The risks of cannulation are identical to those associated with venepuncture. Additionally cannulation carries a small risk of soft tissue infection which will be minimised by an aseptic insertion technique and is easily recognisable and treatable. The short duration of cannulation (a few hours) further minimises this risk.

2. Vaccination

MVA85A has undergone thorough pre-clinical testing and has now been administered to over 2500 healthy human individuals with one possible SAR. The potential known adverse events associated with vaccination are:

Local reaction from aerosol inhaled vaccination

Expected reactions to inhaled vaccination include mild throat discomfort and coughing. Transient bronchospasm (causing wheeze) is possible. In the completed aerosolised trials (TB026 and TB035) there was no significant difference between respiratory adverse events or pulmonary function tests between the group receiving a priming immunisation with aerosol MVA85A and the group receiving a priming immunisation with ID MVA85A. In both groups AEs were infrequent and mild. In TB035, of the 9 subjects who received an aerosol boost vaccination 1 month after ID prime, 7 experienced transient mild to moderate respiratory AEs. All were self-limiting. This only occurred in subjects receiving an aerosol boost 1 month after an ID prime. The last 3 subjects in this group did not therefore receive an aerosol MVA85A boost.

Systemic reactions

Constitutional influenza-like symptoms such as fatigue, headache, malaise, feverishness, and muscle aches can occur with any vaccination and last for 2-3 days. In the case of MVA85A, fatigue and headache are most common, occurring in 50-90% of volunteers, and these are nearly always mild. Presyncopal and syncopal episodes may occur at the time of vaccination which rapidly resolve. As with any other vaccine, temporary ascending paralysis (the Guillain-Barré syndrome, GBS) or immune mediated reactions that can lead to organ damage may occur. For influenza vaccines an excess of approximately 1 GBS case per million persons immunised has been observed. No cases were observed in people under 45 years of age. However, this has never been seen with the MVA85A vaccine or vaccines containing any of its components.

TB specific risks

There is a very small risk of a Koch reaction, or immunopathology at the site of infection occurring when volunteers latently infected with *M.tb* are vaccinated with MVA85A. No such reactions were

seen in previous trial TB007, where MVA85A was administered systemically to volunteers with latent *M.tb* infection. All volunteers will be closely monitored and followed up both clinically and radiologically.

Allergic reactions

Allergic reactions from mild to severe may occur in response to any constituent of a vaccine preparation. Anaphylaxis is extremely rare but can occur in response to any vaccine.

3. Bronchoscopy

Bronchoscopy is a widely and safely used investigative procedure in clinical research studies involving both healthy volunteers and patients with respiratory conditions such as asthma and interstitial lung disease (60, 61). Clinical guidelines for performing investigative bronchoscopy in research studies are well established (62).

The bronchoscopies will be carried out in a dedicated NHS bronchoscopy suite with an excellent safety record by highly skilled and experienced consultant respiratory physicians. Intravenous sedation and topical local anaesthesia are administered prior to bronchoscopy to reduce discomfort, facilitate the procedure, and remove memory of the event (In 98% of cases, subjects have no memory of the procedure). Trained, experienced staff and facilities for resuscitation and drugs for reversal of sedation will be available. No transbronchial or endobronchial biopsies will be taken. To further minimise risk, volunteers will be excluded if they have an abnormal CT chest, a significant smoking history, a history of atopy, or any evidence of lung disease, including asthma (as defined by: a clinical diagnosis of asthma; prescription of asthma medication; airflow obstruction on spirometry; history of nocturnal or exercise-induced wheeze).

The risks of bronchoscopy are discussed at the time of consent and comprise: adverse reaction to sedation or local anaesthetic, sore throat, nose bleed (minimized as scope passed through mouth), laryngospasm, hypoxia, post-procedure flu-like symptoms (1-2 days) and risk of death (<1 in 100,000).

- Bronchoalveolar lavage is a routine procedure in investigative bronchoscopy and carries minimal bleeding risk. The risk of infection or febrile reactions will be minimised by full bronchoscope asepsis and superflush.
- Respiratory depression secondary to sedation is rare. No rescue medication for oversedation has been required for volunteers participating in trials TB026 and TB035 to date.
- Allergic reactions from mild to severe may occur in response to any constituent of the local anaesthetic or sedative agents. Anaphylaxis is extremely rare but can occur.

The Summary of Product Characteristics (SmPC) for the local anaesthetic and sedative agents contain full details of the indications and side effects of these licensed medications.

4. CT thorax

A computed tomography scan of the thorax is a painless radiological investigation which exposes volunteers to approximately 2 milliSieverts of radiation, equivalent to around 9 months of natural background radiation. The additional risk of cancer due to one CT thorax is small, approximately 1 in 9000. The total radiation exposure for this trial (two CT scans) will therefore be 4 milliSieverts, carrying an attributable cancer risk of 1 in 4500.

5. Pulmonary Function Tests

Vigorous respiratory manoeuvres such as forced expiration through the spirometer can occasionally lead to coughing or lightheadedness, but these symptoms are mild and rapidly self-limiting.

Potential benefits

Volunteers are not expected to benefit directly from participation in this trial. Volunteers will gain some information about their general health as a result of the screening history, examination, blood tests, urine tests, CT thorax and pulmonary function tests. They may also gain health information from the bronchoscopy. Volunteers found to have a previously undiagnosed condition thought to require further medical attention will be referred appropriately to their GP or an NHS specialist service for further investigation and treatment.

It is hoped that their contribution will enable the development of a safe and successful vaccine for TB and further our knowledge about mucosal vaccines.

7 OBJECTIVES AND OUTCOME MEASURES

Table 3. Objectives and Outcome Measures

Objectives	**Outcome Measures**	**Timepoint(s) of evaluation of this outcome measure**
Primary Objective To evaluate the safety of MVA85A vaccination by the aerosol inhaled route in healthy adult volunteers who are latently infected with *M.tb*.	Actively and passively collected data on adverse events	At each visit, via diary card for 14 days after vaccination and at the 6 month phone call
Secondary Objectives To evaluate the systemic and mucosal cellular and humoral immunogenicity induced by MVA85A vaccination by the aerosol inhaled route, in healthy volunteers who are latently infected with *M.tb.*	Laboratory markers of cell mediated and humoral immunity, including ex-vivo ELISpot in blood and intracellular cytokine staining in blood and BAL samples	At each visit
Tertiary Objectives To evaluate other exploratory markers of cell-mediated and humoral immunogenicity induced by aerosol MVA85A, including transcriptomic analysis	Laboratory markers of cell mediated and humoral immunity in blood and BAL samples	At each visit

8 PARTICIPANT IDENTIFICATION

8.1 Trial Participants

Healthy UK adults who are latently infected with *M.tb* and have no evidence of active disease.

8.2 Inclusion Criteria

Volunteers must meet all of the following criteria to enter the trial:

- Healthy adult aged 18-55 years
- Resident in or near Oxford, Birmingham or London for the duration of the trial period

- Screening IGRA positive
- Considered low risk of reactivation of their latent infection due to distant contact history
- Chest CT normal; or abnormal with features consistent with primary *M.tb* infection but no features suggestive of active disease
- No relevant findings in medical history or on physical examination
- Allow the Investigators to discuss the individual's medical history with their GP
- Use effective contraception (see below) for the duration of the trial period (females only)
- Refrain from blood donation during the trial.
- Give written informed consent
- Allow the Investigator to register volunteer details with a confidential database (The Over-volunteering Protection Service) to prevent concurrent entry into clinical trials
- Able and willing (in the Investigator's opinion) to comply with all the trial requirements

8.3 Exclusion Criteria

Volunteers must meet none of the following criteria to enter the trial:

- Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned use during the trial period[d]
- Prior vaccination with candidate vaccine MVA85A, candidate vaccine FP85A, any other recombinant MVA vaccine or any other candidate TB vaccine
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned trial vaccination date
- Clinically significant history of skin disorder, allergy, atopy, immunodeficiency (including HIV), cancer (except BCC or CIS), cardiovascular disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder, neurological illness, psychiatric disorder, drug or alcohol abuse
- Concurrent oral or systemic steroid medication or the concurrent use of other immunosuppressive agents
- History of anaphylaxis to vaccination or any allergy likely to be exacerbated by any component of the trial vaccine, sedative drugs, or any local or general anaesthetic agents
- Pregnancy, lactation or intention to become pregnant during trial period
- Any respiratory disease, including asthma
- Current smoker
- Clinically significant abnormality on screening CT thorax[a, b]
- Clinically significant abnormality of pulmonary function tests[b]
- Any nasal, pharyngeal, or laryngeal finding which precludes bronchoscopy
- Current use of any medication taken through the nasal or inhaled route including cocaine or other recreational drugs
- Clinical, radiological, or laboratory evidence of current active TB disease[c]
- Past treatment for TB disease
- Any clinically significant abnormality of screening blood or urine tests[b]

- Positive HBsAg, HCV or HIV antibodies[b]
- Any other significant disease, disorder, or finding, which, in the opinion of the Investigator, may either put the volunteer at risk, affect the volunteer's ability to participate in the trial or impair interpretation of the trial data

[a] Excepting findings felt to be consistent with latent TB infection only.

[b] Volunteers who are excluded from the trial because they have been discovered during screening procedures to be suffering from a previously undiagnosed condition thought to require further medical attention will be referred appropriately to their GP or an NHS specialist service for further investigation and treatment.

[c] If there is any evidence of active TB disease either on clinical or radiological grounds, further investigation and treatment will be offered under the supervision of a consultant physician in respiratory or infectious diseases.

[d] Volunteers will be excluded from the trial if they are concurrently involved in another study or trial that involves regular blood tests or an investigational medicinal product. In order to check this, volunteers will be asked to provide their National Insurance or Passport number (if they are not entitled to a NI number) and will be registered on a national database of participants in clinical trials (www.tops.org.uk).

Effective contraception for female volunteers

Female volunteers are required to use an effective form of contraception during the course of the trial. There is no information about the effect of these vaccines on a fetus. Previous investigations looking at excretion of viral vectors after vaccination in urine for males, demonstrated no detectable virus; therefore males are not required to use barrier contraception whilst taking part in this study as the risk of excretion of the virus is very low.

Acceptable forms of contraception for female volunteers include:

- Established use of oral, injected on implanted hormonal methods of contraception
- Placement of an intrauterine device (IUD) or intrauterine system (IUS)
- Permanent sterilisation or bilateral tubal occlusion
- Barrier methods of contraception (condom; or occlusive cap with spermicide)
- Male sterilisation, if the vasectomised partner is the sole partner for the subject
- True abstinence, when this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence and withdrawal are not acceptable methods of contraception)

9 TRIAL DESIGN AND PROCEDURES

This is a phase I trial to evaluate the safety and immunogenicity of candidate TB vaccine MVA85A administered by the aerosol inhaled route in healthy volunteers who are latently infected with *M.tb*.

9.1 Trial Numbers and Groups

9 volunteers will be enrolled; the first 3 to the starter group, , then 3-6 volunteers into Group A.

Table 4. Trial groups

Arm of trial	Sample size	Intervention

Starter group	3	Aerosol inhaled MVA85A 1 x 10^7 pfu
Group A (Standard Dose)	3-6	Aerosol inhaled MVA85A 5 x 10^7 pfu

9.2 Recruitment

Volunteers considered to be at low risk of reactivation of their latent infection due to distant contact history, will be recruited from TB contact clinics at Oxford University Hospitals, Birmingham Chest Clinic Heart of England, Royal Free Hospital, King's College Hospital, Guy's and St Thomas' Hospital and St George's University Hospital. Patients who fulfil the inclusion criteria will be given a Volunteer Information Sheet Summary at their clinic attendance and asked if they would be interested in finding out more about the trial. If so, they will be given the full Volunteer Information Sheet to take with them to read in their own time. If still interested, and with their permission, the trial team will contact the patient to arrange a screening appointment. Ideally, the volunteer will delay their prospective anti-tuberculous chemoprophylaxis, provided through the usual NHS routes and TB/chest clinics, until after the trial D28 follow-up visit and CT, decision not to enrol, or early withdrawal; whichever occurs first. The study team will keep each volunteer's NHS TB/chest clinic team aware of when these timelines are reached. However, considering the challenges inherent in recruiting latently infected volunteers, if the volunteer does not want their prospective anti-TB chemoprophylaxis delayed they will still be eligible to be enrolled onto the study.

Additionally, we will send a letter to patients seen at the clinic over the previous year with a diagnosis of latent *M.tb* infection who have not been given anti-tuberculous therapy, inviting them to participate in this trial. We will also display posters in GP practices in the hope of attracting interest from latently infected individuals in the community who are no longer attending TB contact clinics.

9.3 Informed Consent

Written informed consent will be obtained at screening by a GCP trained Investigator.

The volunteer must sign and date the latest approved version of the consent form before any trial specific procedures are performed.

The Volunteer Information Sheet will be made available to the volunteer no less than 24 hours prior to attending for screening. The details enclosed will be discussed with the volunteer, including:

- the aims and nature of the trial
- the vaccines used
- the schedule of visits and tests to be carried out
- the implications and constraints of the protocol
- compensation for the volunteer
- known side effects and risks of taking part in the trial

The following general principles will be emphasised:

- Participation is entirely voluntary
- The volunteer may withdraw from the trial at any time for any reason
- Withdrawal or refusal to participate involves no penalty or loss of medical benefits
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the trial and the procedures involved
- There is no direct benefit from participating
- The volunteer's GP will be contacted to corroborate their medical history and confirm that the volunteer does not meet any of the exclusion criteria

- The volunteer will be registered on the TOPS database (The Over-Volunteering Protection System)
- The volunteer's blood samples taken as part of the trial will be stored indefinitely for further research. The volunteer may elect to have their samples destroyed after the period required to meet Good Clinical Practice and regulatory requirements.

The volunteer will then have time to consider whether or not to participate. If the volunteer decides to participate, they will sign and date two copies of the consent form, one for them to take away and keep, and one for the Investigator which will be retained at the trial site. These forms will also be signed and dated by the Investigator or Clinical Research Nurse. This will occur before any trial specific procedures are performed.

9.4 Screening and Eligibility Assessment

Once the volunteer has given their consent to undergo screening, a baseline medical history (including concomitant medication) and physical examination will be performed by a GCP trained doctor. Inclusion and exclusion criteria will be checked using a tabulated format. Demographic and occupational data relating to risk of prior *M.tb* exposure will be collected. Vital signs will be checked and bloods taken including FBC, clotting profile, U&Es, CRP, LFTs, HIV antibodies, HBsAg, HCV antibodies and IGRA. Volunteers will be counselled by one of the Investigators for HIV, Hepatitis B and Hepatitis C testing. Urine will be tested for the presence of clinically significant proteinuria, glycosuria or haematuria. A pregnancy test will be performed for female volunteers. Volunteers will then be scheduled to re-attend for a CT thorax provided no exclusion criteria have been met.

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with Investigator discretion for interpretation of results and the need for repeated tests. In general, volunteers will be excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory AE, according to the site-specific laboratory AE tables, filed in the TMF (Oxford), or Site Master File (Birmingham and London).

The total duration of the screening visit will be around 2 hours.

9.5 Follow up visits

D0 - Vaccination

Any new medical issues or symptoms that have arisen will be assessed. Venepuncture and other samples will be taken according to the Schedule of Trial Procedures in section 4.2. The inclusion and exclusion criteria for the trial will be reviewed; provided that the volunteer still satisfies all inclusion criteria (and no exclusion criteria) and their consent remains valid, the volunteer will be allocated to a group. MVA85A vaccination will be performed by the aerosol route according to the trial SOP. Volunteers will be kept under observation for around 60 minutes after vaccination. The vaccination visit will last approximately 2 hours. Volunteers will also have blood taken for HLA typing.

D7 – Bronchoscopy

The bronchoscopy visit lasts several hours and takes place at the bronchoscopy suite in the OUH NHS Foundation Trust Hospitals by the specialist team there. Details of the procedure are outlined in section 6.1. In exceptional circumstances where volunteers are absolutely unable to travel to Oxford, the bronchoscopy may be performed at the other NHS trust study sites (King's College Hospital NHS Trust, University of Birmingham NHS Trust, Royal Free Hospital NHS Trust). The bronchoscopy will always be performed by a respiratory consultant trained in the procedure and following the trial specific SOP.

D28 – CT thorax

The volunteer will usually have a clinic assessment on the same day, prior to the scan. Any abnormality reported on the CT will be appropriately investigated and followed up.

Other follow-up visits

These will be performed as specified in the Schedule of Trial Procedures (section 4.2). Any new medical issues or symptoms that have arisen will be assessed. Additional procedures or laboratory tests may be carried out at the discretion of the Investigators if deemed clinically necessary. Follow-up visits will last approximately 15-30 minutes. The maximum trial duration will be around 24 weeks after the date of enrolment.

9.6 Timepoints

Screening – trial enrolment time window

Enrolment should take place no longer than 120 days following the date of screening appointment. If more than 120 days elapse, the screening visit should be repeated in full prior to enrolment in order to minimise the risk to participants of any new unidentified health problems having arisen during that period.

Vaccination of first volunteer – subsequent vaccination time window

The first volunteer, who will be allocated to the Starter Group, will be vaccinated with MVA85A by the aerosol inhaled route at least 48 hours before any subsequent volunteers in this group. After safety review, the first volunteer in Group A will be vaccinated at least 48 hours prior to further enrolment.

Vaccination – bronchoscopy time window

According to our clinical research (and in line with pre-clinical findings), the peak effector T cell response detected from PBMC in venous blood samples is around seven days after vaccination with MVA85A. In our previous trial of aerosolised MVA85A, high frequencies of Ag85A-specific cytokines were detected in BAL samples at one week post-vaccination, therefore bronchoscopy will be performed at seven days to match that trial.

Follow up period

The follow up period will be six months in accordance with findings from previous trials in which adequate safety data and reliable markers of immunogenicity have been obtained in this time interval.

9.7 Sample handling

Details regarding samples, volume and frequency of sampling are listed in the Schedule of Trial Procedures (section 4.2). Blood samples will be processed according to local laboratory standard operating procedures. All samples will be in anonymised form at the CCVTM; samples at Birmingham and London sites may contain patient numbers and details as per NHS procedures.

Volunteers will be informed that there may be leftover samples of their blood. With the volunteers' informed consent, any leftover cells and serum, plasma or BAL samples will be frozen for future analysis of *M.tb* and/or vaccine-related responses. This may include human DNA and RNA analysis to search for correlates of vaccine immunogenicity and efficacy. Samples may be shipped to other parties involved in our research in anonymised form for immunological analysis. Volunteers will be able to decide if they will permit such future use of any leftover samples. If they elect not to permit this, all of those leftover samples will be discarded after the required period of storage to meet GCP and regulatory requirements.

9.8 Vaccination Postponement Criteria

Vaccination will not proceed on the scheduled day in any of the following situations:

- The volunteer has a current or recent upper respiratory tract infection, unless they have been symptom-free for at least one week
- The volunteer has a positive pregnancy test
- The volunteer has a temperature > 37.5^{o}C
- The Investigator judges the volunteer to have an acute moderate or severe illness (whether febrile or not)
- The volunteer has received a live non-trial vaccine within the preceding 28 days
- The Investigator has any other concern that vaccination may not be in the volunteer's best interests

In this case the volunteer may be vaccinated at a later date or withdrawn from the trial at the discretion of the Investigator.

9.9 Bronchoscopy Postponement criteria

Bronchoscopy will not proceed on the scheduled day in any of the following situations:

- The volunteer has a temperature > 37.5^{o}C
- The volunteer has a positive pregnancy test
- The Investigator judges the volunteer to have an acute moderate or severe illness (whether febrile or not)
- The Investigator has any other concern that bronchoscopy may not be in the volunteer's best interests

In the event of postponement the bronchoscopy will be rescheduled when possible up to a 3 week window.

9.10 Discontinuation / Withdrawal Criteria

Every reasonable effort will be made to maintain protocol compliance and participation in the trial.

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being (including on the advice of the LSC). In addition the volunteer may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator
- Ineligibility (either arising during the trial or retrospectively, having been overlooked at screening)
- Significant protocol deviation
- Volunteer non-compliance with trial requirements (failure to attend two or more follow-up visits)
- Any AE which requires discontinuation of trial involvement or results in inability to comply with trial procedures
- Confirmed pregnancy during the trial

Any volunteer who becomes pregnant during the trial will be followed up as per the protocol and until the end of the pregnancy. We will not routinely perform venepuncture in a pregnant volunteer.

The reason for withdrawal will be recorded in the CRF. Volunteers withdrawn from the trial may be replaced on the decision of the Investigator. If the volunteer is withdrawn due to an AE, the Investigator will arrange for appropriate specialist management or follow up visits or telephone calls until the AE has resolved or stabilised. The regulatory authorities will be informed in a timely manner. The extent of follow up after premature discontinuation will be determined by the Investigator but will be at least for the whole trial period, and if pregnant, until pregnancy outcome.

If a volunteer withdraws from the trial, samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Long term safety data collection will continue as appropriate if a volunteer has received one or more vaccine doses.

9.11 Safety

Discontinuation of the trial

The trial will be discontinued in the event of any of the following:

- New scientific information is published to indicate that volunteers in the trial are being exposed to undue risks as a result of administration of the IMP, or as a result of the trial procedures or follow-up schedule.
- Serious concerns about the safety of the IMP arise as a result of one or more vaccine related SAE(s) occurring in the volunteers enrolled in this or any other ongoing trial of the MVA85A vaccine.
- For any other reason at the discretion of the Investigator.

9.12 End of Trial Definition

The trial will be completed when the last volunteer enrolled into the trial has completed the final follow up telephone call. An end of trial letter will be sent to each volunteer's GP and their TB clinician.

10 INVESTIGATIONAL MEDICINAL PRODUCT AND DEVICES

10.1 IMP Description

The MVA85A vaccine consists of the attenuated vaccinia virus MVA vector with a 1176 base-pair insert, which is almost the complete *M.tb* gene for Ag85A, with the tissue plasminogen activator (TPA) signal sequence preceding the N terminus and a monoclonal antibody tag (pk) at the C terminus. Expression of the antigen 85A DNA sequence is regulated by the vaccinia P7.5 early/late promoter. MVA85A is manufactured under Good Manufacturing Practice conditions by IDT Biologika GmbH (IDT), Germany.

MVA85A is supplied as a sterile liquid in glass vials. Each vial of lot 0050811 contains 400 µL of vaccine at a concentration of 8.4×10^8 pfu/mL in 10mM Tris buffer 140 mM NaCl; pH 7.7. The dose of MVA85A to be used in the starter group will be 1×10^7 pfu (120 µL of a 1:10 dilution, as per Vaccine Dilution SOP) diluted in saline and administered by aerosol inhalation. The dose of MVA85A to be used in Group A will be 5×10^7pfu (60µl) diluted in saline for aerosol inhalation.

10.2 Storage of IMP

MVA85A will be shipped from IDT directly to the Clinical Biomanufacturing Facility (CBF) on dry ice and in the presence of a temperature logger. The vaccine will be certified and labelled for use in trial TB040 by a qualified person (QP) at the University of Oxford.

The vaccine will be stored at -80°C (nominal temperature) in a secure, temperature-monitored freezer at the Centre for Vaccinology and Tropical Medicine, University of Oxford, Churchill Hospital.

10.3 Dispensing and administration

All movements of vials of the trial vaccine between IDT and the University of Oxford and in or out of the locked freezer will be documented. Vaccine accountability, storage, shipment and handling will be in accordance with local SOPs and other relevant local forms.

On vaccination day, vaccines will be allowed to thaw to room temperature and will be administered within 1 hour. The vaccine will be administered by aerosol inhalation, according to the trial-specific SOP. Volunteers will stay in the unit for 60 minutes (±10 minutes) after vaccination. During the administration of the vaccine, monitoring equipment, oxygen, medicines including bronchodilators and resuscitation equipment will be immediately available for the management of anaphylaxis and bronchospasm according to the site-specific SOP.

In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, a number of measures will be instituted during and following vaccination:

- The dilution process will be carried out in a sealed vial in accordance with the vaccine dilution SOP.
- The aerosol inhaled vaccination will take place in a closed clinic room and not opened until 10 minutes after the end of nebulisation.
- During aerosol inhalation clinical staff will wear appropriate protective clothing as agreed with the GMSC.
- All disposable items including nebuliser parts, needles, vials, dressings, and protective clothing will be disposed of as GMO waste by autoclaving, in accordance with the current approved SOP and the contained use GMO regulations 2014.
- All hard surfaces within the clinic room where vaccination took place will be cleaned and disinfected in accordance with GMSC recommendations.
- Non-disposable parts of the MicroAIR NE-U22 nebuliser will be cleaned and disinfected in accordance with the device documentation and GMSC recommendations.

10.4 Saline

Sterile saline will be used for dilution of MVA85A (as needed, to achieve the correct dose). Once prepared, the vaccine will then be made up to 1ml with saline for administration via the MicroAIR NE-U22 nebuliser.

10.5 MicroAIR NE-U22

This is an approved electromedical device, CE0197, EAN code 40 15672 10142 1. Information about the specifications, usage, and maintenance of the nebuliser device can be found in the device documentation (65). This is the same device used in studies TB026 and TB035.

10.6 Sedative & anaesthetic agents for bronchoscopy

Fentanyl is a licensed opioid used routinely to provide analgesia and sedation during medical procedures. It will be stored, dispensed and administered in accordance with standard NHS procedures and the Summary of Product Characteristics (SmPC).

Midazolam is a licensed benzodiazepine used routinely to provide sedation and amnesia during medical procedures. It will be stored, dispensed and administered in accordance with standard NHS procedures and the SmPC.

Lignocaine is a licensed local anaesthetic used routinely during medical procedures. It will be stored, dispensed and administered in accordance with standard NHS procedures and the SmPC.

Other licensed drugs may be used during bronchoscopy at the discretion of the Investigators.

11 ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

11.1 Interim Safety Review

Prior to dose escalation of the vaccine, the clinical team and Chief Investigator will review the safety data and adverse events in volunteers before proceeding to the next vaccine dose. Interim safety data may also be made available to manufacturers (in coded format) as specified in the contract with the manufacturer(s).

11.2 Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a volunteer, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Each adverse event will be graded by the participant according to the table for grading severity of adverse events (see Section 11.7). Severity gradings may be reviewed and discussed with the participants at the clinic visits.

Adverse Reaction (AR)

An AR is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the reporting medical Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., IB for an unapproved IMP).

Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death.
- Life-threatening event (i.e., the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death.

- Persistent or significant disability or incapacity (i.e., substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation or prolongation of existing hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.
- Congenital anomaly or birth defect.

Serious Adverse Reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting Investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the IB or SmPC.

NB: To avoid confusion or misunderstanding the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

11.3 Foreseeable Adverse Reactions

Foreseeable adverse reactions are listed in section 6.3.

11.4 Expected Serious Adverse Events

No serious adverse events are expected in this study.

11.5 Causality Assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event, the relationship of the event to the time of vaccine administration and the known biology of the vaccine therapy (Table 5). Causality assessment will take place during interim analyses and at the final safety analysis.

Table 5. Guidelines for assessing the relationship of vaccine administration to an AE

0	**No Relationship**	No temporal relationship to trial product; ***and*** Alternate aetiology (clinical state, environment or other interventions); ***and*** Does not follow known pattern of response to vaccine
1	**Unlikely**	Unlikely temporal relationship to trial product; ***and*** Alternate aetiology likely (clinical state, environment or other interventions); ***and*** Does not follow known typical or plausible pattern of response to vaccine
2	**Possible**	Reasonable temporal relationship to trial product; ***or*** Event not readily produced by clinical state, environment or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
3	**Probable**	Reasonable temporal relationship to trial product; ***and*** Event not readily produced by clinical state, environment or other interventions; ***and*** Known pattern of response seen with other vaccines
4	**Definite**	Reasonable temporal relationship to vaccine; ***and*** Event not readily produced by clinical state, environment or other interventions; ***and*** Known pattern of response seen with the vaccine

11.6 Reporting Procedures for All Adverse Events

All AEs occurring in the 14 days following each vaccination observed by the Investigator or reported by the volunteer, whether or not attributed to study medication, will be recorded on electronic diary cards. Data from the diary cards will be extracted at each safety review and following the last volunteer last visit (LVLV). Outside the diary card periods, expected local and systemic AEs (listed in table 7 below) will be specifically solicited at each visit, and graded by severity and causality (as detailed in sections 11.7 and 11.5). All AEs starting after the diary card period, or persisting after this period, will be recorded in the AE line listing of the CRF.

The overall appearance of the lung mucosa will be assessed at the day 7 bronchoscopy.

Any radiological changes to the lungs will be assessed at the day 28 CT thorax.

All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). Serious adverse events (SAEs) will be collected throughout the entire trial period.

Reporting Procedures for Serious AEs (see Safety Reporting SOP)

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported on the SAE forms to members of the study team immediately the Investigators become aware of their occurrence, as described in the site-specific SOP. Copies of all reports will be forwarded for review to the Chief Investigator and Principal Investigators (as the Sponsor's representatives) within 24 hours of the Investigator being aware of the suspected SAE. The local safety committee (LSC) will be notified of SAEs which are deemed possibly, probably or definitely related to study interventions; the LSC will be notified immediately (within 24 hours) of the Investigators' being aware of their occurrence. SAEs will not normally be reported immediately to the ethical committee(s) unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event

that is likely to affect safety of trial volunteers, at the discretion of the Chief Investigator and/or LSC. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

Reporting Procedures for SUSARS
The Chief Investigator will report all SUSARs to the MHRA and ethical committee(s) within required timelines (15 days for all SUSARs, unless life threatening in which case 7 days, with a final report within a further 8 days (total 15)). The Chief Investigator will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

All SUSARs and deaths occurring during the study will be reported to the Sponsor. For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

Development Safety Update Report
A Development Safety Update Report (DSUR) will be submitted by the Sponsor to the competent authority and ethical committee on the anniversary of the first approval date from the regulatory authority for each IMP.

11.7 Assessment of Severity

The severity of clinical AEs will be assessed according to the scale in Tables 6-7:

Table 6. Severity grading criteria for physical observations.

	Grade 1 (mild)	**Grade 2 (moderate)**	**Grade 3 (severe)**
Fever (oral)	37.6°C - 38.0°C	38.1°C – 39.0°C	>39.0°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130
Bradycardia (bpm)**	50 – 54	40 – 49	<40
Systolic hypertension (mmHg)	141 - 159	160 – 179	≥180
Diastolic hypertension (mmHg)	91 - 99	100 – 109	≥110
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80

*Taken after ≥10 minutes at rest; **When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy volunteer populations, for example, conditioned athletes; ***Only if symptomatic (e.g. dizzy/ light-headed)

Table 7. Severity grading criteria for local and systemic AEs399

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible

11.8 Procedures to be followed in the event of abnormal findings

Eligibility for enrolment in the trial in terms of laboratory findings will be assessed as detailed in Section 9.4. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. Laboratory adverse events will be assessed using the site-specific tables in the Site Master File/TMF. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate and with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

11.9 Local Safety Committee

A Local Safety Committee (LSC) will be appointed to provide real-time safety oversight. The LSC will be notified within 24 hours of the Investigators' being aware of the occurrence of SAEs. The LSC has the power to place the study on hold if deemed necessary following a study intervention-related SAE. At the time of writing the LSC will be chaired by Professor Brian Angus, Clinical Tutor in Medicine, Honorary Consultant Physician and Director of the Oxford Centre for Clinical Tropical Medicine at Oxford University. There will be a minimum of two other appropriately qualified committee members. All correspondence between Investigator and LSC will be conveyed by the Investigator to the trial Sponsor. The chair of the LSC will be contacted for advice and independent review by the Investigator or trial Sponsor in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to the trial vaccine.
- Any other situation where the Investigator or Trial Sponsor feels independent advice or review is important.

Safety Profile Review

The safety profile will be assessed on an on-going basis by the Investigators. The Chief Investigator, Principal Investigator, and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

12 STATISTICS

This is primarily a safety trial with descriptive endpoints. Following the first three volunteers enrolled into the starter group, 3-6 volunteers will be recruited into Group A, to receive the target dose. Due to existing difficulties recruiting to this clinical trial we have decided to take out Group B (the comparator arm) to ensure that the numbers recruited will still be sufficient to allow for the primary objective, an assessment of safety. A twofold increase in the magnitude of the cellular immune response (as measured by ELISpot response to 85A peptide stimulation) is considered immunologically

meaningful. This sample size is appropriate for a proof-of-concept Phase I safety study and takes into account the low number of potentially eligible healthy adults with latent *M.tb* infection in the UK and the impact of this on recruitment. The sample size has not been determined with the aim of achieving statistical significance.

It is anticipated that the immunological endpoints will not follow a normal distribution. The one-week and three-month responses will be compared. Medians and interquartile ranges will be used to summarise the data and the Mann Whitney U test will be used for statistical comparisons. An area-under-curve analysis will be used to compare the overall responses over time comparing these aerosol data with historical data from previous trials. This analysis will make use of data collected at all time points.

13 DATA MANAGEMENT

13.1 Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include the CRF itself (history and examination), the volunteer consent form, blood and microbiology results, CT report, GP response letter, copy of bronchoscopy report and any further correspondence relating to the volunteer regarding medical/clinical issues. The CRF will be paper or electronic and paper together with electronic diary cards.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

13.2 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections. All information relating to the trial and its volunteers will be held in strict confidence, and in accordance with ICH E6 GCP and institutional requirements.

13.3 Data Recording and Record Keeping

The Chief Investigator will be responsible for collecting, recording, analysing, and storing all the data accruing from the trial. These tasks may be delegated to other Investigators. Paper CRFs will be stored in a key-locked cabinet at each site, and electronic CRFs on the OpenClinica™ database, which is stored electronically on secure servers that are outsourced by OpenClinica™. Data from paper CRFs will be transcribed onto the OpenClinica™ database. Some data may be duplicated anonymously into an electronic Microsoft Excel™ file on the CCVTM secure server for clinical monitoring through the trial.

Trial records will be held by the Investigator for as long as required by legislation as a minimum (currently until at least 2 years after the last marketing authorisation for the product or 2 years after discontinuation of clinical development of an investigational product) and in order to enable dissemination of study results after publication, and to enable decoding and destruction of anonymized samples if subsequently requested by a volunteer. Data will subsequently be transferred to a secure archive in accordance with the Data Protection Act. Volunteers will be assigned individual unique trial numbers for identification on all trial records, except where the use of identifiable information is unavoidable (including on GP correspondence, registration documents, and consent forms).

14 QUALITY ASSURANCE PROCEDURES

14.1 Quality Assurance

Investigator procedures

Approved site-specific SOPs will be used at all clinical and laboratory sites.

Modification to protocol

No substantial amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any substantial amendments to the trial that appear necessary during the course of the trial must be discussed by the Investigator and Sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the CI and will be made a formal part of the protocol following ethical and regulatory approval.

An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the volunteers' safety, the scientific value of the trial, the conduct of the study or safety of the IMP. An administrative change is a non-substantial amendment and does not require REC approval. However, the REC will be notified whenever an administrative change is made.

The Chief Investigator is responsible for ensuring that changes to an approved trial, during the period for which REC approval has already been given, are not initiated without REC review and approval except to eliminate apparent immediate hazards to the volunteer.

Protocol deviation

All deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

14.2 Monitoring

Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The trial will be conducted in accordance with procedures identified in the protocol. Regular monitoring will be performed according to ICH GCP. According to applicable SOPs, the Monitors will verify that the clinical trial is initiated, conducted and completed, and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

15 SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach. A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree:

- the safety or physical or mental integrity of the volunteers of the trial
- the scientific value of the trial"

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory Authority and the NHS host organisation within 7 calendar days.

16 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki as agreed by the World Medical Association General Assembly (Washington 2002).

16.2 Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with ICH Good Clinical Practice (GCP), the requirements of the Medicines for Human Use (Clinical Trial) Regulations 2004, and local regulatory requirements.

16.3 Approvals

A copy of the protocol, proposed informed consent form, other written volunteer information and the proposed advertising material will be submitted to an independent REC for written approval. The Investigators will submit and, where necessary, obtain approval from the REC for all subsequent substantial amendments to the protocol and informed consent document. The Investigators will notify deviations from the protocol or SAEs occurring at the site to the Sponsor and will notify the REC of these in accordance with local procedures.

16.4 Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

16.5 Volunteer Confidentiality

The trial staff will ensure the volunteers' anonymity is maintained. All documents will be stored securely and only accessible by trial staff and authorised personnel. No information concerning the trial or the data will be released to any unauthorised third party, without prior written approval of the sponsor. The trial will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

16.6 Expenses and Benefits

Volunteers will be compensated *pro rata* for their time, travel and for trial procedures while participating in the trial, amounting to a total of approximately £505-£625, depending on the exact number of visits, site of recruitment and whether any repeat or additional visits are necessary.

17 FINANCE AND INSURANCE

17.1 Funding

This trial will be financed by research grants from TBVI and The Wellcome Trust, held by Professor Helen McShane.

17.2 Indemnity

If any volunteer is harmed as a result of this trial, medical care will be provided under the NHS.

Negligent Harm

Indemnity and/or compensation for negligent harm arising specifically from an accidental injury for which the University is legally liable as the Research Sponsor will be covered by the University of Oxford.

Non-Negligent Harm

Indemnity and/or compensation for harm arising specifically from an accidental injury, and occurring as a consequence of the Research volunteers' participation in the trial for which the University is the Research Sponsor will be covered by the University of Oxford.

17.3 Insurance

The University has a specialist insurance policy in place - Newline Underwriting Management Ltd, at Lloyd's of London - which would operate in the event of any volunteer suffering harm as a result of their involvement in the research.

18 PUBLICATION POLICY

When the trial is complete, a manuscript describing the primary trial results will be written and published in a peer-reviewed, open access journal. International guidelines will be followed regarding authorship. There may also be secondary publications on more exploratory results.

19 REFERENCES

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA : the journal of the American Medical Association. 1999 Aug 18;282(7):677-86. PubMed PMID: 10517722. Epub 1999/10/12. eng.

2. WHO. Global tuberculosis report 2014. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf?ua=1 (accessed 03 March 2015). 2014 Contract No.: WHO/HTM/TB/2014.08

3. Corbett EL, Marston B, Churchyard GJ, De Cock KM. Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. Lancet. 2006 Mar 18;367(9514):926-37. PubMed PMID: 16546541. Epub 2006/03/21. eng.

4. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA : the journal of the American Medical Association. 1994 Mar 2;271(9):698-702. PubMed PMID: 8309034. Epub 1994/03/02. eng.

5. Fine PE. The BCG story: lessons from the past and implications for the future. Rev Infect Dis. 1989 Mar-Apr;11 Suppl 2:S353-9. PubMed PMID: 2652252. Epub 1989/03/01. eng.

6. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. Lancet. 2006 Apr 8;367(9517):1173-80. PubMed PMID: 16616560. Epub 2006/04/18. eng.

7. Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. International journal of epidemiology. 1993 Dec;22(6):1154-8. PubMed PMID: 8144299. Epub 1993/12/01. eng.

8. McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat Med. 2004 Nov;10(11):1240-4. PubMed PMID: 15502839. Epub 2004/10/27. eng.

9. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet. 2013 Mar 23;381(9871):1021-8. PubMed PMID: 23391465.

10. Ndiaye BP, Thienemann F, Ota M, Landry BS, Camara M, Dieye S, et al. Safety, immunogenicity, and efficacy of the candidate tuberculosis vaccine MVA85A in healthy adults infected with HIV-1: a randomised, placebo-controlled, phase 2 trial. The Lancet Respiratory medicine. 2015 Mar;3(3):190-200. PubMed PMID: 25726088.

11. Goonetilleke NP, McShane H, Hannan CM, Anderson RJ, Brookes RH, Hill AV. Enhanced immunogenicity and protective efficacy against Mycobacterium tuberculosis of bacille Calmette-Guerin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. J Immunol. 2003 Aug 1;171(3):1602-9. PubMed PMID: 12874255. Epub 2003/07/23. eng.

12. Santosuosso M, McCormick S, Zhang X, Zganiacz A, Xing Z. Intranasal boosting with an adenovirus-vectored vaccine markedly enhances protection by parenteral Mycobacterium bovis BCG immunization against pulmonary tuberculosis. Infect Immun. 2006 Aug;74(8):4634-43. PubMed PMID: 16861651. Pubmed Central PMCID: 1539608.

13. Xing Z, McFarland CT, Sallenave JM, Izzo A, Wang J, McMurray DN. Intranasal mucosal boosting with an adenovirus-vectored vaccine markedly enhances the protection of BCG-primed

guinea pigs against pulmonary tuberculosis. PLoS One. 2009;4(6):e5856. PubMed PMID: 19516906. Pubmed Central PMCID: 2689939.

14. Xing Z, Lichty BD. Use of recombinant virus-vectored tuberculosis vaccines for respiratory mucosal immunization. Tuberculosis (Edinb). 2006 May-Jul;86(3-4):211-7. PubMed PMID: 16504584.

15. Wang J, Thorson L, Stokes RW, Santosuosso M, Huygen K, Zganiacz A, et al. Single mucosal, but not parenteral, immunization with recombinant adenoviral-based vaccine provides potent protection from pulmonary tuberculosis. J Immunol. 2004 Nov 15;173(10):6357-65. PubMed PMID: 15528375.

16. Corbett M, Bogers WM, Heeney JL, Gerber S, Genin C, Didierlaurent A, et al. Aerosol immunization with NYVAC and MVA vectored vaccines is safe, simple, and immunogenic. Proc Natl Acad Sci U S A. 2008 Feb 12;105(6):2046-51. PubMed PMID: 18270165. Pubmed Central PMCID: 2538878. Epub 2008/02/14. eng.

17. Sabin AB. Immunization against measles by aerosol. Rev Infect Dis. 1983 May-Jun;5(3):514-23. PubMed PMID: 6348918. Epub 1983/05/01. eng.

18. Sabin AB, Flores Arechiga A, Fernandez de Castro J, Sever JL, Madden DL, Shekarchi I, et al. Successful immunization of children with and without maternal antibody by aerosolized measles vaccine. I. Different results with undiluted human diploid cell and chick embryo fibroblast vaccines. JAMA : the journal of the American Medical Association. 1983 May 20;249(19):2651-62. PubMed PMID: 6341638. Epub 1983/05/20. eng.

19. Dilraj A, Cutts FT, de Castro JF, Wheeler JG, Brown D, Roth C, et al. Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomised trial. Lancet. 2000 Mar 4;355(9206):798-803. PubMed PMID: 10711928. Epub 2000/03/11. eng.

20. Dilraj A, Sukhoo R, Cutts FT, Bennett JV. Aerosol and subcutaneous measles vaccine: measles antibody responses 6 years after re-vaccination. Vaccine. 2007 May 22;25(21):4170-4. PubMed PMID: 17408818. Epub 2007/04/06. eng.

21. LiCalsi C, Maniaci MJ, Christensen T, Phillips E, Ward GH, Witham C. A powder formulation of measles vaccine for aerosol delivery. Vaccine. 2001 Mar 21;19(17-19):2629-36. PubMed PMID: 11257402. Epub 2001/03/21. eng.

22. Bellanti JA, Zeligs BJ, Mendez-Inocencio J, Garcia-Garcia ML, Islas-Romero R, Omidvar B, et al. Immunologic studies of specific mucosal and systemic immune responses in Mexican school children after booster aerosol or subcutaneous immunization with measles vaccine. Vaccine. 2004 Mar 12;22(9-10):1214-20. PubMed PMID: 15003650. Epub 2004/03/09. eng.

23. Hiremath GS, Omer SB. A meta-analysis of studies comparing the respiratory route with the subcutaneous route of measles vaccine administration. Hum Vaccin. 2005 Jan-Feb;1(1):30-6. PubMed PMID: 17038828. Epub 2006/10/14. eng.

24. Cutts FT, Clements CJ, Bennett JV. Alternative routes of measles immunization: a review. Biologicals. 1997 Sep;25(3):323-38. PubMed PMID: 9325001. Epub 1997/11/05. eng.

25. Belshe RB, Mendelman PM, Treanor J, King J, Gruber WC, Piedra P, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. The New England journal of medicine. 1998 May 14;338(20):1405-12. PubMed PMID: 9580647. eng.

26. Belshe RB, Edwards KM, Vesikari T, Black SV, Walker RE, Hultquist M, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. The New England journal of medicine. 2007 Feb 15;356(7):685-96. PubMed PMID: 17301299. eng.

27. Gaglani MJ, Piedra PA, Herschler GB, Griffith ME, Kozinetz CA, Riggs MW, et al. Direct and total effectiveness of the intranasal, live-attenuated, trivalent cold-adapted influenza virus vaccine against

the 2000-2001 influenza A(H1N1) and B epidemic in healthy children. Archives of pediatrics & adolescent medicine. 2004 Jan;158(1):65-73. PubMed PMID: 14706961. eng.

28. Langley JM, Halperin SA, McNeil S, Smith B, Jones T, Burt D, et al. Safety and immunogenicity of a Proteosome -trivalent inactivated influenza vaccine, given nasally to healthy adults. Vaccine. 2006 Mar 6;24(10):1601-8. PubMed PMID: 16303215. eng.

29. Stephenson I, Zambon MC, Rudin A, Colegate A, Podda A, Bugarini R, et al. Phase I evaluation of intranasal trivalent inactivated influenza vaccine with nontoxigenic Escherichia coli enterotoxin and novel biovector as mucosal adjuvants, using adult volunteers. Journal of virology. 2006 May;80(10):4962-70. PubMed PMID: 16641287. eng.

30. Langley JM, Aoki F, Ward BJ, McGeer A, Angel JB, Stiver G, et al. A nasally administered trivalent inactivated influenza vaccine is well tolerated, stimulates both mucosal and systemic immunity, and potentially protects against influenza illness. Vaccine. 2011 Feb 24;29(10):1921-8. PubMed PMID: 21219987. Epub 2011/01/12. eng.

31. Omer SB, Hiremath GS, Halsey NA. Respiratory administration of measles vaccine. Lancet. Feb 27;375(9716):706-8. PubMed PMID: 20189011. Epub 2010/03/02. eng.

32. Song K, Bolton DL, Wei CJ, Wilson RL, Camp JV, Bao S, et al. Genetic immunization in the lung induces potent local and systemic immune responses. Proc Natl Acad Sci U S A. 2010 Dec 21;107(51):22213-8. PubMed PMID: 21135247. Pubmed Central PMCID: 3009829.

33. Tucker SN, Tingley DW, Scallan CD. Oral adenoviral-based vaccines: historical perspective and future opportunity. Expert Rev Vaccines. 2008 Feb;7(1):25-31. PubMed PMID: 18251691.

34. Belyakov IM, Moss B, Strober W, Berzofsky JA. Mucosal vaccination overcomes the barrier to recombinant vaccinia immunization caused by preexisting poxvirus immunity. Proc Natl Acad Sci U S A. 1999 Apr 13;96(8):4512-7. PubMed PMID: 10200293. Pubmed Central PMCID: 16363.

35. Satti I, Meyer J, Harris SA, Manjaly Thomas ZR, Griffiths K, Antrobus RD, et al. Safety and immunogenicity of a candidate tuberculosis vaccine MVA85A delivered by aerosol in BCG-vaccinated healthy adults: a phase 1, double-blind, randomised controlled trial. The Lancet Infectious diseases. 2014 Oct;14(10):939-46. PubMed PMID: 25151225. Pubmed Central PMCID: 4178237.

36. Stickl H, Hochstein-Mintzel V, Mayr A, Huber HC, Schafer H, Holzner A. [MVA vaccination against smallpox: clinical tests with an attenuated live vaccinia virus strain (MVA) (author's transl)]. Dtsch Med Wochenschr. 1974 Nov 22;99(47):2386-92. PubMed PMID: 4426258. Epub 1974/11/22. MVA-Stufenimpfung gegen Pocken. Klinische Erprobung des attenuierten Pocken-Lebendimpfstoffes, Stamm MVA. ger.

37. Mayr A, Stickl H, Muller HK, Danner K, Singer H. [The smallpox vaccination strain MVA: marker, genetic structure, experience gained with the parenteral vaccination and behavior in organisms with a debilitated defence mechanism (author's transl)]. Zentralbl Bakteriol B. 1978 Dec;167(5-6):375-90. PubMed PMID: 219640. Epub 1978/12/01. Der Pockenimpfstamm MVA: Marker, genetische Struktur, Erfahrungen mit der parenteralen Schutzimpfung und Verhalten im abwehrgeschwachten Organismus. ger.

38. Hochstein-Mintzel V, Huber HC, Stickl H. [Virulence and immunogenicity of a modified vaccinia virus (strain MVA) (author's transl)]. Z Immunitatsforsch Exp Klin Immunol. 1972 Sep;144(2):104-56. PubMed PMID: 4282933. Epub 1972/09/01. Virulenz und Immunogenitat eines modifizierten Vaccinia-Virus (Stamm MVA). ger.

39. Hochstein-Mintzel V, Hanichen T, Huber HC, Stickl H. [An attenuated strain of vaccinia virus (MVA). Successful intramuscular immunization against vaccinia and variola (author's transl)]. Zentralbl Bakteriol Orig A. 1975;230(3):283-97. PubMed PMID: 1146441. Epub 1975/01/01. Vaccinia- und

variolaprotektive Wirkung des modifizierten Vaccinia-Stammes MVA bei intramuskularer Immunisierung. ger.

40. Goonetilleke N, Moore S, Dally L, Winstone N, Cebere I, Mahmoud A, et al. Induction of multifunctional human immunodeficiency virus type 1 (HIV-1)-specific T cells capable of proliferation in healthy subjects by using a prime-boost regimen of DNA- and modified vaccinia virus Ankara-vectored vaccines expressing HIV-1 Gag coupled to CD8+ T-cell epitopes. Journal of virology. 2006 May;80(10):4717-28. PubMed PMID: 16641265. Pubmed Central PMCID: 1472051. Epub 2006/04/28. eng.

41. Bejon P, Peshu N, Gilbert SC, Lowe BS, Molyneux CS, Forsdyke J, et al. Safety profile of the viral vectors of attenuated fowlpox strain FP9 and modified vaccinia virus Ankara recombinant for either of 2 preerythrocytic malaria antigens, ME-TRAP or the circumsporozoite protein, in children and adults in Kenya. Clin Infect Dis. 2006 Apr 15;42(8):1102-10. PubMed PMID: 16575727. Epub 2006/04/01. eng.

42. Gherardi MM, Esteban M. Recombinant poxviruses as mucosal vaccine vectors. J Gen Virol. 2005 Nov;86(Pt 11):2925-36. PubMed PMID: 16227213. Epub 2005/10/18. eng.

43. Belisle JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, Besra GS. Role of the major antigen of Mycobacterium tuberculosis in cell wall biogenesis. Science. 1997 May 30;276(5317):1420-2. PubMed PMID: 9162010. Epub 1997/05/30. eng.

44. Denis O, Tanghe A, Palfliet K, Jurion F, van den Berg TP, Vanonckelen A, et al. Vaccination with plasmid DNA encoding mycobacterial antigen 85A stimulates a CD4+ and CD8+ T-cell epitopic repertoire broader than that stimulated by Mycobacterium tuberculosis H37Rv infection. Infect Immun. 1998 Apr;66(4):1527-33. PubMed PMID: 9529077. Pubmed Central PMCID: 108084. Epub 1998/04/07. eng.

45. McShane H, Brookes R, Gilbert SC, Hill AV. Enhanced immunogenicity of CD4(+) t-cell responses and protective efficacy of a DNA-modified vaccinia virus Ankara prime-boost vaccination regimen for murine tuberculosis. Infect Immun. 2001 Feb;69(2):681-6. PubMed PMID: 11159955. Pubmed Central PMCID: 97939. Epub 2001/02/13. eng.

46. Vordermeier HM, Rhodes SG, Dean G, Goonetilleke N, Huygen K, Hill AV, et al. Cellular immune responses induced in cattle by heterologous prime-boost vaccination using recombinant viruses and bacille Calmette-Guerin. Immunology. 2004 Jul;112(3):461-70. PubMed PMID: 15196215. Pubmed Central PMCID: 1782509. Epub 2004/06/16. eng.

47. Huygen K, Content J, Denis O, Montgomery DL, Yawman AM, Deck RR, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. Nat Med. 1996 Aug;2(8):893-8. PubMed PMID: 8705859. Epub 1996/08/01. eng.

48. Verreck FA, Vervenne RA, Kondova I, van Kralingen KW, Remarque EJ, Braskamp G, et al. MVA.85A boosting of BCG and an attenuated, phoP deficient M. tuberculosis vaccine both show protective efficacy against tuberculosis in rhesus macaques. PLoS One. 2009;4(4):e5264. PubMed PMID: 19367339. Pubmed Central PMCID: 2666807. Epub 2009/04/16. eng.

49. Williams A, Hatch GJ, Clark SO, Gooch KE, Hatch KA, Hall GA, et al. Evaluation of vaccines in the EU TB Vaccine Cluster using a guinea pig aerosol infection model of tuberculosis. Tuberculosis (Edinb). 2005 Jan-Mar;85(1-2):29-38. PubMed PMID: 15687025. Epub 2005/02/03. eng.

50. Vordermeier HM, Dean GS, Rosenkrands I, Agger EM, Andersen P, Kaveh DA, et al. Adjuvants induce distinct immunological phenotypes in a cattle tuberculosis vaccine model. Clin Vaccine Immunol. 2009 Jul 29. PubMed PMID: 19641101. Epub 2009/07/31. Eng.

51. Scriba TJ, Tameris M, Mansoor N, Smit E, van der Merwe L, Mauff K, et al. Dose-finding study of the novel tuberculosis vaccine, MVA85A, in healthy BCG-vaccinated infants. J Infect Dis. 2011;203(21606542):1832-43.

52. Pathan AA, Minassian AM, Sander CR, Rowland R, Porter DW, Poulton ID, et al. Effect of vaccine dose on the safety and immunogenicity of a candidate TB vaccine, MVA85A, in BCG vaccinated UK adults. Vaccine. 2012 Aug 17;30(38):5616-24. PubMed PMID: 22789508. Pubmed Central PMCID: 3424417.

53. McShane H. Developing an improved vaccine against tuberculosis. Expert Rev Vaccines. 2004 Jun;3(3):299-306. PubMed PMID: 15176946. Epub 2004/06/05. eng.

54. Meyer J, Harris SA, Satti I, Poulton ID, Poyntz HC, Tanner R, et al. Comparing the safety and immunogenicity of a candidate TB vaccine MVA85A administered by intramuscular and intradermal delivery. Vaccine. 2013 Feb 4;31(7):1026-33. PubMed PMID: 23266342.

55. WHO. IVR strategic plan 2010 - 2020. http://whqlibdoc.who.int/hq/2010/WHO_IVB_10.02_eng.pdf?ua=1 (accessed 02 March 2015) 2010.

56. Santosuosso M, McCormick S, Roediger E, Zhang X, Zganiacz A, Lichty BD, et al. Mucosal luminal manipulation of T cell geography switches on protective efficacy by otherwise ineffective parenteral genetic immunization. J Immunol. 2007 Feb 15;178(4):2387-95. PubMed PMID: 17277145. Epub 2007/02/06. eng.

57. White AD, Sibley L, Dennis MJ, Gooch K, Betts G, Edwards N, et al. Evaluation of the safety and immunogenicity of a candidate tuberculosis vaccine, MVA85A, delivered by aerosol to the lungs of macaques. Clin Vaccine Immunol. 2013 May;20(5):663-72. PubMed PMID: 23446219. Pubmed Central PMCID: PMC3647747. Epub 2013/03/01. eng.

58. Flynn JL, Chan J. Immunology of tuberculosis. Annual review of immunology. 2001;19:93-129. PubMed PMID: 11244032. eng.

59. Kongerud J, Madden MC, Hazucha M, Peden D. Nasal responses in asthmatic and nonasthmatic subjects following exposure to diesel exhaust particles. Inhalation toxicology. 2006 Aug;18(9):589-94. PubMed PMID: 16864550. eng.

60. Kariyawasam HH, Aizen M, Kay AB, Robinson DS. Safety and tolerability of three consecutive bronchoscopies after allergen challenge in volunteers with mild asthma. Thorax. 2007 Jun;62(6):557-8. PubMed PMID: 17536034. eng.

61. Elston WJ, Whittaker AJ, Khan LN, Flood-Page P, Ramsay C, Jeffery PK, et al. Safety of research bronchoscopy, biopsy and bronchoalveolar lavage in asthma. Eur Respir J. 2004 Sep;24(3):375-7. PubMed PMID: 15358694. eng.

62. Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B, et al. Investigative bronchoprovocation and bronchoscopy in airway diseases. Am J Respir Crit Care Med. 2005 Oct 1;172(7):807-16. PubMed PMID: 16020805. Pubmed Central PMCID: 2718402. Epub 2005/07/16. eng.

63. Ho LP, Davis M, Denison A, Wood FT, Greening AP. Reduced interleukin-18 levels in BAL specimens from patients with asthma compared to patients with sarcoidosis and healthy control subjects. Chest. 2002 May;121(5):1421-6. PubMed PMID: 12006423. eng.

64. Wong-Chew RM, Islas-Romero R, Garcia-Garcia Mde L, Beeler JA, Audet S, Santos-Preciado JI, et al. Immunogenicity of aerosol measles vaccine given as the primary measles immunization to nine-month-old Mexican children. Vaccine. 2006 Jan 30;24(5):683-90. PubMed PMID: 16154241. Epub 2005/09/13. eng.

65. OMRON. OMRON Microair U22 website http://www.omron-healthcare.com/eu/en/our-products/respiratory-therapy/microair-u22 (accessed 02 March 2015). 2015.