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Evaluation of the safety and immunogenicity of candidate tuberculosis vaccines through Phase I Clinical Trials

by

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ABSTRACT

Tuberculosis (TB) is a disease which has been widespread for centuries and remains a major priority for global control. The existing TB vaccine, Bacille Calmette Guerin (BCG), is the oldest and only currently licensed TB vaccine but its efficacy is variable and a new TB vaccine is urgently needed. The two main approaches in use for candidate TB vaccines are whole organism mycobacteria and subunit vaccines. Replication-deficient recombinant viruses are a promising approach for the delivery of subunit vaccines.

This thesis describes early clinical trials of two recombinant poxviruses, modified Vaccinia virus Ankara (MVA) and Fowlpox virus (FP9) each expressing the mycobacterial antigen, 85A and named MVA85A and FP85A respectively.

The candidate vaccine, MVA85A, was evaluated in adults infected with Human Immunodeficiency Virus (HIV) for the first time in a Phase I clinical trial. MVA85A vaccination was safe and vaccination-induced antigen-specific cellular immune responses were detected by *ex vivo* interferon gamma (IFNγ) Enzyme-Linked Immunosorbent Spot (ELISpot) assay.

The first clinical trial of FP85A vaccination was conducted. In a Phase I clinical trial of single FP85A vaccination and heterologous prime-boost regimes of sequential MVA85A and FP85A vaccination in healthy adults, there were no safety concerns. No FP85A vaccine-induced antigen-specific cellular immune responses were detected by *ex vivo* IFNγ ELISpot assay.

A retrospective review of the cumulative safety data of MVA85A vaccination in healthy adults in the UK was conducted to provide a summary safety profile for further clinical development of MVA85A vaccination. No relationships between objective measures of local and systemic AEs and MVA85A vaccination-induced cellular immune responses were found.

The applicability of institutional reference intervals for the interpretation of commonly tested haematological and biochemical blood analytes in young healthy adults participating in clinical trials were evaluated in a retrospective study.

The work described here supports the continuing clinical development of the candidate TB vaccine MVA85A and contributes towards the accurate and safe interpretation of haematological and biochemical blood analytes in clinical trial subjects.

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DECLARATION OF AUTHORSHIP

I, ***Rosalind Rowland***, declare that the thesis entitled

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and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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Definitions and abbreviations

AdHu5	Human adenovirus 5
AdHu5Ag85A	Candidate TB vaccine: AdHu5 expressing antigen 85A
AE	Adverse event
Aeras	Not-for-profit biotechnology company for TB vaccine development
AERAS-402/ Crucell Ad35	Human adenovirus serotype 35 expressing a fusion protein of antigens 85A, 85B and TB10.4
AERAS-422	Candidate TB vaccine: recombinant BCG with an endosomal escape mechanism and over-expressing antigens 85A, 85B and Rv3407
Ag85A	Antigen 85A
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ART	Antiretroviral treatment
APC	Antigen presenting cells
AS	Adjuvant systems
AUC	Area under the curve
ßHCG	beta Human Chorionic Gonadotrophin
BALB/c	Albino laboratory mouse strain
BCG	Bacille Calmette Guerin
BF	FP85A vaccination in previously BCG-vaccinated subjects
BFM	Sequential FP85A and MVA85A vaccinations in previously BCG-vaccinated subjects (interval four weeks)
BMF	Sequential MVA85A then FP85A vaccinations in previously BCG-vaccinated subjects (interval four weeks)
CAF01	Cationic adjuvant factor
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, UK
CD	Cluster of differentiation
CEF	Chicken embryo fibroblast

CFP-10	10 kDa culture filtrate protein
ChAd	Simian adenovirus
CI	Confidence interval
CV	Coefficient of variation
DC	Dendritic cell
dL	Decilitre (0.1 L)
DOT	Directly observed treatment
DOTS	Directly observed treatment and short course drug therapy
DTP	Diphtheria tetanus polio
EDCTP	European and Developing Countries Clinical Trials Partnership
EDTA	Ethylenediaminetetraacetic acid
ESR	Erythrocyte sedimentation rate
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunosorbent spot assay
EPI	Expanded programme on immunisation
ESAT-6	6 kDa early secretory antigenic target of *M.tb*
FCS	Fetal calf serum
fL	Femtolitres (10^{-15}L)
FP85A	Candidate TB vaccine: FP9 expressing antigen 85A
FP9	Recombinant live attenuated fowlpox virus strain
FT	Foundation Trust
g	Grams
G	Gravitational constant
GCP	Good clinical practice
GMO	Genetically modified organisms
GMP	Good manufacturing practice
GMSC	Genetically modified safety committee

GSK	GlaxoSmithKline
GTAC	Gene Therapy Advisory Committee
H4/AERAS-404+IC31®	Candidate TB vaccine: Antigen 85B-TB10.4 fusion protein formulated in IC31®
H56/AERAS-456+ IC31®	Candidate TB vaccine: Antigen 85B-ESAT-6-Rv2660c fusion protein formulated in IC31®
HAV	Hepatitis A virus
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
Hly	Listeriolysin
HLA	Human Leukocyte Antigen
HPV	Human Papilloma Virus
HSE	Health and safety executive
IB	Investigators Brochure
ICAM-1	Intercellular Adhesion Molecule 1
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	Intracellular staining
id	intradermal
ID	identification
ID93+GLA-SE	Candidate TB vaccine: Fusion protein formulated with GLA-SE
IFNγ	Interferon gamma
IgG	Immunoglobulin G
IGRA	Interferon gamma release assay
IKEPLUS	Candidate TB vaccine: *Mycobacterium smegmatis* with esx-3 virulence gene deleted
IL	Interleukin

IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
IQR	Interquartile range
IRAS	Integrated research application system
IU	International units
kDa	kilo Dalton
kg	kilogram
Km	kilometres
KW	Kruskal Wallis one way analysis of variance by ranks
L	Litre
LAM	Lipoarabinomannan
LFA-1	Lymphocyte function-associated antigen 1
LED	Light-emitting diode
LN	Lymph nodes
LTBI	Latent tuberculosis infection
M72+AS01	Candidate TB vaccine: recombinant M72 polyprotein formulated in AS01
M.bovis	*Mycobacterium bovis*
MDR	Multi-drug resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
MGIA	Mycobacterial growth inhibition assay
MHC I	Major histocompatibility complex class I
MHC II	Major histocompatibility complex class II
MHRA	Medicines and Healthcare Regulatory Authority
MIP	*Mycobacterium indicus pranii*
ml	Millilitre
mm	Millimetre
mmol	Millimoles

mM	Millimolar
MMR	Measles mumps rubella
MPL	monophospholipid
MRC	Medical Research Council
M.tb	*Mycobacterium tuberculosis*
MTBVAC	Candidate TB vaccine: live attenuated *M.tb* with *phoP* and *fadD26*-deleted
MVA	Modified Vaccinia virus Ankara
MVA85A	Candidate TB vaccine: Recombinant MVA expressing antigen 85A
Mw	Non-pathogenic mycobacterium
MWU	Mann Whitney U test
NAAT	Nucleic acid amplification test
NAFLD	Non-alcoholic fatty liver disease
NHP	Non-human primates
NHS	National health service
NK cell	Natural Killer cell
NRES	National research ethics service
NTM	Non-tuberculous mycobacteria
ODN	oligodeoxynucelotide
OETC	Oxford-Emergent Tuberculosis Consortium
OR	Odds Ratio
ORCRB	Old Road Research Campus Building
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate buffered solution
PCR	Polymerase chain reaction
pfu	Plaque forming units
PHA	Phytohaemagglutinin

PPD	Purified protein derivative
qPCR	Quantitative PCR
R&D	Research and Development
rBCG30	Candidate TB vaccine: recombinant BCG over-expressing antigen 85B
RCT	Randomised controlled trial
RD	Region of difference
REC	Research ethics committee
RI	Reference interval
rpm	Revolutions per minute
RUTI®	Candidate TB vaccine: detoxified liposomal cellular fragments of *M.tb* bacilli
SAE	Serious adverse event
SAR	Serious adverse reaction
SATVI	South African TB Vaccine Initiative
sc	subcutaneous
SCC	Short course chemotherapy
SD	Standard deviation
SFC	Spot forming cells
SOP	Standard Operating Procedure
SSI	Statens Serum Institut
STI	Sexually transmitted infection
SUSAR	Serious unexpected serious adverse reaction
TB	Tuberculosis
TBVI	TuBerculosis Vaccine Initiative
TGFß	Tissue Growth Factor beta
Th1	T helper 1
Th2	T helper 2
TLR	Toll-like receptor

TNFα	Tumour necrosis factor alpha
Treg	Regulatory T cell
TST	Tuberculin skin test
U	Units
UK	United Kingdom
µg	micrograms
µl	Microlitres
µmol	Micromoles
UNICEF	United Nations Children's Fund (originally named United Nations International Children's Emergency Fund)
URTI	Upper respiratory tract infection
USA	United States of America
VCAM-1	vascular cell adhesion molecule 1
VIS	Volunteer information sheet
VPM1002	Candidate TB vaccine: recombinant BCG expressing listeriolysin to enable endosomal escape
WHO	World Health Organization
VLA-4	Very Late Antigen-4
WSR	Wilcoxon signed rank test
XDR	Extensively drug resistant
YF	Yellow Fever

Chapter 1 : Introduction

Overview

Tuberculosis (TB) is a disease which has been widespread for centuries and remains a major priority for global control. The existing TB vaccine, Bacille Calmette Guerin (BCG), is the oldest and only currently licensed TB vaccine but its efficacy is variable and a new TB vaccine is urgently needed. In the 20th century, there was a great increase in the number of licensed vaccines and many challenges were overcome, but almost all currently licensed vaccines induce antibody-mediated protective immunity. Recent advances in vaccine research support the drive to develop new T cell inducing vaccines for TB and other infectious diseases. Whilst ethical guidelines for conducting clinical research trailed behind scientific advances in the last century, there are now clear structures and legislation for the ethical approval, regulation and conduct of all clinical trials. A number of candidate TB vaccines are currently being evaluated in clinical trials. MVA85A, one of the leading candidates, is a recombinant viral-vectored vaccine expressing a mycobacterial antigen. A second recombinant viral-vectored candidate TB vaccine, FP85A has also been developed.

The aims of this thesis are to:

1. Evaluate the safety of candidate TB vaccine, MVA85A, in Human Immunodeficiency Virus (HIV)-infected adults in the UK;
2. Evaluate the safety of candidate TB vaccine, FP85A, in healthy adults for the first time;
3. Review the safety of MVA85A from a series of early clinical trials in adults in the UK;
4. Evaluate the applicability of institutional reference intervals (RIs) for the interpretation of commonly tested haematological and biochemical blood analytes for a young healthy adult population of clinical trial subjects.

Tuberculosis control

Early strategies

TB is an ancient disease, which originated in East Africa and was widespread, including in Europe, by the 5th century (1). In the 19th century, Theophile Laennec the inventor of the stethoscope described TB pathogenesis; Jean-Antoine Villemin demonstrated the transmissibility of *Mycobacterium tuberculosis (M.tb)* infection and Robert Koch identified the tubercle bacillus (1). Tuberculin, extracts of *M.tb,* was initially introduced

as an (ineffective) treatment for TB disease, before its diagnostic properties in providing evidence of previous infection were realized (2). Clemens von Pirquet developed the tuberculin skin scratch test in 1903 and identified latent *M.tb* infection (LTBI) (1, 2). The intradermal tuberculin skin test (TST), in which skin hypersensitivity to injected tuberculin indicates past or present *M.tb* infection, was subsequently created by Charles Mantoux (2). Standardized purified protein derivative (PPD) was developed by Florence Seibert in the 1930s and is still used today for TSTs and laboratory experiments (1). Early treatment regimens for TB, such as bed rest and fresh air in sanitoria and pulmonary collapse therapies, were minimally effective and only then for mild disease (1).

The BCG Vaccine

The observation that pulmonary TB was rare in individuals who had previously had scrofula (*M.tb* infection of the lymph nodes in the neck) provided evidence for acquired protective immunity against TB (2). In the context of a global epidemic and a lack of effective treatment, Albert Calmette and Camille Guerin set out to develop a prophylactic TB vaccine. Attempts to attenuate virulent human and bovine bacilli, such as boiling, chemical treatment and incubation in leeches' guts had been unsuccessful until Calmette and Guerin discovered that subculturing tubercle bacilli lowered their virulence (2). BCG, an attenuated strain of *Mycobacterium bovis (M.bovis),* was produced by subculturing an isolate of *M.bovis* every three weeks from 1908 to 1919 (2). The first human was vaccinated with BCG in 1921 and was an infant whose mother had died of TB (2). Cutaneous and subcutaneous routes were unpopular with parents due to the local reaction and the natural route of infection of *M.tb* was believed to be gastrointestinal, so the vaccine was administered orally (2). After a series of 664 infants were vaccinated with oral BCG, the Pasteur Institute, Paris began mass production of BCG in 1924 and, by 1928, over 100,000 infants had been vaccinated (2). Concerns regarding the safety of BCG vaccination were heightened when a large number of vaccinated infants in Lubeck developed TB, but the BCG vaccines were subsequently found to have been contaminated with *M.tb* at the hospital performing the immunizations (2, 3). Following large TB epidemics during the Second World War, immunization campaigns of TST-negative children in Hungary, Poland and Germany were carried out (2, 3). The United Nations International Children's Emergency Fund (UNICEF) then funded prophylactic immunization of 14 million people worldwide (1, 3).

The reasons behind the variable and limited efficacy of BCG are not fully understood, but there are a number of hypotheses.

In small studies evaluating immune responses following BCG vaccination, adolescents and infants living in the UK developed stronger interferon gamma (IFNγ) immune

responses to PPD stimulation following BCG vaccination compared to adolescents and infants living in Malawi (4-6). It is not clear how these findings relate to protective efficacy, but BCG vaccine efficacy does increase with increased distance from the equator. In populations where there are high levels of exposure to nontuberculous mycobacteria (NTM) and poor BCG efficacy, those with lower immune responses to NTM show greater IFNγ responses to BCG vaccination, suggesting NTM may be inhibiting BCG's effectiveness (5, 7, 8). Two possible mechanisms by which NTM may affect the efficacy of BCG are masking, where BCG cannot further boost the background level of immunity induced by NTMs; and blocking, where the ability of BCG to replicate and thereby induce a protective immune response is inhibited by pre-existing immunity to NTMs (6, 9).

Different BCG vaccine strains exist, owing to genetic alterations of the original strain during propagation in different laboratory conditions (10). The World Health Organization (WHO) has stored lyophilized seed lots of each strain since 1956 in order to prevent further alterations from the original BCG, such that 90 % of all BCG vaccinations are the French Pasteur 1173 P2, Danish SSI 1331, Glaxo 1077, or Japanese Tokyo 172 strains (11). The interpretation of studies in animal models is limited by discrepancies between study methods but BCG-Glaxo, BCG-Pasteur and BCG-Denmark were the most protective strains in mice (12). Most studies in humans evaluated differences in sizes of vaccine site scar and PPD TST, which do not correlate with protection (12). Studies evaluating differences in immunogenicity between strains gave conflicting results, with small sample sizes and variations in study design (12). However, recently, a randomised-controlled trial (RCT) in infants showed higher immune responses to the BCG-Denmark and BCG-Japan strains compared to BCG-Russia (13). Whilst it is clear that phenotypic as well as genetic differences between strains do exist, the nature and protective significance of these differences cannot be ascertained until biomarkers of protection against *M.tb* are identified (12).

Chronic helminth infections are common in the regions where BCG is less effective (14). Helminth infections are associated with T helper 2 (Th2)-type immune responses and the secretion of the inhibitory cytokines interleukin (IL)-10 and tissue growth factor beta (TGFß) by regulatory T cells (Tregs) (14). Th2 immune responses are important for barrier and mucosal immunity but counteract the T helper 1 (Th1)-type responses which are required for protective immunity against *M.tb* infection. In one study of antihelminthic chemotherapy three months prior to BCG vaccination, IFNγ-secreting cells were higher in frequency in the treated group and TGFß-producing cells were higher in frequency in the placebo group (14). However, in an RCT in pregnant women and children, there were no differences in cytokine responses detected in

whole blood following BCG in the antihelminthic-treated group compared to the placebo group (15).

BCG remains the only licensed TB vaccine and is the oldest vaccine in current use. It is cost-effective in preventing severe disease in childhood (disseminated and meningeal TB), but prevention of adult pulmonary disease is inconsistent (16, 17). There is no evidence suggesting either BCG revaccination or BCG vaccination of adults provides any benefit in protection against TB disease (18). BCG is contraindicated in people infected with HIV, due to the risk of disseminated BCG infection in immunocompromised hosts (11, 19-21).

TB Diagnosis

In addition to prophylactic vaccination, TB control strategies focussed upon prompt case detection (contact tracing), diagnosis and treatment (22). Sputum smear microscopy and culture are conventional diagnostic methods which have been in use for many years and, in resource-poor settings, sputum smear microscopy is still the primary diagnostic tool (23, 24). Fluorescence microscopy is more sensitive than Ziehl-Neelson microscopy and light emitting diode (LED)-based fluorescence microscopy is now recommended by the WHO (23, 25). Mycobacterial culture is the gold standard for TB diagnosis. Lowenstein-Jensen solid medium is straightforward to use and widely available at low cost, but culture takes up to six weeks and drug susceptibility testing even longer (23). Since being endorsed by the WHO in 2007, automated liquid culture systems are being implemented in resource-poor settings, to reduce the time to bacteriological diagnosis (23, 26). New simpler, less expensive culture and sensitivity testing systems are in development (23). The risk of contamination from nontuberculous mycobacteria (NTM) is greater in liquid compared to solid culture systems, and immunochromatographic detection of the *M.tb* antigen, MPB64, is recommended by the WHO for rapid species identification (23, 26).

Molecular technologies for the diagnosis of TB and detection of multi-drug resistant (MDR) TB are in development and some have been approved by the WHO (27). Line probe assays, such as the GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany), involve DNA amplification by polymerase chain reaction (PCR) and the use of oligonucleotide probes to detect *M.tb* and mutations associated with isoniazid and rifampicin resistance (23, 28-32). The Xpert MTB/RIF assay (Cepheid, Sunnyvale, Canada) is a cartridge-based nucleic acid amplification test (NAAT) which can be used to detect nucleic acid sequences from unprocessed clinical samples, including sputum and urine, is being rapidly adopted by TB-endemic countries (23, 24, 27, 32-35). Other NAATs, with the potential to further reduce the technical expertise required and power usage, are in development (32). Assays for the detection of urinary antigen in patients

with advanced HIV disease, in whom disseminated TB is likely, are being developed and enzyme-linked immunosorbent assays (ELISAs) which detect Lipoarabinomannan (LAM) are commercially available (32, 36, 37).

The TST, or Mantoux test, is widely used for the diagnosis of LTBI (38). However, prior BCG vaccination or exposure to environmental NTM may impair the test's specificity and immunodeficiency due to advanced HIV disease may give rise to false negative results (38). IFNγ release assays (IGRAs) detect IFNγ production in response to stimulation of peripheral blood mononuclear cells (PBMC) with immunodominant *M.tb* antigens (6 kDa early secretory antigenic target [ESAT-6] and 10 kDa culture filtrate protein [CFP-10]). Two commercially available tests are an ELISA, the QuantiFERON test (Qiagen, United States of America [USA]) and an enzyme-linked immunosorbent spot (ELISpot) assay (TSPOT.TB test, Oxford Immunotec, UK) (38). Specificity and sensitivity testing of IGRAs is hampered by the lack of a gold standard diagnostic tool for comparison, but meta-analyses suggest they have far greater specificity than TSTs and are also more sensitive (39, 40). IGRAs are used in many countries which have low TB prevalence, for example in the UK, they are recommended for BCG-vaccinated individuals; as confirmation of positive TSTs; and for contacts of sputum-positive TB cases (41). In consideration of the cost and technical requirements associated with IGRAs and the available evidence, TSTs are still recommended over IGRAs by the WHO (23, 42).

Chemotherapy

Streptomycin, introduced in 1946, was the first effective treatment for TB (43). It soon became apparent that the *M.tb* bacillus rapidly acquired drug resistance and combination chemotherapy was adopted (43). Following the introduction of the BCG vaccine and, in the 1950s, long course combination chemotherapy (streptomycin, para-aminosalicylic acid and isoniazid); TB was almost eradicated from wealthy, industrialized nations (43). Efforts to control the TB epidemic in less-developed countries included the introduction of intermittent directly observed treatment (DOT) and BCG vaccination of infants (11, 44). With the addition of rifampicin, pyrazinamide and ethambutol, effective short course chemotherapy (SCC) regimens were introduced, forming the basis of the modern standard recommended regimen (43, 45-47).

There is still a limited repertoire of available drugs and regimes are expensive and lengthy. New cases of pulmonary TB are treated with two months of isoniazid, rifampicin, ethambutol and pyrazinamide, followed by four months of isoniazid and rifampicin (48). In an attempt to address the problems of drug resistance, ethambutol is now recommended to be continued for the full six months if isoniazid resistance testing is not available and there are high levels of isoniazid resistance (48). Three

times weekly DOT is an alternative to the optimal daily dosing schedule, but is not recommended for the first two months in the context of HIV co-infection, due to the risk of drug resistance (48). Longer regimens, including streptomycin (injected), are recommended for patients receiving retreatment. Other drugs which are used in MDR TB treatment regimens, depending upon drug susceptibility, include aminoglysides (kanamycin or amikacin); fluoroquinolones (levofloxacin or moxifloxacin); ethionamide and *p*-aminosalicylic acid (48).

Current strategies

In 1993 the WHO declared TB to be a global emergency (49). Deteriorating, poorly managed control systems, population growth and TB/HIV co-infection had resulted in the emergence of MDR TB and a major resurgence of TB in HIV-endemic areas (50). The need for SCC provision to at least all sputum smear positive cases of TB, and BCG vaccination of infants in high prevalence countries within the Expanded Programme on Immunisation (EPI) was emphasised (50). The Directly Observed Treatment and Short Course Drug Therapy (DOTS) strategy was launched and the Global Fund for HIV, TB and malaria was established (50). TB incidence did decline, but the rate of decline was inhibited by the HIV epidemic and the emergence of MDR and extensively drug-resistant (XDR) strains of *M.tb* (51, 52). Since world population growth outweighed the decline in incidence, TB cases and mortality continued to rise. The Stop TB Partnership was formed in 2000 and addressed the need for a broader approach with the launch of the Global Plan to Stop TB 2006-2015, which was updated in 2010 (53). The strategy focusses on DOTS "*expansion and enhancement*"; TB-HIV co-infection; MDR/XDR TB; "*poor and vulnerable populations"*; *"health system strengthening based on primary care";* engaging "*all care providers"*; empowering *"people with TB and communities through partnership";* and enabling and promoting research into the development of new diagnostics, drugs and vaccines (53). Although one Millennium Development Goal target, to halt and reverse the TB epidemic, has been achieved, there were still 8.6 million new TB cases and 1.3 million deaths from TB in 2012 (24). The rate of decline needs to increase in order to progress towards eliminating the disease.

The limitations in safety and efficacy of the BCG vaccine and continuing burden of TB disease make a clear case for a new TB vaccine. The effectiveness of BCG in childhood suggests successful vaccination against TB is possible. A number of candidate TB vaccines, designed to replace or enhance BCG are in development.

Vaccines: an overview

The origins of vaccination

Vaccination harnesses the adaptive immune response, by aiming to induce long lasting protection through the presence of residual effector T cells, antibodies and antigen-specific memory T and B cells, which persist in the absence of stimulating antigen (54). The principle of acquired immunity was first described in the first century, when it was observed that smallpox could be transmitted from person to person and that survivors of smallpox did not develop the disease (55). Variolation, inoculation using samples from patients with mild smallpox disease, was practised by the Chinese for centuries and used worldwide by the 18th century, but carried the risk of causing smallpox disease, or initiating epidemics. Edward Jenner's research proving cowpox-induced immunity to smallpox, led to replacement of variolation with cowpox inoculation, '*variolae vaccinae'* (55). Despite widespread opposition to vaccination in the 19th century, scientific research continued. The transmissibility of infectious diseases were described in Koch's postulates and proved by Louis Pasteur (56, 57). Pasteur then produced the first live attenuated vaccine (veterinary anthrax) and in 1884 a live attenuated human rabies vaccine (56, 58). The first bacterial vaccine was Jaime Ferran's cholera vaccine (3). The discoveries by von Behring of diphtheria and tetanus toxins; that small quantities of toxin could induce immunity; and that serum from an immunised animal could cure an infected animal, led to the production of antitoxins and later toxoid vaccines (weakened toxins) (3).

20th century vaccines

By 1930, there were live attenuated or killed vaccines against smallpox, rabies, typhoid, shigella, cholera, plague, diphtheria, tetanus, pertussis, and TB (59). Glenny introduced the first adjuvant, by treating diphtheria toxoid with aluminium salts to enhance the magnitude and duration of the immune response to diphtheria toxoid vaccination (3). Freeze-drying smallpox vaccines and bifurcated needles to improve ease of administration contributed to the effectiveness of mass immunisation campaigns (3).

The next era of vaccine development began with Goodpasture's demonstration of viral growth in cultures derived from embryonated hen's eggs, which enabled mass production of typhus vaccines and the development of the 17D yellow fever vaccine and the first inactivated influenza vaccine (59). These vaccines were funded by the military and driven by the need for vaccination of troops during the Second World War (59). In the latter half of the century, live attenuated and killed poliovirus vaccines; live attenuated measles, mumps, rubella and varicella vaccines; a trivalent inactivated

influenza vaccine; bacterial polysaccharide vaccines against *Streptococcus pneumoniae, Neisseria meningitides* and *Haemophilus influenzae;* recombinant subunit hepatitis B virus (HBV) and Lyme disease vaccines; and a killed hepatitis A virus (HAV) vaccine were licensed (3). Other advances included the concept of combining childhood vaccinations (diphtheria, tetanus and polio [DTP]; measles, mumps and rubella [MMR]); and producing multivalent bacterial polysaccharide vaccines (59). Conjugating polysaccharide vaccines to carrier proteins induced T cell-dependent antibody responses, as opposed to T cell-independent antibody responses, and thus improved their effectiveness in infants (54).

Setbacks and challenges

The benefit of the new vaccines was dramatic, but morbidity and mortality due to vaccine-preventable diseases in developing countries remained high. The EPI was established in 1974 to increase worldwide coverage of BCG, DTP, polio, meningococcal, HBV and *Haemophilus influenzae* vaccines (60). In developed countries, there were real and perceived safety issues with some vaccines, leading to vaccine withdrawal or lack of uptake. In the 1970s, a swine flu vaccination programme was halted after a higher incidence of Guillain Barre Syndrome in vaccinees. Also, reports of pertussis-induced brain damage led to a dramatic fall in DTP uptake, resulting in three whooping cough epidemics (61). MMR uptake remains suboptimal following the now discredited link between MMR and autism, which led to decreased herd immunity and outbreaks of measles (62). The first licensed rotavirus vaccine was withdrawn in the 1990s due to a link with intussusception in infants (3).

Whilst new vaccines continue to be licensed, such as recombinant subunit human papillomavirus (HPV) vaccines (the first licensed cancer vaccines); two live rotavirus vaccines and influenza vaccines, the rate of vaccine-development has now slowed (63). Infectious diseases which are considered to be vaccine-preventable, but against which there are no licensed vaccines include malaria, dengue fever, Hepatitis C virus (HCV) and HIV. New approaches and technologies are required to achieve effective vaccination against these diseases. BCG is the only licensed vaccine whose principal mechanism of protection is T cell-mediated and efforts to develop T cell-inducing vaccines are ongoing (64).

Preclinical animal models for evaluation of candidate TB vaccines include mice, guinea pigs, cattle and non-human primates (NHP). Major differences in immunology, TB pathology and BCG vaccine efficacy between humans and small animals restrict the conclusions which can be drawn from the evaluation of candidate vaccines in small animal models (65, 66). Cattle are a natural host for TB and are a good model for human TB in many respects, although the infecting organism is *M.bovis* rather than

M.tb (67). NHP are considered the best available animal model and an *M.tb* aerosol challenge model has been developed for the evaluation of clinical and immunological endpoints in TB vaccine studies (68). However, experiments involving NHPs require specialist facilities and considerable staff expertise and their use is ethically controversial and limited by the substantial costs involved. Animal models need to be validated against efficacy trials in humans to characterise their predictive value for TB vaccine efficacy in humans. In the meantime, candidate TB vaccines are often moved rapidly from preclinical to clinical trials, in order to evaluate their potential for protective efficacy in humans as efficiently as possible.

TB immunology

Improved understanding of the nature of the host immune response to *M.tb* infection will inform the effective design and evaluation of new TB vaccines.

Primary immune responses

M.tb is an aerobic, acid-fast, non-encapsulated, non-spore forming, obligate intracellular bacillus which is transmitted through droplet inhalation and grows best in oxygen rich tissues, such as the lung (69). *M.tb* bacilli enter the alveoli and are phagocytosed by alveolar macrophages and dendritic cells (DCs), but are able to survive by inhibiting phagolysosome fusion and acidification. Initially, a non-specific innate primary immune response, involving macrophages and neutrophils, is triggered through pattern recognition receptors, such as toll-like receptors (TLRs) and the common adaptor molecule MyD88 (54, 69). It is thought that in a very small proportion of individuals who are exposed to *M.tb* (based on positive TST results), the innate immune response is able to clear the infection (70). In 90 to 95 % of infected individuals, the adaptive immune response arrests the bacilli in a state of dormancy, known as LTBI and in up to 10 %, primary TB disease ensues within one to two years (70, 71).

Adaptive immunity

Adaptive immune responses are induced when DCs transport *M.tb* bacilli to local lymphoid tissues (54). Naïve cells are activated through co-stimulatory molecules on DCs and undergo clonal expansion into effector T cells and antibody-secreting B cells. Cluster of differentiation (CD) 4^+ T cells recognize antigens presented by major histocompatibility complex class II (MHC II) molecules. $CD8^+$ T cells recognize cytoplasmic antigens presented by major histocompatibility complex class I (MHC I) molecules and mycobacterial cell wall lipids presented by CD 1 molecules. Effector T cells and antibodies enter the bloodstream and cells home to the site of infection

through very late antigen 4 (VLA-4) and lymphocyte function-associated antigen 1 (LFA-1) surface receptors, which bind to vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) respectively.

Since *M.tb* bacilli are located within the phagosomes of professional antigen presenting cells (APCs); their antigens are presented through the MHC II pathway and principally induce $CD4^+$ T cells. It has been hypothesised, however, that *M.tb.* can cross prime MHC I-restricted $CD8^+$ T cells by inducing apoptosis of the infected host cell. The apoptotic vesicles contain mycobacterial antigens that are taken up by the bystander APCs. Viruses and intracellular bacteria trigger TLR-signalling which induces DCs to secrete interleukin (IL)-12, initiating Th1 responses, which are essential for granuloma formation. Th1 $CD4^+$ T cells secrete IFNγ; activate macrophages and induce opsonizing antibodies and apoptosis. $CD8^+$ T cells secrete IFNγ and have cytotoxic activity via granzyme, perforin and granulysin secretion (70).

In LTBI, a chronic infectious state, there is equilibrium between the host immune response and the *M.tb* bacilli. The bacilli are located within granulomas in the lung and survive by inhibiting phaglysosomal fusion and acidification, but are rendered metabolically inactive and unable to replicate by the host immune response. In TB disease, the infection reactivates and the dormant *M.tb* bacilli become metabolically active and start replicating again. Granulomas become necrotic and the host immune response can no longer contain the infection, resulting in active TB disease with associated lung damage and the exhalation of infectious droplets. Factors which predispose towards reactivation TB disease include impaired IFNγ-secretion associated with inherited immunodeficiencies; reduced $CD4^+$ T cells associated with advanced HIV infection; tumour necrosis factor alpha (TNFα)-blocking medication; and possibly factors causing a shift towards a Th2-type immune response (72). The annual risk of developing active TB disease for individuals with LTBI is one in 100 for HIV-uninfected, but one in ten for HIV-infected individuals (71). These factors and murine knockout, cell depletion and adoptive transfer studies highlight the importance of Th1 $CD4^+$ T cells in protection against TB (73-78). The roles of other $CD4^+$ T cell subsets are less well characterized. Th17 cells secrete IL-17 which attracts neutrophils to the site of infection and may be beneficial or responsible for immunopathology (79). Tregs secrete IL-10 and TGF-ß and may limit immunopathology or inhibit protective immune responses (79).

Whilst antibodies may play some role in protective immunity against TB, *M.tb* bacilli are protected by their intracellular location. However, vaccine-induced antibodies may be useful for protection against primary *M.tb* infection by neutralizing bacilli within the alveoli, before their uptake by phagocytes (79).

Evaluating the immunogenicity of TB vaccines

Immune biomarkers which accurately predict vaccine-induced protection against TB are yet to be defined (80, 81). The most widely evaluated immunological outcome in clinical trials of TB vaccines is IFNγ production in response to antigen-stimulation (82-88). Typically, PBMCs are extracted from heparinised blood or whole blood samples are used (89). Samples are obtained at baseline and regular intervals post-vaccination and stimulated with vaccine-specific or mycobacterial antigens. The frequencies of antigen-specific IFNγ-producing $CD4^+$ and $CD8^+$ T cells may be evaluated by ELISpot or by intracellular staining (ICS) and flow cytometry (82-86). Serum samples may also be obtained for the detection of soluble cytokines and antibodies using cytokine bead assays or ELISA (83, 84, 86). Flow cytometry is now a standard technique in many laboratories and allows the detection of multifunctional T cells (cells expressing more than one cytokine simultaneously), most commonly IFNγ, TNFα and IL-2-producing cells (82, 85, 90-92). Whilst a correlation between multifunctional $CD4^+$ T cells and protective immunity against TB has been demonstrated in some mouse models, there is no evidence that antigen-specific IFNγ-producing or multifunctional $CD4^+$ T cells correlate with protection against TB in humans (93-96). However, IFNγ production is known to be essential and these assays are reliable and reproducible so are currently the best available methods for indicating vaccine-immunogenicity. Flow cytometric methods also enable a detailed functional and phenotypic evaluation of cell types to be conducted (85). The molecules granzyme, perforin and granulysin indicate cytolytic T cell function; T cell trafficking molecules indicate the tissue to which cells are homing; and surface molecules which correspond to effector, memory and proliferative activity can be identified (83, 85, 92). Alongside efforts to develop validated biomarkers, existing techniques are being harmonized and standardized to facilitate comparisons between different clinical trials, regimes and vaccines (88).

Developing new TB vaccines

Antigen selection

Standard practice is to select antigens which are immunodominant; essential for virulence; highly conserved; contain T cell epitopes; and to which T cell responses are protective in animal models as targets for new TB vaccines. Such T cell epitopes can be identified by systematically testing overlapping synthetic peptides from immunogenic antigens for protective efficacy in animal models; or by reverse immunogenetics to predict possible epitopes within a genetic sequence (54). However, there is an argument that more variable regions of the *M.tb* genome should be explored for the identification of vaccine targets (97). The basis for this is that, although *M.tb* is more

conserved than other organisms, there is evidence of some strain diversity and the organism may have developed strategies for 'subverting' the immune response to immunodominant, conserved antigens (97). The mycobacterial antigens most commonly incorporated into candidate TB vaccines are the major secretory proteins. The antigen 85 complex (A, B and C) constitutes a major portion of the secreted proteins of both *M.tb* and BCG, is highly conserved amongst all mycobacterial species and present in all strains of BCG; is essential for mycobacterial cell wall assembly and is a virulence factor, since it is required for survival in macrophages (98, 99). Antigen 85 components are highly immunogenic in humans and animals and can induce protective immune responses in animal models (100-102).

ESAT-6 is a 6 kilo-Dalton (kDa) protein which is a major target for protective cell mediated immunity in animals and is recognized by T cells from patients with TB disease (103-105). TB10.4, another antigen in the ESAT family, is immunodominant in animals and humans; contains $CD4^+$ and $CD8^+$ T cell epitopes and induces protective immune responses in mice (106-108). Both ESAT-6 and TB10.4 antigens contain genes which are important for immunogenicity and lie within the Region of Difference 1 (RD-1) (109, 110). RD-1 is present in virulent strains of *M.bovis* and *M.tb* but was deleted from BCG (111). It is hypothesised that incorporating antigens containing RD-1 into candidate TB vaccines may induce superior immunogenicity compared to BCG (111, 112). A potential problem with using these antigens in vaccines is that they may reduce the specificity of IGRAs for LTBI diagnosis.

Most of the antigens incorporated into candidate vaccines currently in clinical trials are expressed early in *M.tb* infection and may be down regulated in later stages of infection. Another hypothesis is that for optimal protection against the establishment or reactivation of LTBI, antigens expressed throughout infection are also required (113).

Vaccine carrier selection

New TB vaccines are being developed with the aim of preventing infection (pre-exposure); or preventing primary progression to TB or reactivation of LTBI (post-exposure). Immunotherapeutic TB vaccines aim to shorten the course of, or improve the response to chemotherapy. There are two main approaches to vaccine development; live mycobacterial vaccines and subunit vaccines.

Whole organism mycobacterial vaccines are designed as prophylactic vaccines to replace BCG. Live attenuated vaccines, such as BCG, are usually more potent than killed (inactivated) vaccines and stimulate more $CD4^+$ and $CD8^+$ T cells (54). Targeted attenuation and modification of infectious organisms, such as deletion of specific

virulence genes and overexpression of immunodominant antigens, are now possible (54). The rationale for novel live attenuated mycobacterial vaccines is to build upon the efficacy conferred by BCG against systemic disease whilst addressing the reasons for its limited efficacy against pulmonary disease. The two leading strategies are recombinant BCG vaccines and *M.tb*-based vaccines (83, 114). As BCG has been shown to induce protective and durable protection against TB in some geographical regions, it may be possible to enhance this protection with modifications to the existing vaccine. Alternatively, targeted deletion of specific *M.tb* virulence genes is being used to develop live attenuated strains which retain the breadth of immunodominant antigens in *M.tb*, which are not all present in BCG.

Subunit vaccines may be used in conjunction with BCG, or with a novel mycobacterial vaccine, to boost, enhance or prolong the protection afforded by BCG. Subunit vaccines in clinical evaluation involve the delivery of immunodominant mycobacterial antigens to the immune system, using viral vectors or protein-adjuvant systems (82, 84, 85, 113, 115-117). These regimes would retain BCG vaccination of neonates, overcoming the ethical issues inherent with a replacement BCG vaccine. Subunit acellular vaccines are safer than cellular or whole organism vaccines, but require an adjuvant or carrier. DNA vaccines induce low magnitude T cell responses in humans, but with poor efficacy in clinical trials and no such candidate TB vaccines have been tested in clinical trials (118). The two principal approaches being developed for vaccine delivery are the use of new T cell-inducing adjuvants with protein subunit vaccines or recombinant replication-deficient viral vectored vaccines (82, 84, 85, 113, 115-117).

Protein-based subunit vaccines can be produced to high levels of purity in bulk, but require adjuvants in order to induce a potent and durable immune response (119). Adjuvants may be antigen delivery systems, such as aluminium-based adjuvants or emulsions, or have immunopotentiating properties, such as TLR ligands, saponins, cytokines and bacterial toxins (119). Only aluminium-based adjuvants are licensed for widespread human use and these induce humoral immunity (119). Other adjuvants which are licensed in vaccines include MF59, an oil-in-water emulsion, and monophospholipid A (MPL) (120). A number of candidate immunostimulant adjuvants which induce cell mediated immunity are in development (119-121). Recombinant viral vectors, such as poxviruses and adenoviruses, are a technology with the capacity for cloning large or multiple immunodominant antigens which can be manufactured to high titres, allowing easy scale up of vaccine production (122). Replication-deficient human adenoviruses release prolonged but self-limited high levels of antigens, induce high frequencies of $CD8^+$ T cells and preferentially target the respiratory epithelium (82, 85, 123-127). Live Vaccinia virus vaccination led to the successful worldwide eradication of smallpox but its use as a recombinant viral vector is limited as it is

infectious to humans. Replication-deficient recombinant Modified Vaccinia virus Ankara (MVA) was safely administered to more than 120,000 vaccinees as part of the smallpox eradication program (128).

The leading immunotherapeutic and prophylactic vaccine candidates currently in clinical trials are summarised in **Figure 1-1** and described below.

Figure 1-1 Candidate prophylactic TB vaccines currently in clinical trials

	Phase I	Phase IIa	Phase IIb	Phase III
	safety and immuno-genicity	safety and immuno-genicity	Proof-of-concept efficacy	Multi-site efficacy
Mw				
RUTI®				
VPM1002				
MTBVAC				
M72+AS01				
H4/AERAS-404+IC31®				
H56/AERAS-456+IC31®				
ID93+GLA-SE				
AdHu5Ag85A				
AERAS-402/Crucell Ad35				
MVA85A				

Whole mycobacterial vaccines

Mw

Mw (Department of Biotechnology, India) is derived from *Mycobacterium indicus pranii* (MIP), a rapid growing, non-pathogenic atypical mycobacteria which enhances Th1 responses. Given alongside standard chemotherapy for leprosy, Mw was associated with enhanced clinical responses, bacteriological clearance and reduced treatment time (129). In sputum smear-positive TB patients, intradermal Mw given every 15 days alongside standard chemotherapy was associated with decreased time to sputum conversion and improved cure rates (130). Further Phase III randomised safety and efficacy clinical trials of Mw alongside standard chemotherapy in new TB patients and TB patients undergoing retreatment have been conducted (trial identifications [IDs] NCT0065226 and NCT00341328) (131). Mw is currently being investigated as an adjunctive treatment for TB pericarditis in an RCT with prednisolone (132).

RUTI®

RUTI® (Archivel Farma) is a non-live polyantigenic vaccine containing detoxified liposomal cellular fragments of *M.tb* bacilli. It is being developed with the principal aim of shortening the course of chemotherapy for treating LTBI (133). RUTI® has been evaluated in a number of small animal models for a range of applications, including as a prophylactic vaccine to replace or boost BCG; as a prophylactic post-exposure vaccine; and as a therapeutic vaccine (134-138). In a double blind placebo-controlled Phase I RCT in BCG-naïve healthy men in Spain, RUTI® was well tolerated and associated with modestly enhanced IFNγ ELISpot responses to PPD and mycobacterial antigens, including ESAT-6 and 85B (139). The next stage is Phase I studies evaluating the RUTI® vaccine in LTBI and future studies may evaluate its potential for treating active TB (140).

VPM1002

Three recombinant BCG vaccines reached early clinical evaluation and were engineered to improve $CD8^+$ T cell induction (ΔureC hly^+ rBCG, Max Planck institute for Infection Biology, Berlin, Germany) or over-express immunodominant antigens (rBCG30, University of California, Los Angeles, USA), or combine both strategies (research strain AFRO-1, AERAS 422, Aeras) (83, 141, 142). Only ΔureC hly^+ rBCG (now named VPM1002) is continuing in clinical development (83). VPM1002 expresses listeriolysin (Hly) derived from *Listeria monocytogenes* and enables BCG to escape from the endosome. This strain has been made urease-C deficient to provide optimal pH for Hly activity (143). As BCG only induces weak apoptosis, so does not cross-prime $CD8^+$ T

cells, the aim of VPM1002 is to enhance $CD8^+$ T cell production by improving presentation of BCG antigens via the MHC I pathway. BALB/c mice were significantly better protected against *M.tb* aerosol challenge by VPM1002 than by the parental BCG strain, Danish 1331, likely related to improved cross-priming (143, 144). Protection was mediated by Th1-type cytokines and IL-17 (145). VPM1002 induced multifunctional, IFNγ-producing T cells in BCG-naïve and BCG-vaccinated adults (83). The safety and immunogenicity of VPM1002 in neonates is now being compared with that of BCG in an RCT in South Africa (trial ID NCT01479972) (131).

Live attenuated *M.tb* strains

MTBVAC (University of Zaragoza, Spain), a *phoP* and *fadD26*–deleted live attenuated *M.tb* strain, is being evaluated in a clinical trial in Switzerland (trial ID NCT02013245) (114, 131). The *phoP* inactivated SO2 strain protected against TB in guinea pigs and NHPs and induced polyfunctional and sustained memory $CD4^+$ T cells (146-149).

Another group is developing mutants of *M.tb* strain H37Rv, in which genes important for replication, persistence and immune evasion have been deleted or modified (150, 151). Promisingly, IKEPLUS (Colorado State University, USA), a mutant strain of *Mycobacterium smegmatis* with deleted esx-3 virulence gene, induced protective bactericidal $CD4^+$ memory T cell responses in mice (152).

Subunit vaccines

M72+ASO1

MPL is a TLR4 agonist which induces potent Th1 immune responses and is present in the GlaxoSmithKline (GSK) Adjuvant Systems (AS) AS01, AS02 and AS04. AS04, MPL combined with alum, is licensed for use with HBV and HPV vaccines (121). AS01, an oil-in-water emulsion, and AS02, a liposomal emulsion, each containing MPL and QS21 (a detergent purified from the bark of *Quillaja saponaria)* have been tested principally with the leading candidate malaria vaccine, RTS,S (120, 153). The polyprotein, Mtb72F, was produced by fusion of two proteins, Mtb32 and Mtb39, which were selected based on their ability to induce IFNγ production by both $CD4^+$ and $CD8^+$ T cells in healthy, PPD TST-positive people (154). Mtb72F, formulated in AS02A, protected NHP against TB and was safe in PPD TST-negative and positive adults (84, 155, 156). A recombinant polyprotein with improved stability, M72, was well tolerated and induced significantly higher vaccine-specific $CD4^+$ T cell responses when formulated in AS01 compared to M72/AS02 or Mtb72F/AS02 (157). M72+AS01 (GSK, Aeras) was well-tolerated and immunogenic in BCG-vaccinated adolescents and adults in South Africa (158). The vaccine is being evaluated in infants in The Gambia; adults with TB disease in Estonia

and Taiwan; and adults with HIV-infection and a Phase IIb efficacy trial is planned (trial IDs NCT01098474, NCT01424501, NCT00707967 and NCT01755598) (131, 159).

Hybrid 1

Hybrid 1 (Statens Serum Institut [SSI], Denmark) is a fusion protein of immunodominant antigens 85B and ESAT-6, which provides protection against TB in mice, guinea pigs and NHP (160-162). Hybrid 1 formulated with IC31® (Valneva) was safe and induced durable immune responses when evaluated in PPD-negative, BCG vaccinated and *M.tb*-infected healthy adults (86, 163). IC31® is a synergistic combination of a single stranded oligodeoxynucelotide (ODN) and an immunopotentiating peptide ($KLKL_5KLK$), which induces potent antigen-specific cellular immunity via the TLR9/MyD88 signalling pathway (164). Hybrid 1 has also been formulated with a novel liposomal adjuvant, cationic adjuvant formulation (CAF01, SSI), giving rise to durable multifunctional Th1 T cell responses and protection against *M.tb* aerosol challenge in mice (165-167). A Phase I clinical trial of Hybrid1/CAF01 in TST-negative, BCG naïve adults was recently completed (trial ID NCT00922363) (131). A concern with incorporating antigen ESAT-6 into a TB vaccine is its use in IGRAs. Three of the 15 subjects vaccinated with Hybrid 1+IC31® developed transiently positive ESAT-6/CFP-10 QuantiFERON-TB Gold diagnostic test results following vaccination (86, 163).

H4/AERAS-404+IC31®

In HyVac4 (SSI), antigen ESAT-6 was replaced with the *M.tb*-specific antigen, TB10.4, to create an 85B-10.4 fusion protein (116). HyVac4+IC31® boosted responses to BCG and enhanced protection to *M.tb* aerosol challenge compared to BCG alone in murine and guinea pig models (168, 169). This vaccine is now designated H4/AERAS-404+IC31® and a series of Phase I clinical trials in BCG naïve and BCG-vaccinated adults in Europe and South Africa have been completed (159). A safety and immunogenicity study in BCG-vaccinated infants in South Africa is underway (trial ID NCT01861730) (131, 159).

H56/AERAS-456+IC31®

Hybrid 56 (H56, SSI) is a multistage subunit vaccine, in which the early expressed antigens 85B and ESAT-6 have been combined with the latency-associated protein Rv2660c, which is expressed throughout infection (113). It is hoped that this vaccine will protect against reactivation in people with LTBI. H56+IC31® vaccination of BCG-primed NHPs contained *M.tb* infection; reduced clinical disease; and protected against anti-TNF antibody-induced LTBI reactivation (170). The first clinical trials of H56/AERAS-456+IC31® are now being conducted in adults with and without LTBI in South Africa (trial IDs NCT01865487 and NCT01967134) (131, 159).

ID93+GLA-SE

ID93+GLA-SE is a fusion protein of four antigens from *M.tb*, formulated with a novel adjuvant, GLA-SE, a synthetic TLR-4 agonist in an oil and water emulsion (115, 171). The vaccine was designed for use in LTBI and active TB disease in the Infectious Disease Research Institute, Seattle. Blood from LTBI subjects was screened for immunodominant T cell antigens in order to select three virulence proteins (Rv2608, Rv3619, Rv3620) and a latency antigen (Rv1813) for inclusion in the fusion protein. ID93+GLA-SE boosted BCG and induced multi-antigen polyfunctional T cell responses in small animal models and NHPs (115). In mouse and NHP models of active TB disease, ID93+GLA-SE immunotherapy as an adjunct to chemotherapy was associated with reduced bacterial burden; reduced lung pathology and reduced treatment duration compared to chemotherapy alone (171). The candidate is now in Phase I safety, tolerability and immunogenicity studies in healthy adults in the USA and South Africa (trial IDs NCT01599897 and NCT01927159) (131, 159).

AdHu5Ag85A

AdHu5Ag85A is human adenovirus 5 (AdHu5) expressing antigen 85A (Ag85A), developed at McMaster University, Canada (127). Boosting BCG with intranasal AdHu5Ag85A in mice and intranasal or intramuscular AdHu5 Ag85A in guinea pigs improved the survival rates following *M.tb* aerosol challenge compared to BCG alone (172, 173). Neutralizing AdHu5 antibodies are frequent, especially in developing countries where BCG is less effective, with significant levels detected in up to 90 % of the sub-Saharan African population and 45 % of the population in the USA (174). There were also concerns that AdHu5-specific T cells may have increased HIV-1 susceptibility in the STEP study of an AdHu5 HIV-1 vaccine (175). However, this hypothesis has not been supported by subsequent studies, which have shown no correlation between the frequency of baseline AdHu5-specific $CD4^+$ T cells and AdHu5 serostatus and no differences in the magnitude of post-vaccination AdHu5-specific $CD4^+$ T cell responses between AdHu5 seropositive and seronegative subjects (176, 177). In a first-in-human clinical trial of AdHu5Ag85A in Canada, the vaccine was well tolerated and immunogenic in both BCG-naïve and BCG-vaccinated subjects, although polyfunctional $CD4^+$ and $CD8^+$ T cell responses were of greater magnitude in BCG-vaccinated subjects (82). Pre-existing anti-AdHu5 immunoglobulin G (IgG) antibodies were identified in most subjects, but antibody titres did not correlate with vaccine-specific T cell responses (82).

AERAS-402/Crucell Ad35

AERAS-402/Crucell Ad35 is a non-replicating Ad35 expressing a fusion protein of mycobacterial antigens 85A, 85B and TB10.4 (178, 179). Ad35 was selected due to low levels of pre-existing immunity and low frequency of neutralizing antibodies in developing countries and HIV-infected subjects; and low cross-reactivity with Ad5 antibodies (123, 180, 181). Intramuscular or intranasal vaccination protected mice against intranasal *M.tb* challenge, mediated by IFNγ-producing $CD4^+$ and $CD8^+$ T cells (178). AERAS-402/Crucell Ad35 boosted BCG vaccine responses in healthy adults in TB endemic and non-endemic areas, inducing robust, polyfunctional $CD4^+$ and $CD8^+$ T cell responses and the highest frequency of $CD8^+$ T cell responses previously reported in a clinical trial of a candidate TB vaccine (85, 182). AERAS-402/Crucell Ad35 is currently being evaluated in HIV-infected adults in South Africa and in a multicentre Phase II RCT in BCG-vaccinated healthy infants in Kenya, Mozambique and South Africa (trial ID NCT01198366) (131, 159, 183).

MVA85A

MVA85A (University of Oxford, UK) is a recombinant poxvirus, MVA, expressing Ag85A. MVA is a highly attenuated strain of Vaccinia virus, produced by more than 570 passages through chicken embryo fibroblast cells, during which multiple, fully characterised deletions accumulated (184). MVA is unable to replicate efficiently in human cell lines and most mammalian cells, but viral replication is blocked at a late stage of virion assembly, so protein synthesis is unimpaired (185). MVA85A boosts antigen-specific IFNγ T cell responses in mice, cattle and NHP (186-188). Protective efficacy of BCG-prime, MVA85A-boost vaccination regimes has been demonstrated in mice, guinea pigs, cattle and NHP (148, 189-191).

Due to the theoretical risk of the Koch reaction (vaccine-induced immunopathology in persons previously exposed to mycobacteria) the first clinical trials of MVA85A were conducted in TST-negative adults in the UK (117). Trials were then conducted in subjects with gradually increasing mycobacterial exposure. In the UK, trials in BCG-vaccinated adults, followed by adults with LTBI were conducted, as well as dose-finding studies (192-194). The first trials of MVA85A in countries with a high incidence of TB (The Gambia and South Africa) were conducted in *M.tb*-uninfected adults, before adults with LTBI were recruited (195-197). Gradual age de-escalation was performed through clinical evaluation of MVA85A in adolescents and children in South Africa, followed by trials in infants in South Africa and The Gambia (198-201). The candidate vaccine, MVA85A has now been safely administered to BCG-vaccinated asymptomatic adults, adolescents, children and infants and to adults infected with HIV and/or *M.tb*. In adults, MVA85A induces high frequencies of antigen-specific IFNγ-producing

polyfunctional $CD4^+$ T cells, including expansion of a memory population and antigen-specific, IFNγ-producing $CD8^+$ T cells have also been detected (92, 202). In a recently completed Phase IIb efficacy trial in infants in South Africa, BCG-prime, MVA85A boost did not offer significantly increased protection against TB or *M.tb* infection compared to BCG vaccination alone (200, 203). MVA85A is currently being evaluated in HIV-infected adults and HIV-exposed infants in South Africa (trial IDs NCT01151189 and NCT01650389); in combination with the carrier protein IMX313 (trial ID NCT01879163); and administered by aerosol (trial IDs NCT01497769 and NCT01954563) (131, 159).

A second subunit vaccine, FP85A, using a recombinant avian poxvirus, FP9, as a carrier for Ag85A, has recently been developed (190). Two early clinical trials evaluating the candidate TB vaccines MVA85A and FP85A in adult subjects in the UK will be described in this thesis.

Conducting Clinical Trials

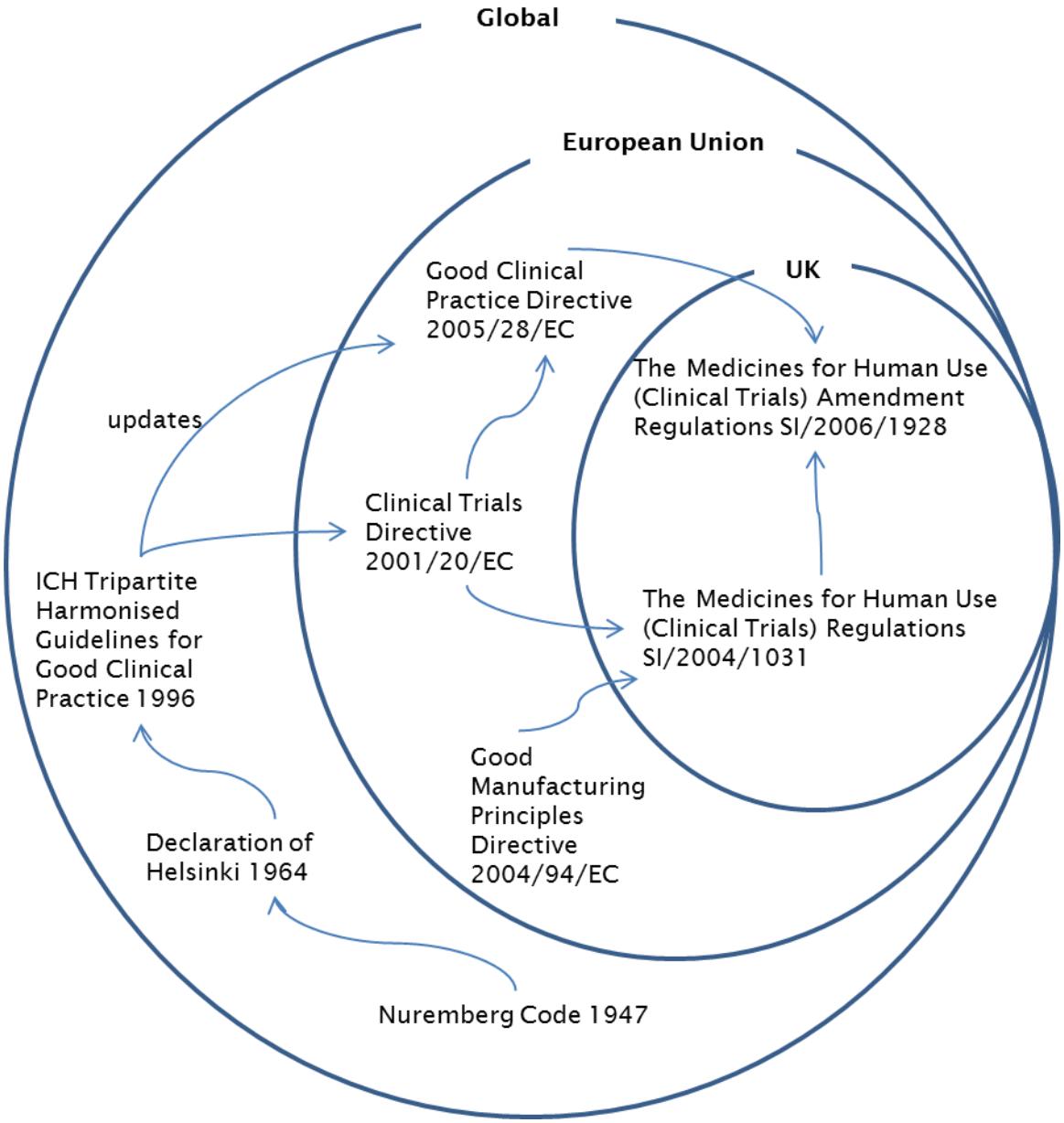
National, European and global ethical and regulatory requirements

Alongside advances in vaccine development, it has been necessary to define ethical principles and implement legislations and regulations governing the conduct of research involving human subjects. Nazi doctors and scientists responsible for conducting biomedical research using prisoners in concentration camps during the Second World War were judged against the ten principles of the Nuremberg code, which included informed consent; beneficence; absence of coercion and correctly designed scientific experiments (204). The Declaration of Helsinki, a statement of the ethical duties of physicians undertaking clinical research by the World Medical Association, was based upon these principles (205). The concepts of detailed research study protocols and oversight by an independent research ethics committee (REC) were introduced in subsequent amendments (205). Disasters, such as with thalidomide in Europe, led to increasing national regulations for new medicinal products prior to marketing, but harmonisation was needed to facilitate international access to new products (206).

Following a WHO conference of regulatory authorities, The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was formed in 1990, bringing together the regulatory authorities and pharmaceutical industry of Europe, Japan and the USA (206). ICH Tri-partite Harmonised Guidelines and recommendations for safety, quality and efficacy are continuously developed and updated. Good Clinical Practice (GCP) is *"an international ethical and scientific quality standard for designing, conducting, recording and*

reporting trials that involve the participation of human subjects" based upon the Declaration of Helsinki (207). The ICH GCP guidelines were implemented firstly into European Directives, which set minimum requirements for member states, and subsequently into UK Law **(Figure 1-2)** (208-212).

Figure 1-2 UK clinical trial regulations and their relationship with European Directives and Global Guidelines



Good Clinical Practice

The first principle of ICH GCP is that *"Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s)"* (207). The ICH GCP principles relating to specific aspects of clinical trials are summarised in **Table 1-1**.

Table 1-1 The principles of ICH GCP

Activity	Summaries of the ICH GCP principles (207)
Planning	A risk-benefit analysis should be conducted, in which the *"rights, safety and well-being*" of trial subjects are protected and prioritised and *"anticipated benefits justify the risks"*; The trial should be supported by sufficient *"non-clinical and clinical information*" on the investigational medicinal product (IMP); The clinical trial design should be *"scientifically sound"* and "*described in a clear, detailed protocol".*
Approval	The clinical trial protocol should be approved by a REC prior to commencement of the trial.
Conduct	Clinical trials should be *" conducted in accordance with the ethical principles…that are consistent with GCP and…regulatory requirements";* "*Freely given informed consent should be obtained from each subject prior to clinical trial participation;* The trial should comply with the approved clinical trial protocol.
Investigators	*"The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician";* *"Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s)".*
Records	*"All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification";* *"The confidentiality of records that could identify subjects should be protected…in accordance with the…regulatory requirements."*
IMPs	The manufacture, handling and storage of IMPs *"should be in accordance with Good Manufacturing Practice* (GMP) *and…the approved protocol*."
Standard Operating Procedures (SOPs)	*"Systems with procedures that assure the quality of every aspect of the trial should be implemented".*

The practicalities of conducting clinical trials in the UK

The clinical trial Sponsor, an "*individual, company, institution or organisation which takes responsibility for the initiation, management and/or financing of a clinical trial",* is usually a pharmaceutical company, or academic institution (209). Investigators conduct the clinical trial, overseen by a Chief Investigator. Sponsors and investigators have defined responsibilities, although a sponsor may allocate or delegate certain roles or responsibilities to individuals or organisations (207, 213). Peer review of the proposed research question is recommended as good practice and is included in the process of funding applications for non-commercial trials (213). National Health Service (NHS) Research and Development (R&D) departments are consulted regarding clinical trials recruiting NHS patients.

A number of trial documents must be prepared and approved by the Sponsor in order to secure indemnity and insurance for the clinical trial. A trial protocol which *"describes the objectives, design, methodology, statistical considerations and organisation of a clinical trial*" and an investigator's brochure (IB), a *"document containing a summary of the clinical and nonclinical data relating to an* IMP *which are relevant to the study of the product in human subjects*" are regulatory requirements (210). The trial protocol is also submitted for ethical approval, along with volunteer information sheets, consent forms and case report forms for data collection. Trial master files and systems for trial management, monitoring and pharmacoviligance are also established. All clinical trials conducted in the European Community, which commenced after 1st May 2004, are recorded in a database called EudraCT and a unique EudraCT number is assigned to each trial prior to applications for clinical trial approvals (214). Honorary research contracts with the NHS organisation are required for clinical research staff without an NHS contract (213).

Integrated Research Application System (IRAS) is a single system which now exists for applying for permissions and approvals (215). Application for ethical approval of the clinical trial is made to a REC. Site specific approval was also obtained, until 2009, from RECs, but this is now the responsibility of NHS R&D offices (213). The Gene Therapy Advisory Committee (GTAC) was the UK national REC for gene therapy products, defined as *"the deliberate introduction of genetic material into human somatic cells for therapeutic, prophylactic or diagnostic purposes".* In 2012, this was replaced by the National Research Ethics Service (NRES) committee, London-West London and GTAC (216). A proposed clinical trial considered *"low genetic risk"* may be transferred by NRES Committee, London-West London and GTAC, to other NRES committees. Each Member State in Europe has a Competent Authority from whom a Clinical Trial Authorisation must be obtained. For clinical trials of IMPs under UK

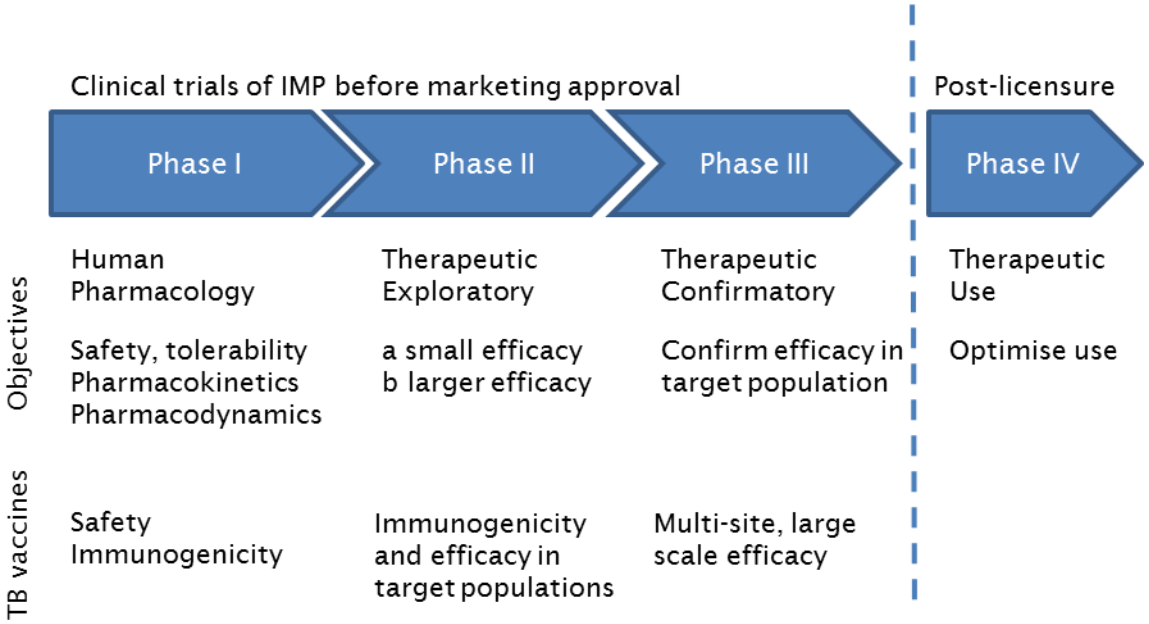
regulations, Clinical Trial Authorisation applications are now completed within the IRAS and submitted to the Medicines and Healthcare Regulatory Authority (MHRA) (217). IMPs must be manufactured to GMP standards by a manufacturer with an IMP manufacturing authorisation. Before release, each product batch must be certified as meeting the requirements in the Clinical Trials Directive by a Qualified Person (209, 213). Preclinical toxicity and bio-distribution studies in animals are required prior to administration of any IMP to human subjects. There are also regulations for labelling of IMPs (213). The use of IMPs which are genetically modified organisms (GMO), such as MVA85A, must comply with the GMO (Contained Use) Regulations 2000 and their subsequent amendments, which are enforced by local Genetically Modified Organism Safety Committees (GMSC) on behalf of the Health and Safety Executive (HSE) (218).

In 2006, a single dose of a CD28 monoclonal antibody called TGN1412, which was administered to asymptomatic adult males in a Phase I clinical trial, caused cytokine storm-induced multi-organ failure in all six subjects (219). Preclinical testing, whilst conducted according to regulatory requirements, had failed to predict the cytokine storm and novel in vitro techniques were developed for future trials of monoclonal antibodies (220). The introduction of an accreditation system for first-in-human clinical trials was recommended by the Expert Scientific Group who reviewed Phase One clinical trials following the TGN1412 incident and consequently introduced by the European Medicines Agency (221). The system effectively ensures that expertise and resources are concentrated in a limited number of different clinical trial centres, with highly experienced study personnel and well established safeguarding systems.

The classification of clinical trials

Clinical trials are often classified into one of four temporal Phases **(Figure 1-2)**. For regulatory purposes, a Phase I trial "*means a clinical trial to study the pharmacology of an IMP when administered to humans, where the sponsor and investigator have no knowledge of any evidence that the product has effects likely to be beneficial to the subjects of the trial"* (210). However, this classification system is not a requirement and has limitations. Whilst there are typical objectives for each Phase, these are not well defined and may differ between IMPs and institutions. Classifying clinical trials according to their objective was recommended in an ICH harmonised tripartite guideline **(Figure 1-3)** (222).

Figure 1-3 Classification systems for clinical trials of IMPs, with candidate TB vaccines as an example (222)



Monitoring vaccine safety

The safety of an investigational vaccine used in a clinical trial is evaluated throughout the clinical trial. Pharmacovigilance refers to the written recording and reporting of adverse events (AEs), which is the responsibility of the sponsor. There are set European Community definitions for the terms used in safety reporting **(Table 1-2)** (209). The Principal Investigator assesses and records the causality and seriousness of each AE as it is reported. An Annual Safety Report is submitted to the REC and the Competent Authority (MHRA for clinical trials conducted in the UK). In addition, the protocol specifies the criteria for which expedited reporting of a serious AE (SAE) is required. The sponsor is notified within 24 hours of any SAE which fits the criteria for expedited reporting. Causality is assessed and recorded. SAEs classified as related to the IMP are defined as serious adverse reactions (SARs) and the expectedness of the SAR is then assessed. A serious unexpected SAR (SUSAR) is reported to the REC and Competent Authority, whilst an expected SAR is recorded and followed up. All SAEs and SARs are followed up until their resolution.

The Brighton Collaboration is a global research network for the promotion of safe immunization (223). Whilst there are legal requirements governing the conduct of generic clinical trials, this network sets research standards which are specific for vaccine research. Standardized case definitions of AEs are available to all members, aiming towards international systematized safety reporting of vaccines in clinical trials (224). A Brighton Collaboration methods working group was formed to develop guidelines for vaccine safety data collection, analysis and presentation (225). For example, AEs should be reported regardless of the time between vaccination and AE onset (225). This network also provides the capacity for linking databases and rapid reporting and dissemination of important safety findings (223).

Table 1-2 Definitions for use in IMP safety surveillance (209)

Term	Definition
AE	"*any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment"*
adverse reaction	*"all untoward and unintended responses to an IMP related to any dose administered"*
SAE/SAR	*"any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect"*
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 unauthorised investigational product or summary of product characteristics for an authorised product)"*

Aims of this thesis

The aims of the research described in this thesis were to investigate various clinical aspects of early clinical trials of candidate TB vaccines.

Two clinical trials of candidate TB vaccines, MVA85A and FP85A, were conducted to evaluate:

1. Safety and immunogenicity of MVA85A vaccine in HIV-infected adults in the UK
2. Safety and immunogenicity of FP85A vaccine in healthy human adults in the UK

Safety data of MVA85A vaccination from a series of Phase I clinical trials were reviewed in order to:

3. Conduct a cumulative analysis of the safety of MVA85A vaccination in healthy adult subjects in the UK

Blood results from healthy human subjects volunteering to participate in clinical trials were analysed to:

4. Evaluate within-person and between-person variability in a healthy human population and analyse the applicability of standard hospital laboratory RIs to the study population
5. Further evaluate the safety of MVA85A vaccination by comparing pre- and post-vaccination results

Chapter 2 : Materials and Methods

Phase I clinical trial of MVA85A vaccination in HIV-infected adults

Trial Design

This was an open label, non-randomised Phase I dose-escalation clinical trial of MVA85A vaccination in HIV-infected adults. The planned sample size was 20 subjects, designed to detect frequently occurring AEs. Subjects were enrolled sequentially into two equally sized groups, for MVA85A vaccine dose escalation in Group two after an interim safety analysis of Group one. The roles and responsibilities of the clinical investigators for the trial are detailed in **Table 2-1**.

Table 2-1 Clinical investigators for Phase I clinical trial of MVA85A vaccination in HIV-infected adults

Chief Investigator	Prof Helen McShane	Lead for trial design, conduct and reporting; staff recruitment and supervision.
Principal Investigator	Dr Guy Rooney	Referral of patient volunteers from the Great Western Hospitals NHS FT.
Principal Investigator	Prof Jonathan Ross	Referral of patient volunteers from the University Hospitals Birmingham NHS FT.
Lead Investigator	Dr Angela Minassian	Trial design; preparation of clinical trial protocol, volunteer information sheet and application forms; submissions for MHRA, GTAC, GMSC and R&D approvals; amendments to inclusion and exclusion criteria; addition of new clinical trial site in London; subject screening, vaccination and follow up appointments; Annual Progress Reports.
Investigator	**Dr Rosalind Rowland (author)**	**Clinical trial amendments; trial maintenance; subject recruitment; screening, vaccination and follow up appointments; maintenance of trial master files; addition of new clinical trial site in Birmingham; electronic safety data capture; Annual Progress Reports; Annual Safety Reports; safety data analysis; statistical analyses; End of Trial Notification; Final Report.**
Investigator	Mr Ian Poulton	Trial maintenance; subject follow up appointments.
Investigator	Dr Matthew Hamill	Subject screening, vaccination and follow up appointments.

Ethics

The initial applications for ethical, regulatory and GMSC approvals and subsequent substantial amendments were submitted to GTAC, Oxfordshire REC (site specific assessment) the GMSC and the MHRA. Applications for NHS R&D approvals were submitted to Oxford Radcliffe Hospitals NHS Trust, Oxford, UK; Great Western Hospitals NHS FT, Swindon, UK; and Imperial College Healthcare NHS Trust, London, UK. Applications for substantial amendments were submitted to GTAC, local RECs, local R&D departments and the MHRA.

The trial was conducted in accordance with the Declaration of Helsinki. Recruitment began after all approvals had been obtained. Written informed consent by subjects and corroborative medical histories from subjects' HIV specialists were obtained before any study procedures were performed.

Annual Progress Reports for the clinical trial and Annual Safety Reports for the candidate vaccine were submitted to GTAC, local RECs, local R&D departments and the MHRA.

On completion of the clinical trial, an End of Trial Notification and Final Report were submitted to GTAC, local RECs, local R&D departments and the MHRA.

Study sites

The main clinical trial site for recruitment, enrolment and follow up of clinical trial subjects was the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Oxford, UK. Additional clinical trial sites for the recruitment and follow up of subjects were Oxford Radcliffe Hospitals NHS Trust, Oxford, UK; Great Western Hospitals NHS FT, Swindon, UK; Imperial College Healthcare NHS Trust, London, UK and University Hospitals Birmingham NHS FT, Birmingham, UK.

Subjects

Subjects were HIV-infected adults, who were otherwise healthy, were not established on antiretroviral treatment (ART) and were not showing signs of HIV disease progression. Subjects were recruited from specialist HIV clinics at the sites listed above. Generic inclusion and exclusion criteria are listed in **Table 2-2**. Inclusion and exclusion criteria specific to this clinical trial are listed in **Table 2-3.**

Table 2-2 Generic inclusion and exclusion criteria for both clinical trials

Inclusion Criteria
Agreement to practice barrier contraception from the start of the study until three months after the final vaccination; For females, a negative pregnancy test on the day of vaccination and agreement to practice effective contraception for the entire duration of the study; Agreement to refrain from blood donation during the course of the study; Written informed consent; Willing to allow the investigators to discuss the volunteer's medical history with their Primary Care Practitioner (healthy subjects) or HIV specialist (HIV-infected subjects).
Exclusion Criteria
Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period; Prior receipt of a recombinant MVA or fowlpox vaccine; Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate; Administration of chronic (defined as more than 14 days) immunosuppressive drugs or other immune modifying drugs within six months of vaccination (for corticosteroids, this will mean prednisolone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed); History of allergic disease or reactions likely to be exacerbated by any component of the vaccine, e.g. egg products; Any history of anaphylaxis in reaction to vaccination; History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ) History of serious psychiatric condition; Any other chronic illness requiring hospital specialist supervision; Suspected or known current injecting drug or alcohol abuse (as defined by an alcohol intake of greater than 42 units every week); Seropositive for hepatitis B surface antigen (HBsAg); Seropositive for HCV antibodies; For females, pregnancy, lactation or willingness/intention to become pregnant during the study; Any other significant disease, disorder or finding, which, in the opinion of the Investigators, may either put the volunteer at risk because of participation in the study, or may influence the result of the study, or the volunteer's ability to participate in the study; Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis.

Table 2-3 Trial-specific inclusion and exclusion criteria for Phase I clinical trial of MVA85A vaccination in HIV-infected adults

Inclusion Criteria
Adults aged 18 to 55 years; HIV antibody positive; diagnosed at least six months previously; $CD4^+$ T cell count greater than 350 cells/μl; nadir $CD4^+$ T cell count not less than 300 cells/μl; HIV viral load not greater than 100,000 copies/ml.
Exclusion Criteria
Any ART within the past six months; Any AIDS defining illness; Chest X Ray showing TB or evidence of other active infection.

Intervention

The candidate TB vaccine MVA85A had previously been constructed (188). In brief, the Ag85A DNA sequence was derived from *M.tb* strain H37Rv. The Ag85A sequence was ligated after the P7.5 promoter in the Vaccinia shuttle vector pSC11. Recombinant viruses were prepared by *in vitro* recombination of the shuttle vector encoding 85A with MVA in primary cultures of chicken embryo fibroblasts (CEFs) and selected by repeated plaque purification in CEF monolayers.

Clinical grade MVA85A vaccine (lot number 010402) was manufactured under GMP conditions by Impfstoffwerk Dessau-Tornau (IDT) Biologika GmbH, Dessau-Rosslau, Germany, for Oxford University. The vaccine was supplied as liquid in glass vials.

Clinical trial interventions were performed at the CCVTM. The vaccinations were administered on the day of enrolment. The vaccine was delivered by intradermal injection into the deltoid area of the arm. The MVA85A vaccine dose for subjects in Group one was $5x10^7$ pfu of MVA85A. The MVA85A vaccine dose for subjects in Group two was $1x10^8$ pfu of MVA85A (administered as two injections, one in each arm).

The minimum interval between vaccination of the first and second subjects in each group was 24 hours.

Trial Procedures

The trial schedule is shown in **Table 2-4.**

Table 2-4 Schedule of procedures for Phase I clinical trial of MVA85A vaccination in HIV-infected adults

	Screening	Enrolment	Follow up							
Attendance number	1	2	3	4	5	6	7	8	9	10
Timeline (weeks+days)		0	0+2	1	2	4	8	12	24	52
Informed consent	✓									
Vital observations	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Medical History	✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Physical Examination	✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Urinalysis	✓									
β-HCG urine test	✓	✓								
Chest X ray	✓									
Inclusion / Exclusion criteria	✓	✓								
Venepuncture:										
HLA typing		✓								
HBV,HCV serology	✓									
$CD4^+$ T cell count, HIV-1 RNA viral load	✓			✓	✓	✓	✓	✓	✓	✓
Haematology[a]	✓				✓			✓		
Biochemistry[b]	✓				✓			✓		
Exploratory Immunology	✓	✓		✓	✓	✓	✓	✓	✓	✓
Vaccination		✓								
Vaccine site inspection		✓	✓	✓	✓	✓	✓	✓	✓	✓
Diary cards provided		✓								
Diary cards collected				✓						
AEs data collection		✓	✓	✓	✓	✓	✓	✓	✓	✓

[a]Full blood count; [b]sodium, potassium, urea, creatinine, albumin, bilirubin, alanine aminotransferase (ALT), alkaline phosphatase (ALP)

Monitoring

An external monitor (Appledown Clinical Research Limited, Buckinghamshire, UK) conducted regular independent monitoring prior to; during; and following completion of each trial.

Phase I clinical trial of new candidate vaccine, FP85A, alone and in prime-boost regimes with MVA85A

Trial Design

This was an open label, non-randomised, Phase I safety and immunogenicity clinical trial of FP85A vaccination in healthy, previously BCG-vaccinated, adult subjects. Subjects were enrolled sequentially into three groups, each corresponding to a different vaccine regime. The planned sample size was 36 subjects, designed to detect frequently occurring AEs. The roles and responsibilities of the clinical investigators for the trial are detailed in **Table 2-5**.

Table 2-5 Clinical investigators for Phase I clinical trial of FP85A and MVA85A vaccination in healthy adults

Chief Investigator	Prof Helen McShane	Lead for trial design, conduct and reporting; staff recruitment and supervision.
Lead investigator	**Dr Rosalind Rowland (author)**	**Trial design; preparation of clinical trial protocol; volunteer information sheet; investigator's brochure, IMP dossier (IMPD) and application forms; submissions for MHRA, GTAC, GMSC and R&D approvals; clinical trial amendments; trial maintenance; subject recruitment; screening, vaccination and follow up appointments; maintenance of trial master files; data analysis; Annual Progress Reports; Annual Safety Reports; End of Trial Notification; Final Report.**
Investigator	Mr Ian Poulton	Trial maintenance; subject follow up appointments.
Investigator	Mrs Cynthia Bateman,	Subject follow up appointments.

Ethics

The initial applications for ethical, regulatory and GMSC approvals and subsequent substantial amendments were submitted to GTAC, Oxfordshire REC (site specific assessment), the GMSC and the MHRA respectively.

The trial was conducted in accordance with the Declaration of Helsinki. Recruitment began after all approvals had been obtained. Written informed consent by subjects and corroborative medical histories from subjects' primary care practitioners were obtained before any study procedures were performed.

Annual Progress Reports for the clinical trial and Annual Safety Reports for the candidate vaccine were submitted to GTAC, local RECs, local R&D departments and the MHRA.

On completion of the clinical trial, an End of Trial Notification and Final Report were submitted to GTAC, local RECs, local R&D departments and the MHRA.

Study site

The clinical trial site for recruitment, enrolment and follow up of clinical trial subjects was the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Oxford, UK.

Subjects

Subjects were healthy, BCG-vaccinated adults, who were recruited from the Oxford area by the use of poster campaigns and information stands. Laura Dinsmore assisted with subject recruitment. Generic inclusion and exclusion criteria are listed in **Table 2-2**. Inclusion and exclusion criteria specific to this clinical trial are listed in **Table 2-6.**

Table 2-6 Inclusion and exclusion criteria for Phase I clinical trial of FP85A and MVA85A vaccinations in healthy adults

Inclusion Criteria
Healthy adult aged 18 to 50 years; Resident in or near Oxford for the duration of the vaccination study; Immunization with BCG greater than 12 months prior to enrolment in the study.
Exclusion Criteria
Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections; Close contact with fowl during the study period (e.g. chicken farming); Mantoux skin test equal to or greater than 15 ml; Screening ELISpot positive in any ESAT-6 peptide or CFP-10 peptide pool.

Interventions

The candidate TB vaccine, FP85A, had previously been constructed as described (226). In brief, the Ag85A DNA sequence was derived from *M.tb* strain H37Rv. The Ag85A sequence was ligated after the P7.5 promoter in the Fowlpox shuttle vector pEFL29. Recombinant viruses were prepared by *in vitro* recombination of the shuttle vector encoding 85A with FP9 in primary cultures of chicken embryo fibroblasts (CEFs) and selected by repeated plaque purification in CEF monolayers.

Clinical grade FP85A vaccine (lot number 010604) was manufactured under GMP conditions by Impfstoffwerk Dessau-Tornau (IDT) Biologika GmbH, Dessau-Rosslau, Germany, for Oxford University. The vaccine was supplied as liquid in glass vials.

The candidate TB vaccine, MVA85A, has been described above.

Clinical trial interventions were performed at the CCVTM. The vaccines were delivered by intradermal injection into the deltoid area of the arm. The dose for all vaccinations was $5x10^7$ pfu. The first vaccinations were administered on the day of enrolment and the second vaccinations after an interval of four weeks.

Subjects in Group one were immunised with FP85A vaccine on the day of enrolment (regime BF). Subjects in Group two were immunised with MVA85A vaccine on the day of enrolment and FP85A vaccine at week four (regime BMF). Subjects in Group three were immunised with FP85A vaccine on the day of enrolment and MVA85A vaccine at week four (regime BFM). The minimum interval between vaccination of the first and second subjects was 24 hours.

Trial Procedures

The trial schedule for Group one is shown in **Table 2-7** and for Groups two and three in **Table 2-8.**

Table 2-7 Schedule of procedures for Phase I clinical trial of FP85A and MVA85A vaccinations in healthy adults (Group one)

	Screening		Enrolment	Follow up						
Attendance number	1	2	3	4	5	6	7	8	9	10
Timeline (weeks+days)			0	0+2	1	4	8	12	24	52
Informed consent	✓									
Vital observations	✓		✓	✓	✓	✓	✓	✓	✓	✓
Medical History	✓		(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Physical Examination	✓		(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Urinalysis	✓									
β-HCG urine test	✓		✓							
Mantoux test	✓									
Mantoux result		✓								
Inclusion / Exclusion criteria	✓		✓							
Venepuncture:										
HLA typing			✓							
HBV,HCV,HIV serology	✓									
Haematology[a]	✓				✓			✓		
Biochemistry[b]	✓				✓			✓		
Exploratory Immunology	✓		✓	✓	✓	✓	✓	✓	✓	✓
Vaccination			✓							
Vaccine site inspection			✓	✓	✓	✓	✓	✓	✓	✓
Diary cards provided			✓							
Diary cards collected				✓						
AEs data collection			✓	✓	✓	✓	✓	✓	✓	✓

[a]Full blood count; [b]sodium, potassium, urea, creatinine, albumin, bilirubin, ALT, ALP

Table 2-8 Schedule of procedures for Phase I clinical trial of FP85A and MVA85A vaccinations in healthy adults (Group two)

	Screening		**Enrolment**	**Follow up**								
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12
Timeline (weeks+days)			0	0+2	1	4	4+2	5	8	12	24	52
Informed consent	✓											
Vital observations	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Medical History	✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Physical Examination	✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
Urinalysis	✓											
β-HCG urine test	✓		✓			✓						
Inclusion / Exclusion criteria	✓		✓			✓						
Venepuncture:												
HLA typing			✓									
HBV,HCV,HIV serology	✓											
Haematology[a]	✓				✓	✓		✓		✓		
Biochemistry[b]	✓				✓	✓		✓		✓		
Exploratory Immunology	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Vaccination			✓			✓						
Vaccine site inspection			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diary cards provided			✓			✓						
Diary cards collected					✓			✓				
AEs data collection			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

[a]Full blood count; [b]sodium, potassium, urea, creatinine, albumin, bilirubin, ALT, ALP

Monitoring

An external monitor (Appledown Clinical Research Limited, Buckinghamshire, UK) conducted regular independent monitoring prior to; during; and following completion of each trial.

Clinical Trial Outcome measures

Evaluation of vaccine safety

The safety profiles of the interventions were evaluated by active and passive AE collection for the duration of follow up, using paper diary cards, completed by subjects, and paper case report forms, completed by investigators **(Table 2-9)**. Documented fever was defined as body temperature greater than 38.0 ˚C. Diameters of erythema and induration were measured in millimetres (mm). The definition of an SAE was an AE which was life threatening, caused persistent or significant disability or incapacity, or resulted in admission to hospital (excluding day case procedures). AEs were assigned a severity score according to standardised criteria **(Table 2-10)**. Standard criteria were also used for determining AE causality **Table 2-11.**

AE analysis was largely descriptive, evaluating the frequency, severity and causality of AEs after each vaccine. Safety data from paper records (diary cards and case report forms) were entered onto an electronic spreadsheet (Microsoft Excel). One hundred per cent cross-checking of source documents was conducted. Statistical analyses were conducted using Stata Statistical Software, Release 9.0, 2005. Continuous variables were summarised using median and interquartile ranges (IQR). Continuous variables between groups (diameters of local reactions) were compared using Mann Whitney U test (MWU) and 95 % confidence intervals (CIs) were computed. Paired samples (diameters of local reactions after sequential vaccinations) were compared using Wilcoxon signed rank tests (WSR). Paired analyses (WSR) of pre- and post-vaccination $CD4^+$ T cell counts and HIV-1 RNA viral loads were also conducted. A p value less than 0.05 was considered statistically significant.

Table 2-9 Solicited AEs

	Diary card	Case report form
Local AEs	Diameter of erythema	Diameter of erythema
	Diameter of induration	Diameter of induration
	Vaccine-site scaling	Vaccine-site scaling
	Vaccine-site pruritus	Vaccine-site pruritus
	Vaccine-site tenderness	Vaccine-site tenderness
	Vaccine-site warmth	
Systemic AEs	Body temperature	Recent illnesses
	'Felt feverish' (feverish symptoms)	Concomitant medications
	'Joint pains' (arthralgia)	Body temperature
	'Generalised muscle ache' (myalgia)	Pulse rate
	'Felt unwell' (malaise)	Blood Pressure
	'Felt more tired than usual' (fatigue)	Haematology/biochemistry blood tests
	Headache	$CD4^+$ T cell/HIV-1 RNA viral load analyses (HIV-infected subjects only)
	'Felt nauseous or vomited'	

Table 2-10 Criteria for AE severity classification

Scale	Description	Definition	Diameter of erythema	Diameter of induration
0		Absence of the indicated symptom	0	0
1	Mild	Awareness of a symptom but the symptom is easily tolerated	<50 mm	<25 mm
2	Moderate	Discomfort enough to cause interference with usual activity	50-99 mm	25-49 mm
3	Severe	Incapacitating; unable to perform usual activities; requires absenteeism or bed rest	>=100 mm	>=50 mm
4	Serious	Life-threatening		

Table 2-11 Criteria for determining AE causality

Relationship	Definition
No Relationship	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
Possible	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
Probable	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
Definite	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

Evaluation of vaccine immunogenicity

Lithium-heparinised blood (55 millilitres [ml]) and serum samples (5 ml) were transported 0.5 kilometres (km) to the Jenner Laboratories, Old Road Research Campus Building (ORCRB), Oxford, UK for analysis.

Exploratory immunology analyses described in this thesis were conducted by Dr Ansar Pathan, Dr Iman Satti and Stephanie Harris, according to established protocols (193). The principal method for evaluating the effect of vaccination on cell mediated immune responses to Ag85A was by *ex vivo* IFNγ ELISpot assay. ELISpot plates (MAIP S4510 Millipore) were coated with 15 micrograms (ug)/ml of anti-IFN antibody (Mabtech, Sweden) over night, washed with phosphate buffered solution (PBS) and later blocked with R10 (RPMI media plus 10 % fetal calf serum [FCS], 2 millimolar [mM] glutamine, 100 units [U]/ml penicillin, 100 µg/ml streptomycin sulfate, and 1 mM sodium pyruvate). PBMCs were isolated from fresh lithium heparinized blood using a direct Ficoll gradient method. PBMC (300,000 cells/well in 100 µl R10) and antigens were then plated in duplicate wells. A single pool of all 66 Ag85A peptides (final concentration of each peptide 2 µg/ml) was used. In addition seven pools, each containing nine to ten 15mer Ag85A peptides overlapping by ten amino acids (final concentration of each peptide 10 µg/ml) were used. Other antigens used were recombinant 85A (r85A, Lionex, Germany, 10 ug/ml) and purified protein derivative (PPD, 20 ug/ml). For detection of LTBI at screening, wells were plated with three pools of five to six peptides of ESAT-6 (Peptide Protein Research, Hampshire, UK) and three pools of six peptides of CFP-10 (Peptide Protein Research) (final concentration of each peptide 10ug/ml). Negative controls for each plate were unstimulated PBMC. Positive controls for each plate were streptokinase (250 U/ml) / streptodornase (12.5 U/ml) and phytohemagglutinin (PHA, 10 µg/ml). The plates were incubated for 18-20 hours at 37 ˚C in a 5 % CO_2 incubator, developed and read on an ELISpot plate reader (Autoimmun Diagnostika GmbH, Germany). The mean numbers of spot forming cells (SFC) of the negative control wells were subtracted from the mean SFC of the antigen-stimulated wells. The threshold for a positive well was defined as double and at least five SFC greater than the negative control wells. The maximum quantifiable SFC/well was 500. The results of the seven pools of overlapping Ag85A peptides were summed. Cryopreserved PBMC were stored for use in subsequent further exploratory immunology analyses.

Statistical analyses were conducted by Dr Rosalind Rowland using Stata Statistical Software, Release 9.0, 2005. Continuous variables were summarised using median and IQR. Paired analyses (WSR) were used to compare the peak (one week post-vaccination) or plateau (52 weeks post-vaccination) responses with pre-vaccination (baseline)

responses. The overall magnitudes of vaccine-induced IFNγ ELISpot responses were summarised using the area under the curve (AUC). Between-group comparisons (MWU) of the AUC of IFNγ ELISpot responses were conducted. The Bonferroni method was used for correction of multiple comparisons. A p value less than 0.05 was considered statistically significant. Correlations between pre-vaccination $CD4^+$ T cell count or HIV-1 RNA viral load and post-vaccination IFNγ ELISpot responses were evaluated using the Spearman test for non-parametric data.

Review of the safety of MVA85A vaccination in healthy adults in the UK

This was a retrospective review of the cumulative safety data of MVA85A vaccination in healthy adults in the UK. Safety data from six completed open label; single arm; non-placebo-controlled safety clinical trials of MVA85A vaccination in healthy adults in the UK were analysed, including the clinical trial described in **Chapter 4** (117, 192-194, 227).

An electronic spreadsheet (Microsoft Excel) for capturing AEs was designed for retrospective analysis and for prospective use in future clinical trials. Dr Nathaniel Brittain assisted with retrospective electronic data entry from paper case report forms. At least 10 % source checking was conducted.

Data were grouped according to pre-enrolment BCG vaccination status; pre-enrolment LTBI status; MVA85A vaccination regime; and MVA85A vaccine dose. The frequency and severity of local reactions and systemic AEs within each group were described. A paired analysis (WSR) of the peak diameters of local reactions after first and second vaccinations with MVA85A was conducted. The peak diameters of local reactions after MVA85A vaccinations were compared between middle dose and upper dose groups.

Data for all subjects who had received the middle dose of MVA85A vaccine were combined to provide a summary profile of AEs after middle dose MVA85A vaccination. AE frequency and severity; day of onset; and duration were summarised. All unsolicited AEs were listed.

The reactogenicity of two licensed vaccines, BCG and a Yellow Fever vaccine, were reviewed in order to provide comparative data for MVA85A. An electronic database (medline) was searched for systematic reviews, meta-analyses and prospective clinical trials recruiting healthy adult subjects in Europe or North America. The following search terms were entered into medline: “BCG vaccine AND humans AND tuberculosis” (limits: clinical trial); “BCG” (limits: meta-analysis); “BCG” (limits: review); “Yellow fever

vaccine AND humans" (limits: clinical trial); "Yellow fever vaccine AND systematic review" and "Yellow fever vaccine" (limits: humans, meta-analysis).

Correlations between peak diameters of erythema or maximum recorded body temperatures and post-vaccination IFNγ ELISpot responses were evaluated using the Spearman test for non-parametric data.

A retrospective study to determine the validity of hospital laboratory reference intervals for healthy young adults participating in early clinical trials of candidate vaccines

A retrospective analysis of screening attendances, screening outcomes and laboratory results included all clinical trials recruiting healthy adult subjects at the Jenner Institute, Oxford, UK between 1999 and 2009. The Principal Investigators who had conducted the clinical trials were Professor Adrian Hill (malaria and influenza vaccine clinical trials), Professor Helen McShane (TB vaccine clinical trials) and Professor Paul Klenerman and Dr Eleanor Barnes (Hepatitis C vaccine clinical trials). Professor Sarah Gilbert was the lead researcher for influenza vaccine clinical trials.

An electronic spreadsheet (Microsoft Excel) for capture of data from paper case report forms was designed. Dr Geraldine O'Hara, Dr Matthew Hamill, Laura Dinsmore and Hannah Donaldson assisted with electronic data entry. Accuracy of electronic data entry was verified by double data entry and crosschecking against paper records.

Most blood samples had been obtained between 07:30 am and 11:00 am and were non-fasting. Biochemistry samples were taken in lithium heparin and haematology samples in potassium ethylenediaminetetraacetic acid (EDTA) using the Beckton Dickinson (BD, Oxford, UK) vacutainer collection system. Laboratory specimens were collected within two hours of phlebotomy and transported two miles to the Oxford Radcliffe Hospital NHS Trust Laboratories for same day analysis. Each blood tube was labelled with a unique study-specific ID number and laboratory reports were generated for these study IDs.

All samples were analysed by Oxford Radcliffe Hospital NHS Trust Laboratories staff. All methods and instrumentation used were commonly used standardised methods. Albumin (bromocresol green method), ALP (DEA buffered enzymatic method), ALT (enzymatic method), bilirubin (vanadate oxidation method), creatinine (Jaffe method), sodium and potassium (both ion selective electrode methods) and urea (urease method) were undertaken on three different clinical chemistry analysers over the period studied. Samples analysed before February 2000 utilised the Bayer Axon

analyser (Bayer Diagnostics, Basingstoke, UK); samples from March 2000 and March 2006 were analysed on an Abbott Aeroset (Abbott diagnostics, Maidenhead, UK); and samples obtained from April 2006 to December 2009 were analysed on a Siemens ADVIA 2400 (Siemens Diagnostics, Frimley, UK). Institutional RIs remained consistent despite these two changes of instrument. Haematological parameters were analysed on the Sysmex XE-2100 haematology analyser. The longitudinal stability of all assays was controlled through internal quality control and external quality assurance participation.

Electronic results data were retrieved by Professor Brian Shine. Expert advice regarding methodology and interpretation was provided by Professor Brian Shine and Mr Timothy James. Dr Geraldine O'Hara, Professor Paul Klenerman and Dr Eleanor Barnes assisted with the development of clinical recommendations.

Laboratory data were excluded pre-analysis if medical assessment had revealed significant past medical or psychiatric history; illicit drug use; excessive alcohol intake or if infection with a blood borne virus was detected at screening.

Between-person analyses

Results for each analyte were filtered to include one pre-intervention result per subject and were sub-grouped by gender. The distributions of the data were visually reviewed using histograms, box plots, normal probability plots (for evaluating central normality) and normal quantile plots (for identifying deviations from normality at the tails of the data). Data were assessed for normality using the Kolmogorov-Smirnov test against a theoretical normal distribution and the Shapiro Wilk test for skewness and kurtosis. Differences in the distribution of data between instrument subgroups were compared using Kolmogorov-Smirnov. The clinical significance of any statistically significant differences was assessed by visual data review using histograms.

Subjects over 40 years of age were excluded and subjects 40 years and under were partitioned into age tertiles. Differences between age tertiles were evaluated by Kruskal Wallis one way analysis of variance by ranks (KW). Where differences were detected, a non-parametric test for trend across ordered groups was performed. Where no differences between age tertiles were detected, 95 % ranges for combined data from subjects of all ages 40 years and under were calculated.

The 95 % range for each analyte was calculated as follows using a previously described method (228-231). Normality of data within each subgroup was assessed graphically by normal probability plots and normal quantile plots. The Shapiro-Wilk test for skewness and kurtosis and the Kolmogorov-Smirnov test against an empirical normal distribution were performed. Where data were not normally distributed, Box-Cox

transformations within each group or subgroup were performed. The effect of normalizing transformation was assessed graphically and statistically as above. The upper (Q1) and lower (Q3) quartiles and IQR (Q1-Q3) of the transformed data were calculated. Data points lying outside the range (Q1 – 1.5 X IQR) to (Q3 + 1.5 X IQR) were identified as outliers and excluded (229). Non-parametric 95 % ranges were derived by obtaining the 2.5^{th} and 97.5^{th} percentiles of the raw data after exclusion of outliers. Parametric 95 % ranges were derived by calculating mean ± 1.96 X SDs of the raw data after exclusion of outliers.

The seasonality of potassium results were evaluated by comparing median monthly potassium results against average monthly temperatures. Potassium results from subjects aged 40 years or under were analysed. Data were grouped by the month the sample was obtained. Monthly average temperatures in Oxford over a 30 year period from 1971-2001 were obtained from the Meterological Office (232). The association between median monthly potassium and mean monthly temperature was evaluated by Spearman's rho correlation coefficient.

Analysis of within-person variation

The analysis of within-person variation included all clinical trial subjects 40 years and under for whom at least two sequential results per analyte were available. If a subject had participated in more than one clinical trial, samples were taken from the first clinical trial in which they had participated. The first (r1) and last (r2) retrieved results for each subject were included in the analysis. This minimised any effect of intervention on the analysis, since the first samples for each subject were pre-intervention and the last samples were taken at least 12 weeks post-intervention. The within-person coefficient of variation (CV) was calculated using a previously described method (233). The square of the CV (CV^2) for each subject was calculated by [*(within subject variance)/ (mean)*^{2}], where within subject variance was [$(r1-r2)^2 / 2$] and mean result was *[(r1+r2)/2*]. The within-person CV for the sample was obtained by calculating the root mean square; [$(\sqrt{(mean\ CV^2)})*100$]. The standard error was calculated by [*(SD of* CV^2*s)/ (*$\sqrt{}$*sample size)*].

Paired pre- and post-intervention results were analysed for the candidate TB vaccine MVA85A (trial ID of an unpublished trial: NCT00548444) (117, 192-194, 227). The latest pre-intervention and earliest post-intervention results available were included in the analysis. Results were excluded if samples were obtained greater than one month (31 days) post-intervention, since vaccine-related AEs and cellular immune responses peak one to two weeks post-vaccination (117, 234-236).

Chapter 3 : Phase I clinical trial of MVA85A vaccination in HIV-infected adults

Introduction

Of the 8.6 million people who developed TB in 2012, 1.1 million (13 %) were infected with HIV and 75 % of TB cases in HIV-infected individuals were in Africa (24). The median incidence rate ratio (probability of developing TB in people infected with HIV) was 14 (IQR 12-20) (24). The WHO recommendations for collaborative TB/HIV activities, introduced in 2004, have saved lives and are continuing to be scaled up (237). Whilst ART reduces the risk of HIV-associated TB, the risk of TB is still higher in HIV-infected compared to uninfected individuals and, to be effective, ART must be commenced whilst $CD4^+$ T cell counts are relatively high (238). However, prophylactic vaccination against TB in HIV-infected individuals cannot be implemented due to the risk of disseminated BCG disease associated with BCG vaccination in people infected with HIV and in infants at risk of HIV (20, 21, 239). There are logistical problems inherent in testing for HIV before vaccinating against TB and maternal antibodies prevent meaningful HIV testing in babies born to mothers infected with HIV (240). It is essential that a new TB vaccine can be safely administered to people infected with HIV. The availability of such a vaccine would enable inclusion of prophylactic vaccination against TB as a collaborative TB/HIV activity.

Live vaccines or vectors are usually contraindicated in the immunosuppressed. MVA, although live, is unable to replicate in mammalian cells and is avirulent in animal models, including immunosuppressed NHP (185, 241). Recombinant MVA vaccines have been administered to people infected with HIV on ART in a number of clinical trials (242-247). In addition, seven individuals infected with HIV but not on ART were safely immunised with an MVA vaccine (248). There were no safety concerns when MVA85A was evaluated in healthy adult subjects at doses of $1x10^7$ pfu, $5x10^7$ pfu and $1x10^8$ pfu (117, 192).

In the study presented here, the safety of MVA85A vaccination in adult subjects infected with HIV was evaluated. A Phase I clinical trial of MVA85A vaccination was conducted. Since BCG-vaccinated healthy adults and adults with LTBI have been safely vaccinated with MVA85A, BCG vaccination and LTBI were not exclusion criteria for this trial (117, 193).

Aims

The primary objective was to evaluate the safety of a single immunisation of candidate TB vaccine, MVA85A, in HIV-infected adults by analysing:

1. The frequency and severity of local reactions;
2. The frequency and severity of systemic AEs;
3. The effect of vaccination on subjects' $CD4^+$ T cell counts and HIV-1 RNA viral loads.

The secondary objective was to evaluate the immunogenicity of MVA85A vaccination in HIV-infected adults by analysing *ex vivo* IFNγ ELISpot responses to Ag85A single peptide pool; summed Ag85A peptide pools; recombinant Ag85A; and PPD and comparing:

1. Baseline (pre-vaccination) responses with peak (week one) and plateau (week 52) responses;
2. The AUCs of responses to Ag85A single peptide pool in Group one (dose $5x10^7$ pfu) with those in Group two (dose $1x10^8$ pfu);
3. The AUCs of summed responses to Ag85A peptide pools in HIV-infected subjects in this trial with those in HIV-uninfected subjects in previous trials.

Results

Approvals prior to commencement of the clinical trial

Ethical approval was obtained from GTAC; approval for contained use of a GMO was obtained from the Oxford Radcliffe Hospital GMSC; and regulatory approval was obtained from the MHRA. Site-specific approvals and R&D approvals were obtained for Oxford Radcliffe Hospitals NHS Trust, Oxford, UK and Great Western Hospitals NHS FT, Swindon, UK.

Substantial amendments

The following substantial amendments to the protocol were approved by GTAC, MHRA, local RECs and local R&D departments after commencement of the clinical trial.

1. The exclusion criterion "Any current or previous ART" was amended to "Any ART within the last six months". The rationale was to avoid excluding females who may have received ART during pregnancy.
2. A third site for subject recruitment at Imperial College Healthcare NHS Trust, London, UK, was added in order to increase the size of population from which to recruit.
3. Two inclusion criteria were amended in order to improve recruitment rates:

   a. Prior BCG vaccination was removed as an inclusion criteria;
   b. The maximum age for inclusion was increased from 50 to 55 years.
4. Following interim safety and immunogenicity analyses of the middle dose of MVA85A vaccine, $5x10^7$ pfu, the protocol was amended to split the planned twenty subjects into two groups of ten. In Group two, the MVA85A vaccine dose was escalated to $1x10^8$ pfu.
5. A fourth site for subject recruitment at University Hospitals Birmingham NHS FT, Birmingham, UK was added, following cessation in recruitment from the sites in Oxford, Swindon and London.

Monitoring

The trial master files were monitored prior to commencement of the trial. Regular monitoring visits were conducted during the course of the trial, including clinical trial sites, source documents and subject case report forms, as well as ongoing trial master file management. A close out monitoring visit was conducted following completion of the clinical trial.

Reports

Annual Progress Reports were submitted to GTAC. In addition, Annual Safety Reports for the IMP, MVA85A, were submitted to GTAC and the MHRA. On completion of the clinical trial, an End of Trial Notification and subsequently a Final Report were submitted to GTAC and the MHRA.

Subject recruitment

Thirty five subjects from four clinical trial sites in Oxford, Swindon, London and Birmingham were screened. Eleven subjects did not meet the clinical trial inclusion criteria and four declined to participate **(Figure 3-1)**. Twenty subjects from three clinical trial sites in Oxford, London and Birmingham were included in the clinical trial. The first ten subjects enrolled were allocated to Group one and the final ten subjects screened were allocated to Group two. All enrolled subjects were followed up for one year following enrolment and were included in the final analysis.

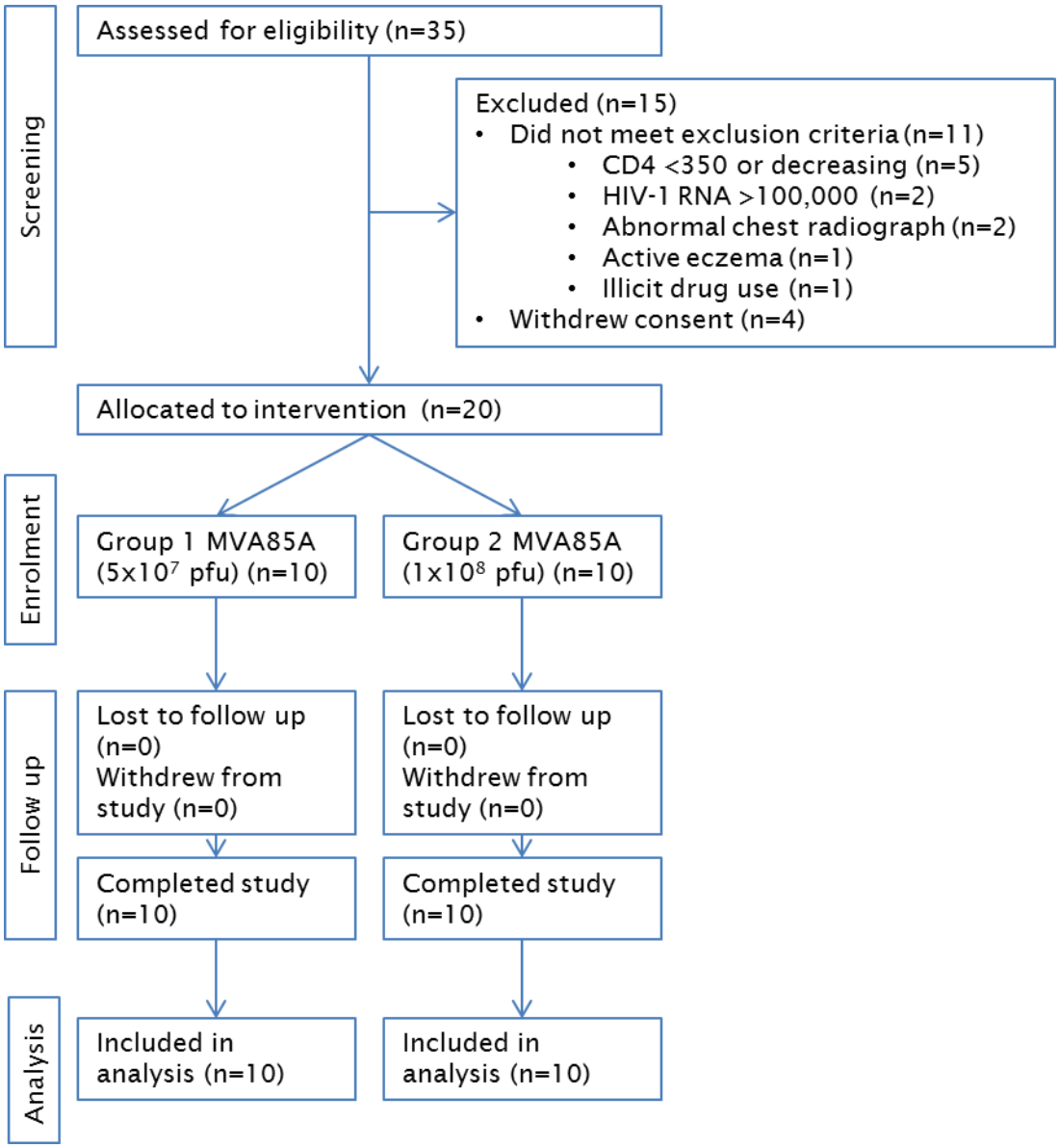
Protocol deviations

Following enrolment of subject 012, a pre-vaccination $CD4^+$ T cell count of 280 cells/μl was noted in one location in the subjects' paper records. This result was below the nadir $CD4^+$ T cell count specified for inclusion, but was not on the hospital database and was not recorded in the hospital records reviewed at the time of screening. The result was also out of keeping with all other results for the subject and was thought, by

the subjects' consultant physician responsible to be spurious. However, this was recorded as a protocol deviation and the standard pre-enrolment letter was amended in order to increase clarity when verifying nadir $CD4^+$ T cell counts at screening.

The final 12 month follow up appointment of subject 008 was outside the specified window as the subject made an unplanned trip outside the UK for some months. Contact was maintained with the subject and the final appointment was conducted on the subjects' return 14 months after enrolment.

Figure 3-1 Flow of subjects



Baseline characteristics of enrolled subjects

The baseline characteristics of enrolled subjects are summarised in **Table 3-1.** The median age of enrolled subjects was 35 years and 85 % of enrolled subjects were males. Subjects in Group one were recruited from Oxford, London and Birmingham, but all subjects in Group two were recruited from Birmingham. Forty five per cent of enrolled subjects were born in Europe and 40 % were born in Africa. Prior BCG vaccination in childhood was confirmed by a clear history or a visible scar in 85 % of enrolled subjects. T cell responses to *M.tb*-specific antigens (CFP-10 or ESAT-6) were detected in subjects 001 and 003 in Group one and subjects 020, 025 and 035 in Group two. Baseline IFNγ ELISpot responses to PPD; $CD4^+$ T cell counts and HIV-1 RNA viral loads were similar between groups. Pre-vaccination within person variability in $CD4^+$ T cell count was 10 % in Group one and 19 % in Group two. Pre-vaccination within person variability in HIV-1 RNA viral load was 47 % in both groups. Subject 003, who was enrolled into Group one, had a persistently undetectable viral load and well preserved $CD4^+$ T cell count.

Table 3-1 Baseline characteristics

		Group 1	Group 2
MVA85A vaccine dose		$5x10^7$ pfu	$1x10^8$ pfu
Number of subjects		10	10
Age, median (IQR)		35 (33 to 46)	35 (30 to 39)
Males		9 (90 %)	8 (80 %)
Continent of birth:	Africa	4 (40 %)	4 (40 %)
	Asia	0	1 (10 %)
	Europe	4 (40 %)	5 (50 %)
	N. America	1 (10 %)	0
	S. America	1 (10 %)	0
Prior BCG vaccination:	definite	7 (70 %)	10 (100 %)
	uncertain	3 (30 %)	
BCG vaccination age, median (IQR)[a]		0 (0 to 12)	10 (0 to 13)
Years since BCG vaccination, median (IQR)[a]		34 (19 to 49)	25 (24 to 33)
LTBI indicated by IFNγ ELISpot responses to CFP-10 / ESAT-6		2 (20 %)	3 (30 %)
Baseline IFNγ ELISpot responses to PPD, median SFC/million PBMC (IQR)		18 (0 to 90)	18 (3 to 27)
Baseline $CD4^+$ T cell count, median cells/μl (IQR)		570 (530 to 770)	625 (410 to 650)
Baseline CD4 %, median (IQR)		30 (24 to 34)	27 (25 to 30)
Baseline HIV-1 RNA viral load, median copies/ml (IQR)		6069 (450 to 22677)	14805 (3138 to 44089)
Recruitment site:	Oxford	5 (50 %)	0
	Swindon	0	0
	London	4 (40 %)	0
	Birmingham	1 (10 %)	10 (100 %)

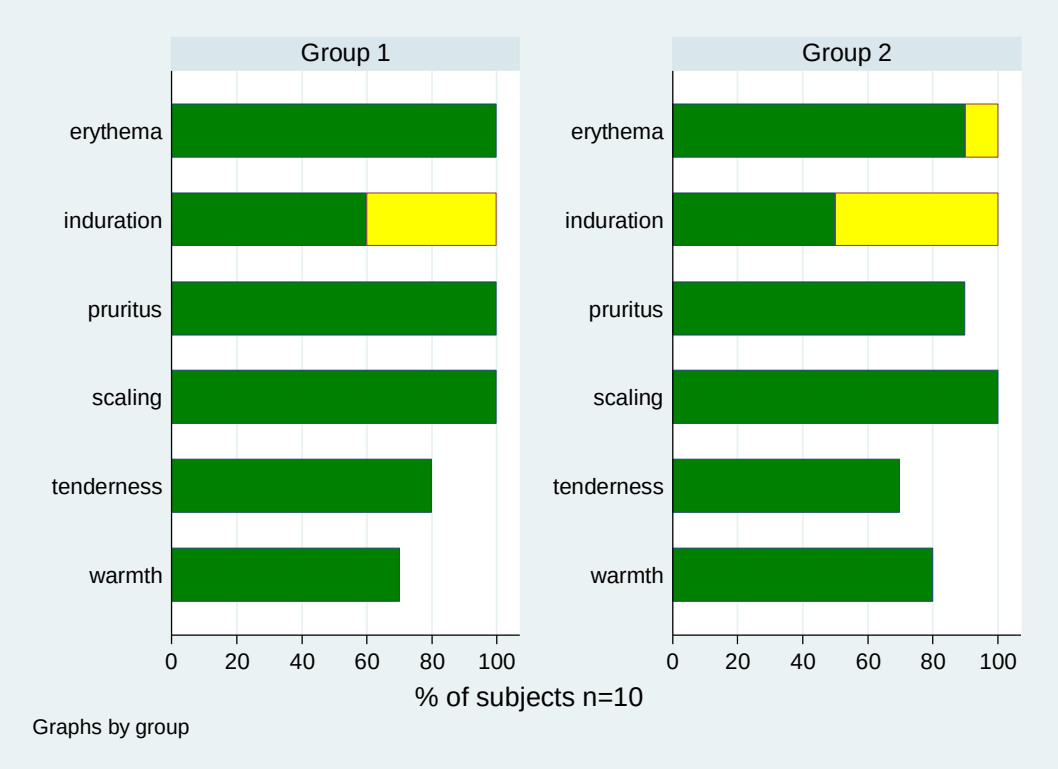
[a]For one subject in Group one, BCG vaccination was administered in childhood, but the year of vaccination was uncertain.

Local reactions

Following vaccination with MVA85A, all subjects in both groups developed a local reaction, consisting of erythema, induration and scaling **(Figure 3-2)**. Four of 67 (6 %) local reactions in Group one were moderate and 63 (94 %) were mild. Five of 64 (8 %) local reactions in Group two were moderate and 59 (92 %) were mild.

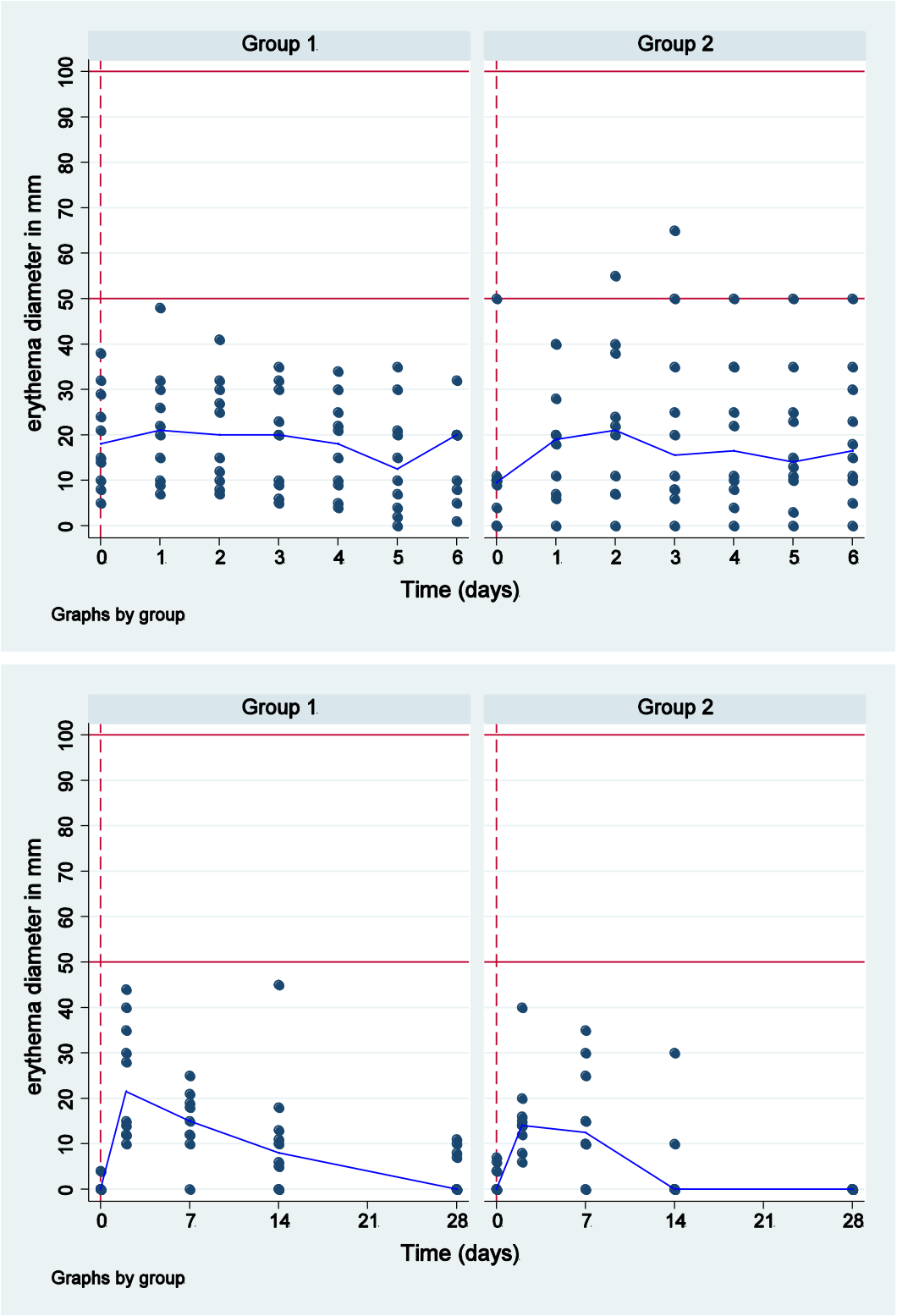
Erythema was classified as mild, according to the diameter of the largest reaction measured, in all subjects in Group one and in nine (90 %) subjects in Group two. Induration was classified as moderate, according to the diameter of the largest reaction measured, in four (40 %) subjects in Group one and in five (50 %) subjects in Group two. **Figures 3-3 and 3-4** show the individual measurements of diameters of vaccine site erythema and induration. One subject (035) had moderate erythema and induration. Other subjects with moderate induration were subjects 003, 009 and 019 in Group one and subjects 022, 029, 030 and 036 in Group two. There were no differences in local reaction diameters between Groups one and two **(Table 3-2)**. Warmth at the vaccination site was reported in seven (70 %) subjects in Group one and eight (80 %) subjects in Group two. Subjects also reported mild vaccination site symptoms of pruritus (100 % in Group one; 90 % in Group two) and tenderness (80 % in Group one; 70 % in Group two). There were no unsolicited local reactions and no local reactions were classified as severe.

Figure 3-2 Percentage of subjects with each local AE



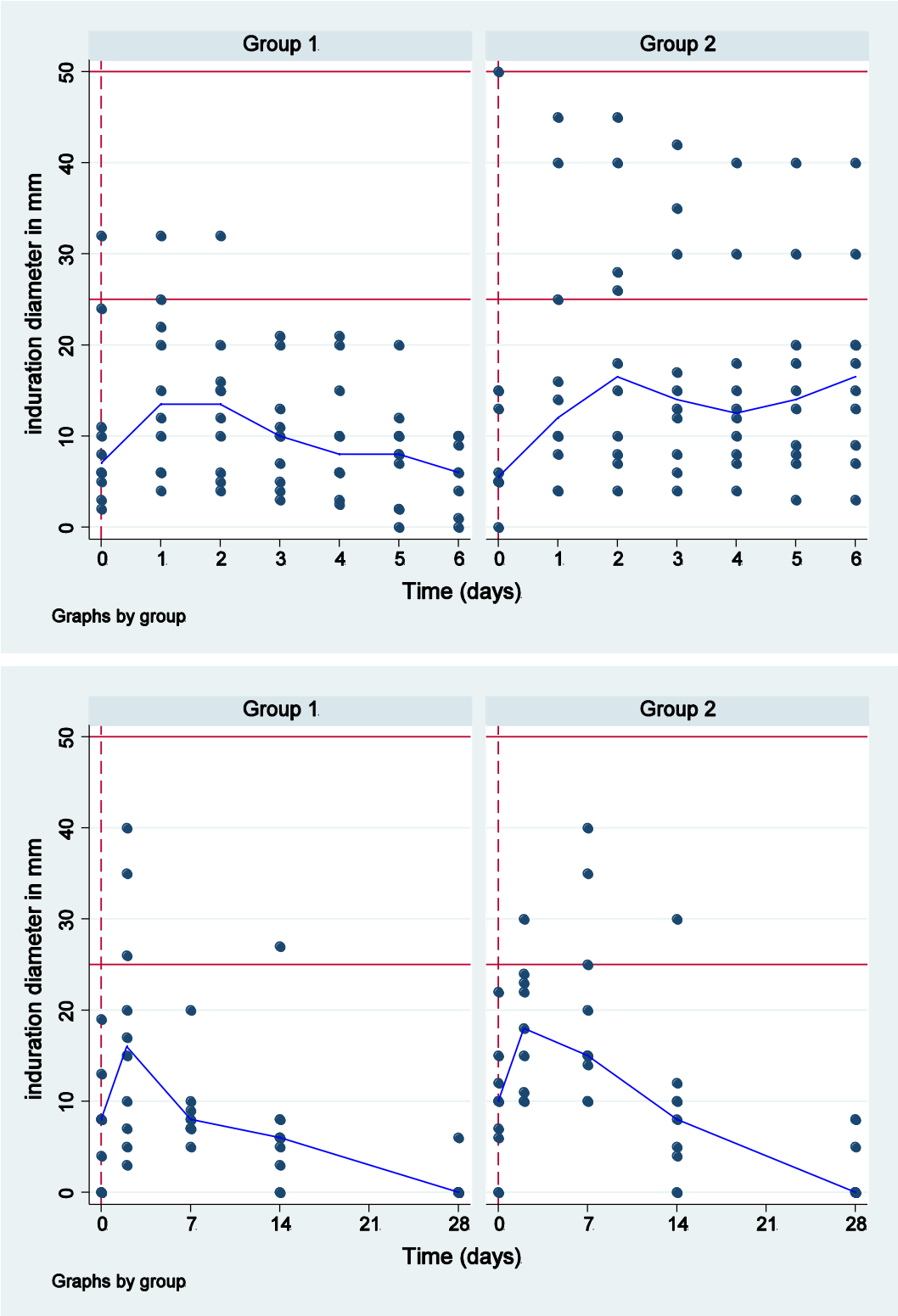
MVA85A vaccine dose: Group 1=5x10^{7} pfu, Group 2=1x10^{8} pfu. Coloured bars=possibly, probably or definitely related to MVA85A vaccination; green=mild, yellow=moderate.

Figure 3-3 Diameters of local erythema following MVA85A vaccination



Upper panel=diary card data (subjects' measurements); lower panel=case report form data (investigators' measurements). MVA85A vaccine dose: Group 1=$5x10^7$ pfu; Group 2=$1x10^8$ pfu. Dots=individual data points; blue lines=medians; y lines=thresholds for classification as moderate (50 mm) and severe (100 mm); dashed x lines=MVA85A vaccinations.

Figure 3-4 Diameters of local induration following MVA85A vaccination



Upper panel=diary card data (subjects' measurements); lower panel=case report form data (investigators' measurements). MVA85A vaccine dose: Group 1=$5x10^7$ pfu; Group 2=$1x10^8$ pfu. Dots=individual data points; blue lines=medians; y lines=thresholds for classification as moderate (25 mm) and severe (50 mm); dashed x lines=MVA85A vaccinations.

Table 3-2 Comparison of local reaction diameters between groups

	Source	Group 1 diameter mm median (IQR)	Group 2 diameter mm median (IQR)	Difference in medians (95 % CI)	p value
Erythema	Diary card	21 (10 to 30)	20 (7 to 33)	2 (-8 to 10)	0.59
	Case report form	22 (12 to 35)	14 (12 to 16)	6 (-3 to 22)	0.29
Induration	Diary card	14 (6 to 20)	15 (8 to 27)	-2 (-10 to 4)	0.51
	Case report form	16 (7 to 26)	18 (11 to 23)	19 (-11 to 10)	0.65

Diary card recordings (subjects' measurements) from days one and two post-vaccination were included in the analysis. Case report form recordings (investigators' measurements) from day two post-vaccination were included in the analysis.

Systemic AEs

AEs classified as possibly, probably or definitely vaccine-related were reported by eight (80 %) subjects in each group. The most frequently reported vaccine-related systemic AEs were headache, fatigue, arthralgia, malaise and feverish symptoms **(Figure 3-5)**. An oral body temperature of 38.1 ˚C on day five was reported by one subject in Group one. There were no other fevers documented. Four unsolicited AEs were classified as possibly, probably or definitely vaccine-related **(Table 3-3)**. The most frequently reported unsolicited AEs were upper respiratory tract infections (URTI) and sexually transmitted infections (STI).

All 28 possibly, probably or definitely vaccine-related AEs in Group one were mild and 22 of 23 (96 %) in Group two were mild. There were no vaccine related or unrelated severe systemic AEs.

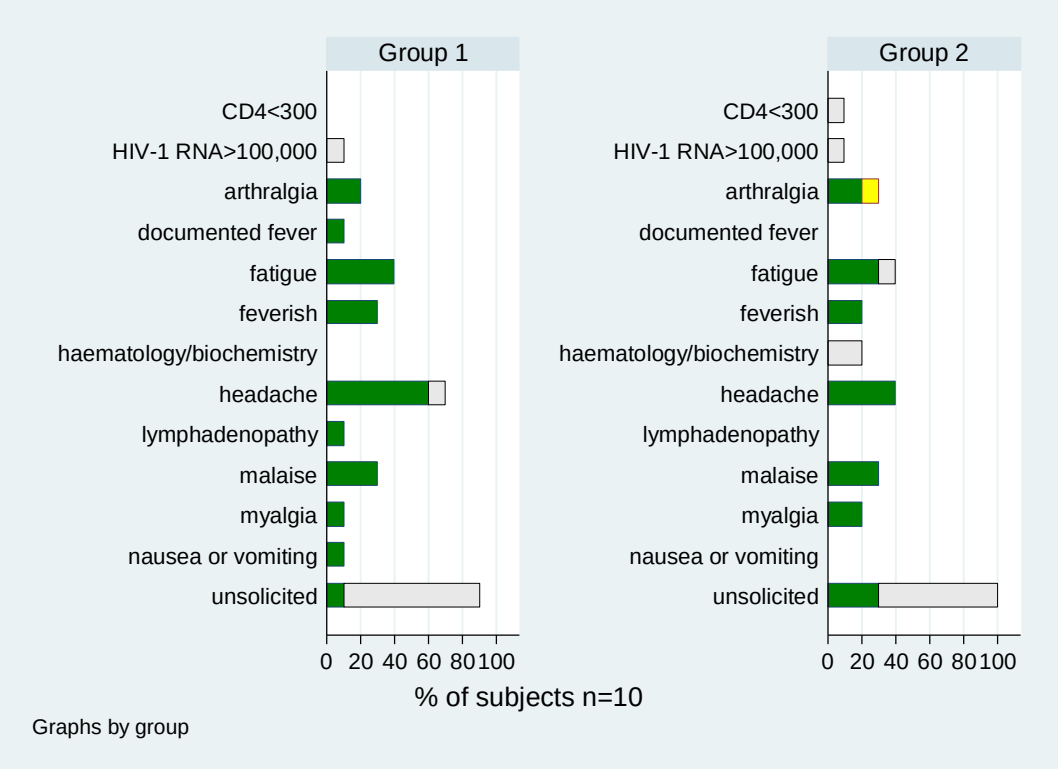
Moderate joint pains in ankles, knees and hands were reported by a female subject (022) in Group two on day 15 after vaccination and resolved without treatment within six weeks. This was associated with a transiently raised erythrocyte sedimentation rate (ESR) of 66 mm/hour and anaemia, both diagnosed 25 days post-vaccination. The anaemia was treated with ferrous sulphate and resolved. In Group one, moderate AEs classified as not related to MVA85A vaccination were headache (onset seven weeks after MVA85A vaccination), retinal haemorrhage (onset 11 months) and gonorrhoea (onset 13 months). Moderate unrelated AEs in Group two were fatigue (onset three weeks after MVA85A vaccination), influenza-like symptoms (onset six months) and productive cough (onset six months).

One subject in Group one (008) and two subjects in Group two (025 and 031) commenced ART six to 12 months after MVA85A vaccination, following declining $CD4^+$ T cell counts or increased HIV-1 RNA viral loads **(Figures 3-6 and 3-7)**. One of these (025) was a male subject who had pre-vaccination neutropenia ($1.69x10^9$/L), which transiently dropped to $0.9x10^9$/L three months after vaccination. Declining $CD4^+$ T cell count and rising HIV-1 RNA viral load were observed in a third subject in Group two (030) six months after MVA85A vaccination **(Figure 3-7)**.

SAEs

There were no SAEs. One male subject (030) was hospitalised for elective reduction of gynaecomastia over ten months after MVA85A vaccination. Initially this episode was reported as an SAE, but following independent assessment, was downgraded as being a pre-existing condition and therefore not an SAE. The condition was longstanding, predating the clinical trial, and surgery was elective and without complications.

Figure 3-5 Percentages of subjects with each systemic AE

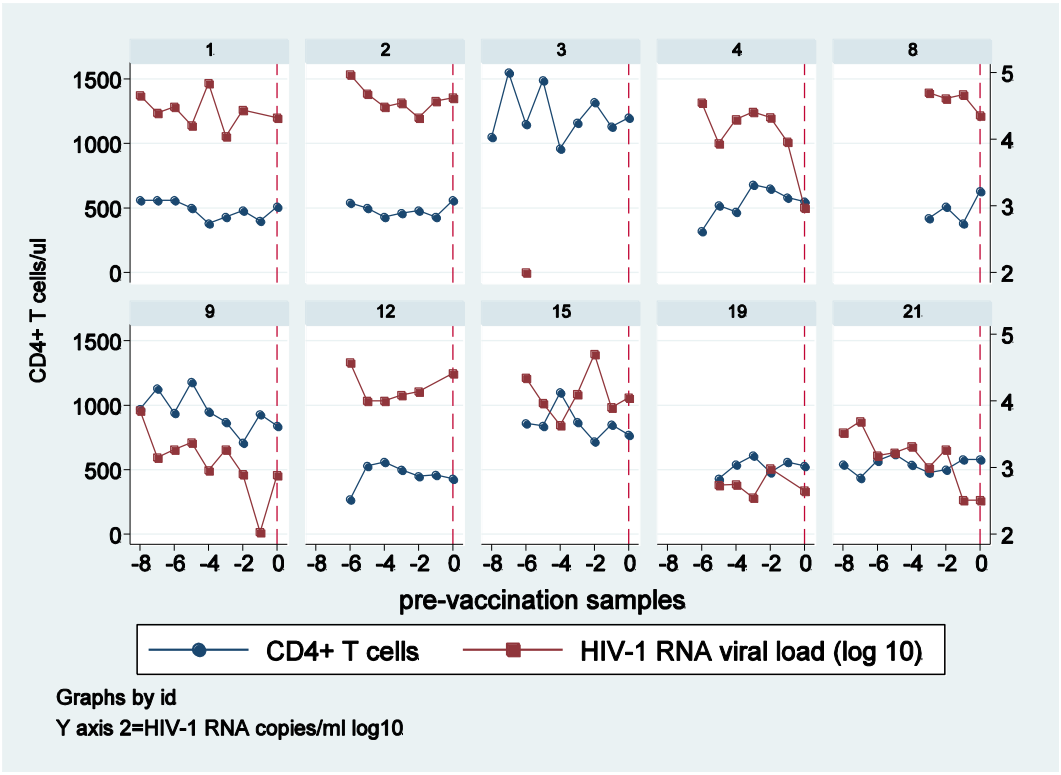


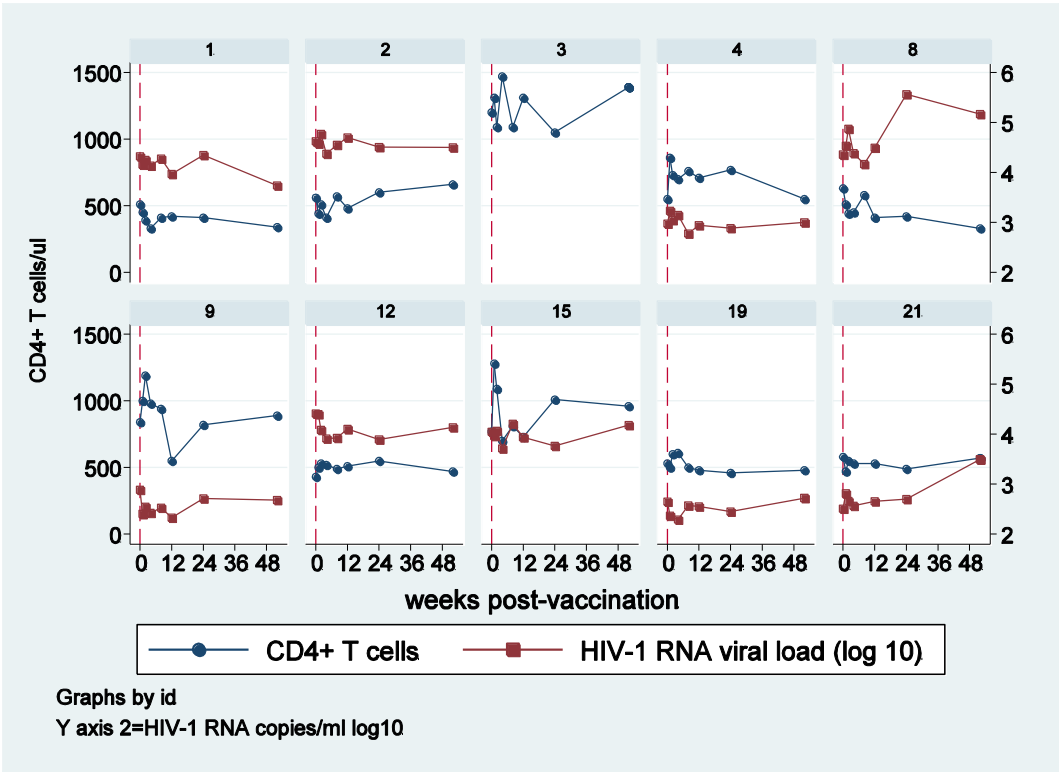
MVA85A vaccine dose: Group 1=5x10^{7} pfu; Group 2=1x10^{8} pfu. Coloured bars=possibly, probably or definitely related to MVA85A vaccination; green=mild; yellow=moderate; grey bars=not related to MVA85A vaccination.

Table 3-3 Unsolicited AEs

Relationship to MVA85A	AE	Group 1	Group 2
Related	diarrhoea	1 (5 %)	0
	shortness of breath	0	1 (5 %)
	shoulder pain	0	1 (5 %)
	vasovagal pre-syncope	0	1 (5 %)
Not related	STI	1 (5 %)	3 (15 %)
	URTI	5 (50 %)	3 (15 %)
	UTI	1 (5 %)	0
	abdominal pain	0	1 (5 %)
	abrasion	1 (5 %)	0
	depression	0	1 (5 %)
	ear infection	0	1 (5 %)
	eczema	2 (10 %)	0
	eye infection	1 (5 %)	2 (10 %)
	hip pain	0	1 (5 %)
	insomnia	1 (5 %)	0
	localised arthralgia	0	1 (5 %)
	retinal haemorrhage	1 (5 %)	0
	seasonal rhinitis	2 (10 %)	0
	sinusitis	0	2 (10 %)
	sore throat	1 (5 %)	1
	surgical procedure	0	1 (5 %)
	swollen hand	0	1 (5 %)
	vasovagal pre-syncope	1 (5 %)	0

Figure 3-6 CD4⁺ T cell counts and HIV-1 RNA viral loads in Group one

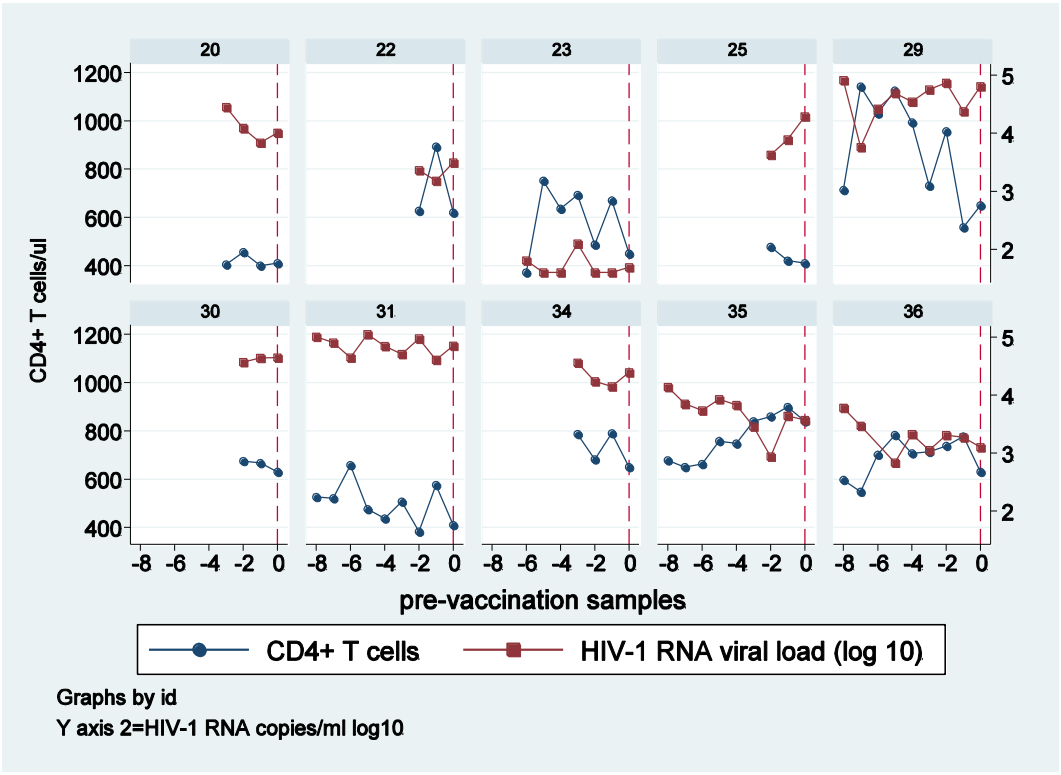


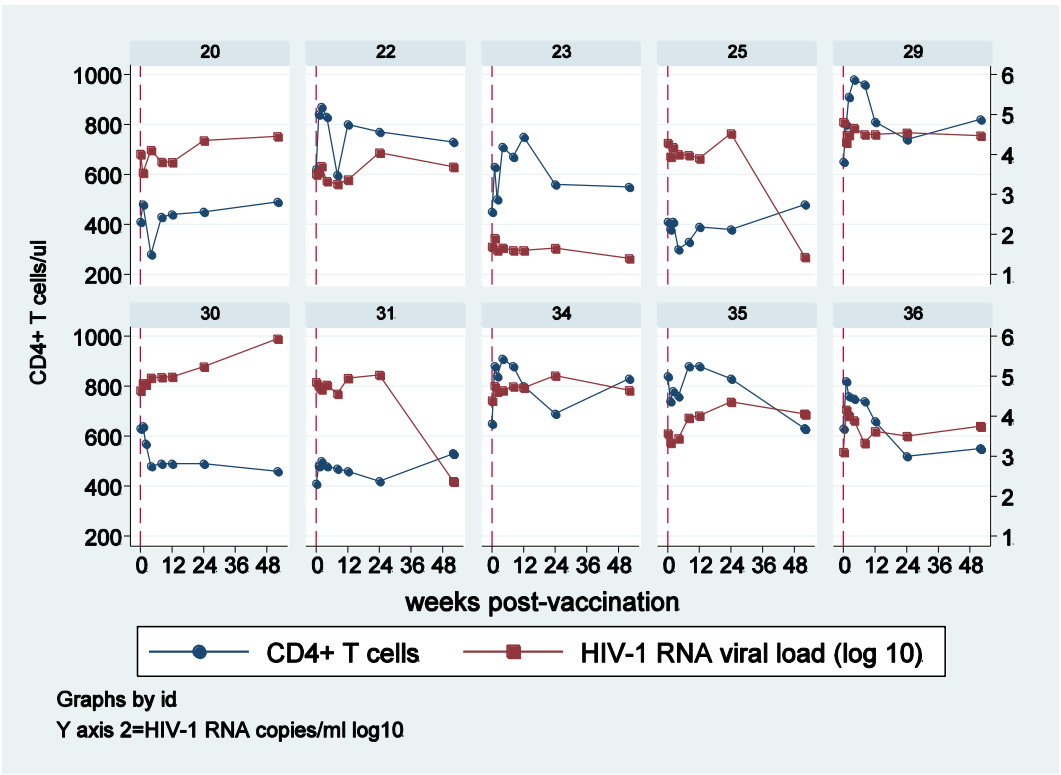


HIV-1 RNA copies/ml log10	1	2	3	4	5	6
HIV-1 RNA viral load (copies/ml)	10	100	1000	10000	100000	1000000

Upper panel=pre-enrolment variability in CD4⁺ T cell count and HIV-1 RNA viral load (samples are charted in chronological order but time intervals are variable). Lower panel=post-vaccination results. Dashed x lines=MVA85A vaccinations.
Table=conversion between HIV-1 RNA copies/ml log10 and HIV-1 RNA copies/ml.

Figure 3-7 CD4⁺ T cell counts and HIV-1 RNA viral loads in Group two





HIV-1 RNA copies/ml log10	1	2	3	4	5	6
HIV-1 RNA viral load (copies/ml)	10	100	1000	10000	100000	1000000

Upper panel=pre-enrolment variability in CD4+ T cell count and HIV-1 RNA viral load (samples are charted in chronological order but time intervals are variable). Lower panel=post-vaccination results. Dashed x lines=MVA85A vaccinations.
Table=conversion between HIV-1 RNA copies/ml log10 and HIV-1 RNA copies/ml.

Table 3-4 Paired analysis comparing pre- and post-vaccination CD4⁺ T cell counts and HIV-1 Viral Loads within Groups one and two

One week post-vaccination compared to pre-vaccination					
	Group	**Pre-vaccination median (IQR)**	**Week 1 median (IQR)**	**Median difference (IQR)**	**p value (WSR)**
CD4⁺ T cell count (cells/μl)	1	570 (530 to 770)	505 (470 to 1000)	20 (-110 to 160)	0.48
	2	625 (410 to 650)	690 (480 to 820)	110 (10 to 190)	0.04
HIV-1 RNA viral load (copies/ml)	1	6069 (450 to 22677)	5471 (253 to 24945)	-365 (-1966 to 326)	0.36
	2	14805 (3138 to 44089)	11538 (3495 to 55877)	-756 (-10737 to 13152)	0.88
Twenty four weeks post-vaccination compared to pre-vaccination					
	Group	**Pre-vaccination median (IQR)**	**Week 24 median (IQR)**	**Median difference (IQR)**	**p value (WSR)**
CD4⁺ T cell count (cells/μl)	1	570 (530-770)	575 (460 to 820)	-45 (-100 to 120)	0.96
	2	625 (410-650)	540 (450 to 740)	25 (-30 to 90)	0.51
HIV-1 RNA viral load (copies/ml)	1	6069 (450-22677)	3281 (506 to 22193)	-169 (-5407 to 181)	0.48
	2	14805 (3138-44089)	28360 (11024 to 103169)	13225 (1952 to 36884)	0.05

IFNγ ELISpot responses to Ag85A

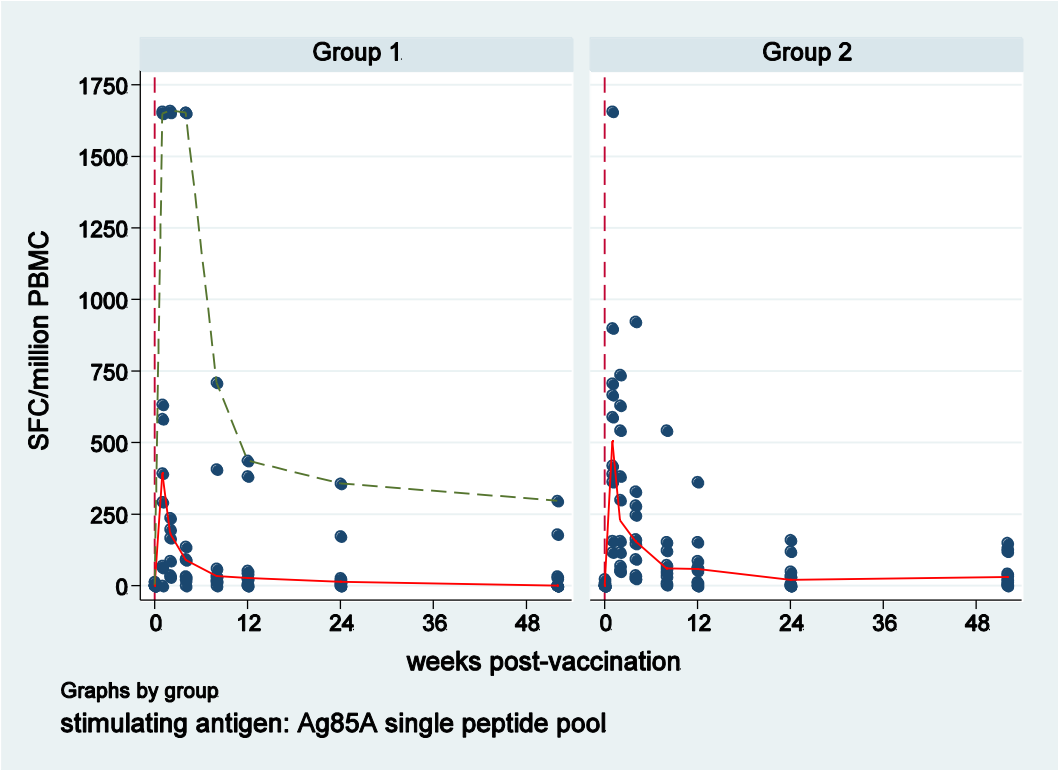
The kinetics and magnitude of the antigen-specific T cell responses following MVA85A vaccination were evaluated by IFNγ ELISpot assay, following PBMC stimulation with Ag85A peptides, PPD and recombinant Ag85A protein **(Figures 3-8 and 3-9)**.

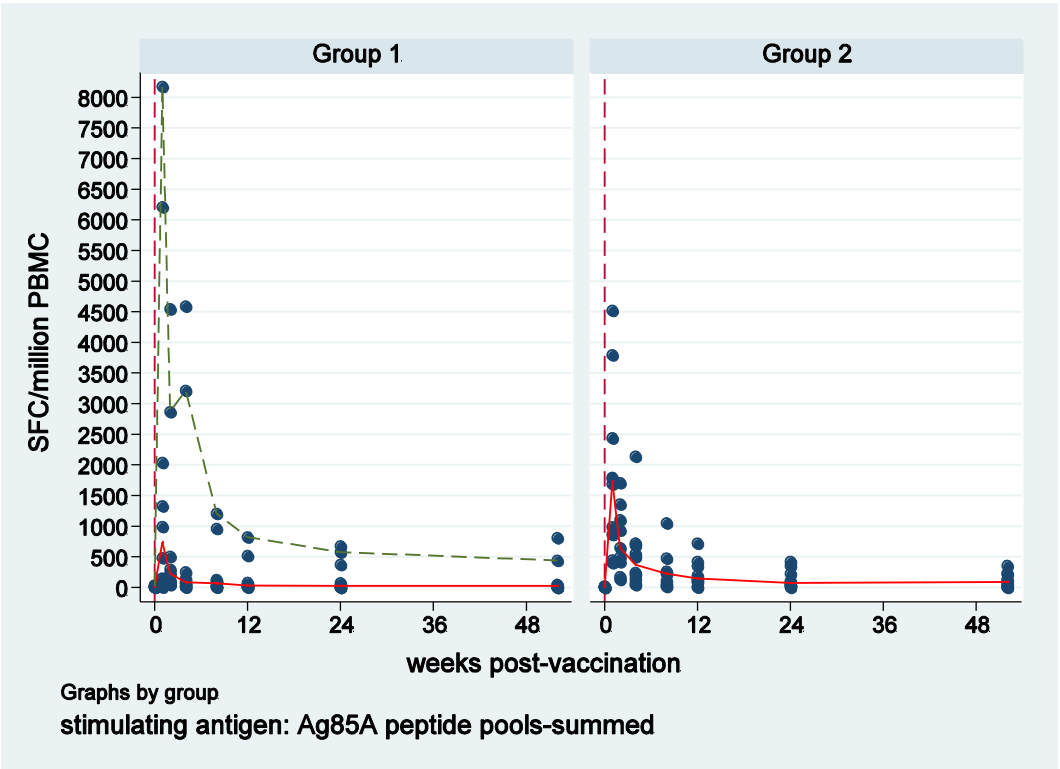
In both groups, peak (week one) IFNγ ELISpot responses to all antigens were significantly greater than baseline **(Tables 3-5 and 3-6).** Responses to Ag85A peptides in Group two, but not in Group one, remained significantly higher than baseline at week 52 **(Table 3-5)**. Responses to PPD and recombinant Ag85A were not maintained until week 52 in either group **(Table 3-6)**. The AUC of responses to a single pool of Ag85A were calculated and there was no significant difference between the AUC of responses in Group one and Group two **(Table 3-7)**.

The summed IFNγ ELISpot responses to pools of Ag85A peptides were compared with dose matched HIV-uninfected subjects from previous trials. For both doses, the AUC of responses were significantly higher in the HIV-uninfected subjects than in HIV-infected subjects **(Table 3-8)**.

In Group one, there was a positive correlation between the pre-vaccination $CD4^+$ T cell count and AUC (R=0.73, p=0.02) and a negative association between baseline HIV-1 RNA viral loads and AUC (R=-0.68, p=0.04) **(Figure 3-10)**. There were no significant correlations between $CD4^+$ T cell count and AUC (R=0.35, p=0.33) or HIV-1 RNA viral load and AUC (R=-0.48, p=0.16) in Group two **(Figure 3-10)**.

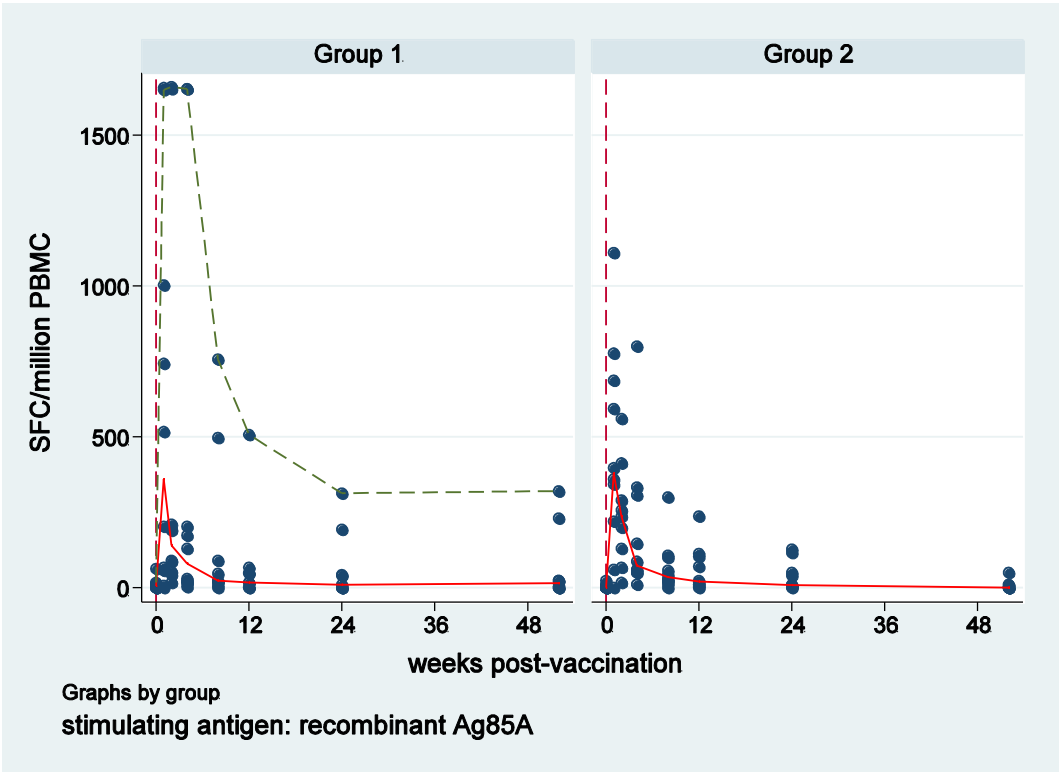
Figure 3-8 IFNγ ELISpot responses to Ag85A peptides

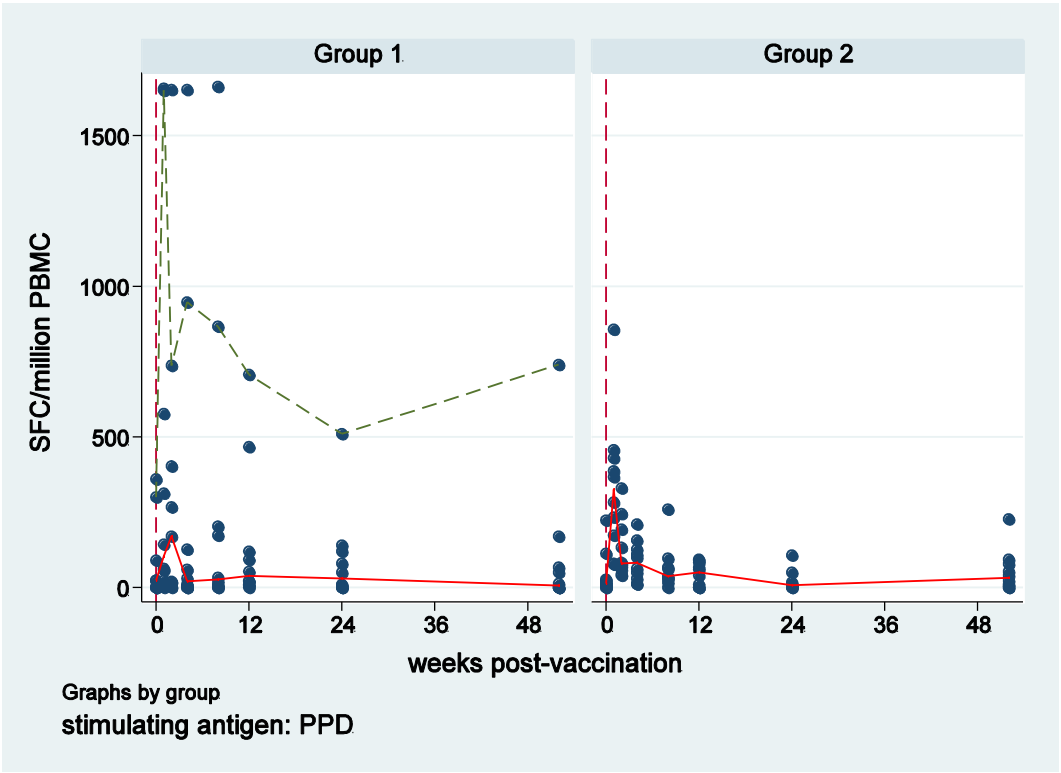




MVA85A vaccine dose: Group 1=5x10^{7} pfu; Group 2=1x10^{8} pfu. Dots=individual data points; red lines=connected medians; dashed x lines=MVA85A vaccinations; grey dashed line=connected data points for subject 003 (undetectable HIV-1 RNA viral load and preserved CD4^{+} T cell count).

Figure 3-9 IFNγ ELISpot responses to recombinant Ag85A and PPD





MVA85A vaccine dose: Group 1=5x10^{7} pfu; Group 2=1x10^{8} pfu. Dots=individual data points; red lines=connected medians; dashed x lines=MVA85A vaccinations; grey dashed lines=connected data points for subject 003 (undetectable HIV-1 RNA viral load and preserved CD4^{+} T cell count).

Table 3-5 Comparison of pre-vaccination (baseline) IFNγ ELISpot responses with peak and plateau responses to Ag85A peptides within Group one and Group two

Ag85A:	Single peptide pool		Summed peptide pools	
Group:	**1 (n=9)**	**2 (n=10)**	**1 (n=10)**	**2 (n=10)**
Pre-vaccination median (IQR)	0 (1 to 3)	0 (0 to 7)	10 (3 to 17)	3 (0 to 13)
Peak response week 1 median (IQR)	393 (70 to 900)	502 (363 to 667)	738 (140 to 3793)	1730 (862 to 2037)
Median difference between peak and baseline responses (IQR)	393 (63 to 900)	502 (340 to 667)	730 (140 to 3783)	1721 (842 to 2034)
p value (WSR)	0.009	0.005	0.007	0.005
Plateau response week 52 median (IQR)	27 (0 to 43)	20 (3 to 120)	25 (0 to 123)	55 (10 to 222)
Median difference between peak and baseline responses (IQR)	27 (0 to 43)	20 (3 to 113)	17 (-6 to 113)	52 (10 to 209)
p value (WSR)	0.06	0.008	0.11	0.008

Table 3-6 Comparison of pre-vaccination (baseline) IFNγ ELISpot responses with peak and plateau responses to PPD and recombinant Ag85A within Group one and Group two

Antigen:	PPD		Recombinant 85A	
Group	**1 (n=10)**	**2 (n=10)**	**1 (n=10)**	**2 (n=10)**
Pre-vaccination median (IQR)	18 (0 to 90)	18 (3 to 27)	5 (0 to 13)	0 (0 to 7)
Peak response week 1 median (IQR)	103 (23 to 857)	298 (214 to 387)	360 (57 to 1003)	379 (220 to 687)
Median difference between plateau and baseline responses (IQR)	89 (10 to 487)	314 (77 to 387)	350 (57 to 1003)	379 (220 to 664)
p value (WSR)	0.04	0.005	0.007	<0.001
Plateau response week 52 median (IQR)	18 (0 to 67)	25 (0 to 78)	12 (0 to 127)	0 (0 to 10)
Median difference between peak and baseline responses (IQR)	0 (-23 to 13)	5 (-6 to 40)	5 (0 to 23)	0 (0 to 7)
p value (WSR)	0.80	0.48	0.13	0.39

Table 3-7 Comparison of the AUC of responses to Ag85A single peptide pool between Groups one and two

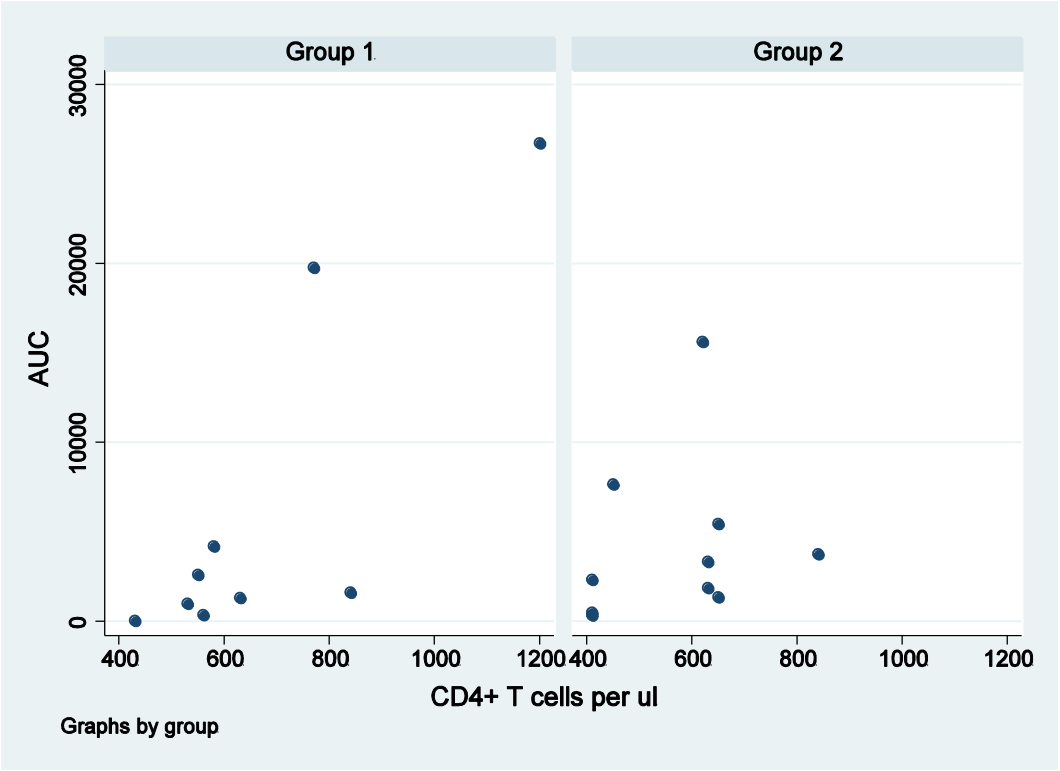
Group 1 (n=9)	Group 2 (n=10)	Difference in medians (95 % CI)	p value (MWU)
1623 (996 to 4203)	2839 (1357 to 5455)	-407 (-3297 to 3705)	0.68

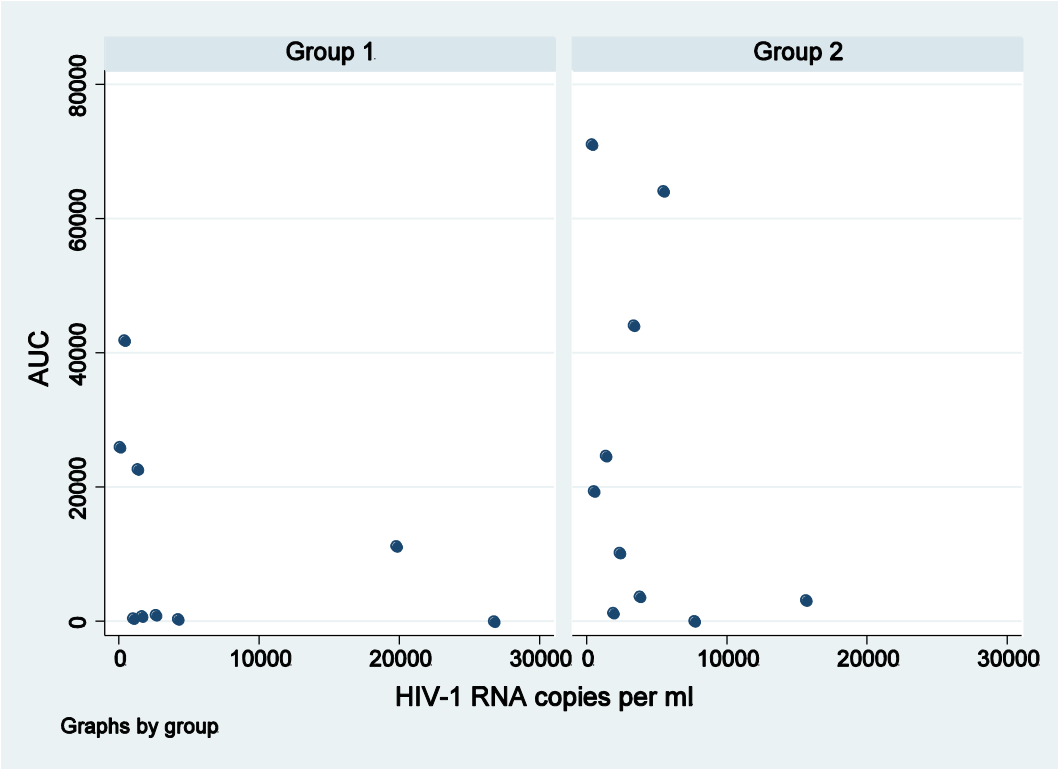
Table 3-8 Comparison of the AUC of responses to summed peptide pools of Ag85A between HIV-infected subjects and HIV-uninfected subjects

	HIV-infected (n=10)	HIV uninfected	Difference in medians (95 % CI)	p value (MWU)
$5x10^7$ pfu	Group 1: 2790 (870 to 9692)	15786 (9831 to 30383) (n=31)[a]	11078 (5261 to 17130)	0.005
$1x10^8$ pfu	Group 2: 5929 (3824 to 11316)	41575 (26919 to 53807) (n=12)[b]	31384 (20778 to 45504)	0.0001

[a] Data for HIV uninfected subjects, MVA85A vaccine dose $5x10^7$ pfu, was obtained from previously published clinical trials (117, 194); [b] Data for HIV uninfected subjects, MVA85A vaccine dose $1x10^8$ pfu, was obtained from a previously published clinical trial (192)

Figure 3-10 Relationships between AUC and pre-vaccination $CD4^+$ T cell count or HIV-1 RNA viral load





MVA85A vaccine dose: Group 1=$5x10^7$ pfu; Group 2=$1x10^8$ pfu. AUC=area under the curve, calculated using IFNγ ELISpot responses to a single pool of Ag85A peptides; dots=individual data points.

Discussion

This was the first study evaluating the safety and immunogenicity of MVA85A in healthy adults infected with HIV.

Safety

Local reactions were observed in 100 % of subjects. This was comparable to the frequencies of local reactions in healthy adult subjects or subjects with LTBI vaccinated with $5x10^7$ pfu or $1x10^8$ pfu of MVA85A (117, 192, 193). In a recent trial of MVA as a smallpox vaccine, the frequency of local reactions was lower in the HIV-infected compared to the HIV-uninfected group (249). Systemic AE profiles were similar between the two dose groups of HIV-infected subjects in this trial and between HIV-infected subjects in this trial and healthy subjects receiving the same doses of MVA85A in previous trials (117, 192, 193). HIV-uninfected adults with LTBI have previously been safely vaccinated with MVA85A (193). In this clinical trial, the sample size of subjects with LTBI was small but there were no obvious safety concerns associated with MVA85A vaccination of HIV-infected adults with LTBI.

In other studies, $CD4^+$ T cell counts have been shown to fluctuate by 60 to 120 cells/μl over weeks, with greater variation if $CD4^+$ T cell counts are greater than 400 cells/μl (250, 251). The increase in $CD4^+$ T cell count one week after vaccination in Group two may therefore be due to physiological fluctuation, although a non-specific increase in $CD4^+$ T cells in response to vaccination should also be considered. HIV disease progression was observed in four subjects in total, but in all cases the interval between vaccination and decline in $CD4^+$ T cell count or rise in viral load was greater than six months and was considered unlikely to be related to MVA85A vaccination. In exploratory studies using stored samples from this clinical trial, there was no evidence that extensive HIV infection of antigen-specific MVA85A vaccine-induced $CD4^+$ T cells took place (252). In quantitative PCR (qPCR) studies, HIV *gag* DNA was detected in Ag85A-specific $CD4^+$ T cells in samples from one of eleven subjects and there was no change in expression of the HIV co-receptor, CCR5, on $CD4^+$ T cells after MVA85A vaccination (252).

This trial builds upon previous experience of MVA and recombinant MVAs in HIV-infected subjects (242-244, 246-249). All but ten of the subjects recruited in the other trials cited were taking ART, but this trial recruited asymptomatic HIV-infected subjects not taking ART, giving important additional safety data. That there were no SAEs or severe AEs further supports the future evaluation of recombinant MVA-based vaccines in subjects with HIV.

Immunogenicity

Peak post-vaccination IFNγ ELISpot responses in the HIV-infected population in this study were significantly greater than pre-vaccination responses, but were lower in magnitude compared to healthy HIV-uninfected subjects in previous trials (117, 192). In healthy HIV-uninfected adults, IFNγ ELISpot responses to Ag85A peptides following MVA85A vaccine doses of $1x10^7$, $5x10^7$ and $1x10^8$ pfu were maintained above baseline levels for at least 52 weeks (117, 192, 193). In this study, responses in HIV-infected subjects, who had received $1x10^8$ pfu of MVA85A, but not those receiving $5x10^7$ pfu, were maintained above baseline levels until week 52. In healthy adults, $1x10^8$ pfu MVA85A induced a significantly greater magnitude of IFNγ ELISpot responses compared to $5x10^7$ pfu MVA85A but there was no significant difference between the AUC of responses after $5x10^7$ pfu and $1x10^8$ pfu MVA85A in HIV-infected subjects (192). A subsequent Phase IIa clinical trial in South Africa has also demonstrated reduced immunogenicity of MVA85A in HIV-infected compared to HIV-uninfected subjects (195).

Subjects with a reduced $CD4^+$ T cell count are likely to have a limited capacity for an increased immune response with increased vaccine dose. The greatest magnitude of response was in subject 003 in Group one, who had a preserved $CD4^+$ T cell count and undetectable HIV-1 RNA viral load. The possibility that results may have been confounded by the inclusion of a subject (003) with preserved $CD4^+$ T cell count in Group one was considered. However, even if subject 003 was removed from the analysis, there was no significant difference in the magnitudes of responses between Groups one and two (data not shown). Pre-vaccination $CD4^+$ T cell counts and HIV-1 RNA viral loads for Group one (but not Group two) correlated with post-vaccination IFNγ ELISpot responses positively and negatively respectively. A plausible explanation could be that differences in $CD4^+$ T cell counts and HIV-1 RNA viral loads do not affect the magnitude of vaccine-induced response unless $CD4^+$ T cell counts approach the levels seen in healthy adults.

Clinical trials of other MVA-based vaccines have also demonstrated immunogenicity in HIV-infected subjects (242, 244, 245, 247, 249, 253). A trend towards lower antibody responses in HIV-infected compared to uninfected subjects and weaker $CD4^+$ T cell compared to $CD8^+$ T cell responses in HIV-infected subjects have been reported (247, 249).

Limitations

This was an observational clinical trial, primarily aiming to detect frequently occurring AEs and major safety concerns.

Subjects were not randomised into the two groups as this was a dose-escalation trial. However, baseline parameters were similar between the two groups and there was no evidence for an increase in AE frequency or severity at the higher dose. The AE profile was as expected, based upon previous experience with MVA85A in HIV-uninfected adults and reports of other MVA vaccines in HIV-infected adults (117, 192, 194, 242-244, 247, 253).

A larger sample size would have enabled division of subjects into subgroups according to LTBI and BCG status and a control group would be useful in drawing conclusions regarding AE causality. However, the population of adults with a diagnosis of HIV infection, with a preserved $CD4^+$ T cell count and not taking ART was very small. Oxford and Swindon have small populations of patients infected with HIV. The population in London was considerably larger, but recruitment potential was limited by other clinical trials with similar inclusion criteria. The protocol amendment for the addition of the fourth clinical trial site in Birmingham enabled the target sample size of twenty subjects to be achieved. This sample size was sufficient for division of the subjects into subgroups for dose escalation, such that subsequent studies in HIV-infected adults can be conducted using the upper dose of MVA85A vaccine ($1x10^8$ pfu).

An age-matched control group of HIV-uninfected subjects would strengthen the comparison between vaccine responses in HIV-infected and uninfected individuals. As expected, the immune responses in HIV-infected individuals in this trial were lower than those consistently observed in HIV-uninfected adults. Immunogenicity comparisons between HIV-infected subjects in this trial and dose-matched HIV-uninfected subjects from previous trials were confounded by the trials being conducted months to years apart and the younger age of HIV-uninfected subjects in previous trials (117, 192, 194). However, the main aim of measuring IFNγ ELISpot responses was to identify any detectable immune responses in the HIV-infected population.

Obtaining the summed IFNγ ELISpot responses to the seven pools of Ag85A peptides may have artificially augmented immunogenicity by duplicate counting of any T cells responding to epitopes contained within the regions of overlap. However, it was useful to evaluate the summed responses in order to be consistent with previous clinical trials of MVA85A (117, 192, 194). Responses to a single pool of Ag85A peptides were also evaluated, for a more realistic estimation of the magnitude of IFNγ ELISpot responses.

Future work

The results of this trial support further evaluation of MVA85A in HIV-infected adults. In a Phase II clinical trial in South Africa, MVA85A vaccine (dose $5x10^7$ pfu) was safely

administered to HIV-infected and HIV/*M.tb*-coinfected adults (195). Following the safety of the upper dose of MVA85A vaccine ($1x10^8$ pfu) in the trial described here, the same dose was used in a Phase I clinical trial of ART-naïve and ART-established HIV-infected subjects in Senegal (254). Antigen-specific IFNγ ELISpot responses were lower in ART-naïve subjects compared to subjects on ART after the first vaccination but comparable after a second dose at 12 months (254). The safety and immunogenicity of MVA85A in HIV-infected subjects is now being evaluated in a proof of concept Phase II efficacy trial in South Africa (trial ID NCT01151189) (131, 159). Another trial is evaluating MVA85A vaccination of HIV-exposed infants, in whom BCG testing is delayed until HIV can be excluded (trial ID NCT01650389) (131, 159).

The safety of MVA85A and other recombinant MVA candidate vaccines in HIV-infected subjects could be further investigated by assessing for any subtle effects on HIV disease, such as the dynamics of the HIV-1 RNA viral load in the first week after vaccination.

Although MVA85A vaccination did not show improved efficacy in infants compared to BCG vaccination alone, evaluation of the efficacy of MVA85A vaccination compared to placebo in HIV-infected adults, who cannot be safely vaccinated with BCG, is warranted.

Conclusions

This was the first clinical trial of candidate TB vaccine, MVA85A, in asymptomatic, HIV-infected adults. MVA85A vaccination was safe in adult subjects infected with HIV living in the UK. For both doses of $5x10^7$ pfu and $1x10^8$ pfu of MVA85A, most AEs were mild and there were no severe AEs. IFNγ ELISpot responses to Ag85A increased significantly one week after vaccination in both groups and were maintained above baseline for 52 weeks in Group two. These results support the inclusion of HIV-infected subjects in the ongoing clinical evaluation of candidate TB vaccine, MVA85A, in particular with respect to inclusion of HIV-infected subjects in larger scale safety and efficacy clinical trials.

Chapter 4 : Phase I clinical trial of new candidate tuberculosis vaccine, FP85A, alone and in prime-boost regimes with MVA85A

Introduction

MVA85A vaccination protects against TB in animal models and has consistently induced a high frequency of IFNγ-secreting T cells in early clinical trials in healthy adult subjects (117, 148, 189, 191-194). Larger scale efficacy trials will help to determine the applicability of animal models and to define biomarkers of protection in humans, but this will take some time. The lack of efficacy of a BCG-prime, MVA85A-boost vaccination regime in infants compared to vaccination with BCG alone highlights the need for the continuing development of novel vaccine candidates and regimes alongside efficacy trials of the most advanced candidates (203). Only moderate immunogenicity is achieved by the BCG-prime, MVA85A-boost regime in infants. Regimes which enhance vaccine immunogenicity in target populations are needed.

Viruses are being investigated as vaccine vectors for malaria, HIV-1, influenza, dengue fever, Japanese encephalitis, HCV and cancer vaccines, as well as for TB vaccines (255-257). Enhanced immunogenicity has been demonstrated for heterologous compared to homologous prime-boost regimes (236). FP9 is a live, attenuated form of a European strain of fowlpox virus with a fully characterized genome, developed through multiple passages in avian cells, initially for use as a vaccine for poultry (258, 259). FP9 is not infectious in non-avian species, but the discovery that it is able to express antigens and induce protective immunity in mammalian species led to its identification as a candidate vector for use in humans (260-262). Recombinant FP9 candidate malaria vaccines, when used in prime-boost regimes with recombinant MVA vaccines, enhanced immunogenicity and induced sterile immunity in two subjects in the UK (236, 263, 264). A recombinant FP9 expressing Ag85A, named FP85A, has been developed. Enhanced protection against aerosol *M.tb* challenge was observed when guinea pigs were vaccinated sequentially with BCG, MVA85A and FP85A, compared to BCG vaccination alone (190).

A Phase I clinical trial was conducted to evaluate the safety of FP85A vaccination in healthy human subjects for the first time. Three regimes were evaluated in previously BCG-vaccinated subjects: FP85A vaccination (regime BF); MVA85A vaccine prime, FP85A vaccine boost (regime BMF); and FP85A vaccine prime, MVA85A vaccine boost (regime BFM).

Aims

The primary objective was to evaluate the safety of FP85A vaccination alone and in prime-boost regimes with MVA85A vaccination in BCG-vaccinated subjects (regimes BF, BMF and BFM) by analysing:

1. The frequency and severity of local reactions;
2. The frequency and severity of systemic AEs.

The secondary objective was to evaluate the immunogenicity of FP85A vaccination alone and in prime-boost regimes with MVA85A vaccination in BCG-vaccinated subjects (regimes BF, BMF and BFM) by analysing IFNγ ELISpot responses to Ag85A peptides, PPD and recombinant Ag85A protein and comparing:

1. Baseline (pre-vaccination and week four) responses with post-vaccination (weeks one and five) responses within each group;
2. The AUCs of responses between different heterologous prime-boost regimes.

Results

Approvals prior to commencement of the trial

Ethical approval was obtained from GTAC. Approval for contained use of a GMO was obtained from the Oxford Radcliffe Hospital GMSC. Regulatory approval was obtained from the MHRA. A site-specific approval was obtained from Oxfordshire REC.

Substantial amendments after commencement of the trial

The following substantial amendments to the protocol were approved by GTAC, MHRA and Oxfordshire REC after commencement of the clinical trial.

1. Trial recruitment closed early prior to enrolling the planned sample size. This was due to the FP85A vaccine failing to meet the pass criteria for the annual vaccine potency test.

Monitoring

Monitoring was delegated to an external agency and 100 % document monitoring was conducted. The trial master files and approvals were monitored prior to commencement of the trial. Regular monitoring visits were conducted during the course of the trial, including clinical trial sites, source documents and subject case report forms, as well as ongoing trial master file management. A close out monitoring visit was conducted following completion of the clinical trial.

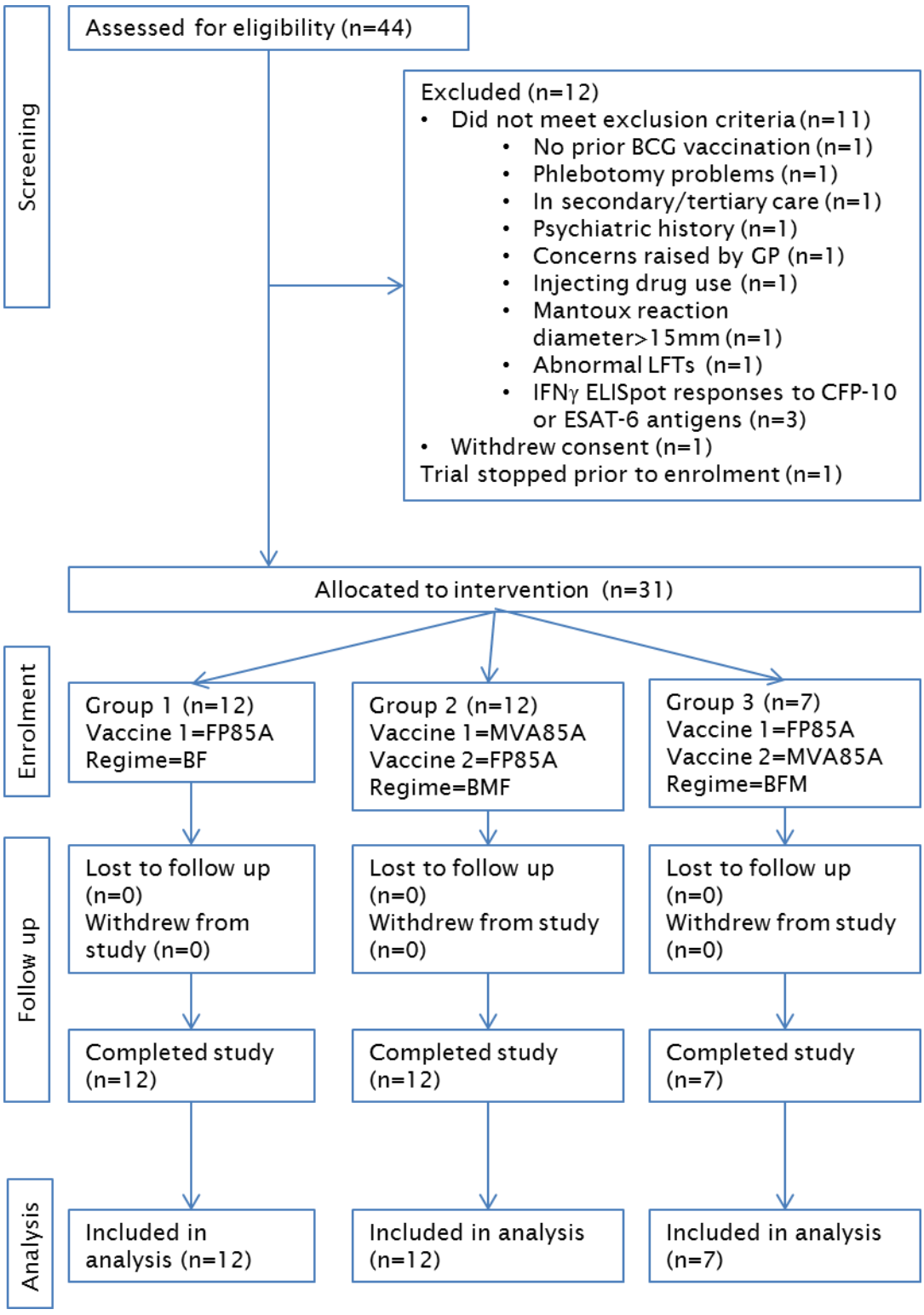
Reports

Annual Progress Reports were submitted to GTAC. In addition, Annual Safety Reports for the IMPs, FP85A and MVA85A, were submitted to GTAC and the MHRA. On completion of the clinical trial, an End of Trial Notification and subsequently a Final Report were submitted to GTAC and the MHRA.

Subject recruitment

Forty four healthy adult volunteers were screened and 31 subjects were enrolled **(Figure 4-1)**. The first 12 subjects enrolled were allocated to Group one (regime BF) and the next 12 subjects enrolled were allocated to Group two (regime BMF). The final seven subjects enrolled were allocated into Group three (regime BFM). Recruitment ended before the planned sample size of 36 subjects had been enrolled, since it was not possible to extend the expiry date for the FP85A vaccine.

Figure 4-1 Flow of subjects



Baseline characteristics

The baseline characteristics of subjects within each group are summarised in **Table 4-1**. More females than males were enrolled and in Group one, 90 % of subjects were female. Similar proportions of male and female subjects were enrolled in Groups two and three. Most subjects in each group were young adults, born in Europe. In most cases BCG vaccination had been administered in childhood or adolescence, with an interval of over ten years between BCG vaccination and vaccination with the candidate TB vaccines in this trial. Pre-vaccination IFNγ ELISpot responses to PPD were of a similar magnitude between groups.

Table 4-1 Baseline characteristics of enrolled subjects

		Group 1	Group 2	Group 3
Regime		**BF**	**BMF**	**BFM**
Vaccines administered		**FP85A**	**MVA85A,FP85A[a]**	**FP85A,MVA85A[a]**
Number enrolled		12	12	7
Males		1 (8 %)	5 (42 %)	3 (43 %)
Age, median years (IQR)		23 (22 to 37)	26 (23 to 31)	29 (27 to 46)
Continent of birth:	Europe	11 (92 %)	10 (83 %)	7 (100 %)
	Asia	1 (8 %)	2 (17 %)	0
Prior BCG vaccination		12 (100 %)	12 (100 %)	7 (100 %)
BCG vaccination/enrolment interval, median years (IQR)[b]		10 (10 to 25)	12 (9 to 20)	15 (10 to 34)
Visible BCG scar		10 (83 %)	8 (67 %)	6 (86 %)
Mantoux reaction: induration diameter, median mm (IQR)		4 (0.5 to 5.5)	2 (0 to 9)	3 (0 to 6)
Pre-vaccination IFNγ ELISpot responses to PPD, median SFC (IQR)		133 (50 to 283)	117 (32 to 225)	183 (107 to 263)

[a]Four week interval between vaccinations; [b]For two subjects in Group one and two subjects in Group two, the year of BCG vaccination was unknown.

Safety of FP85A vaccination (Group one, regime BF)

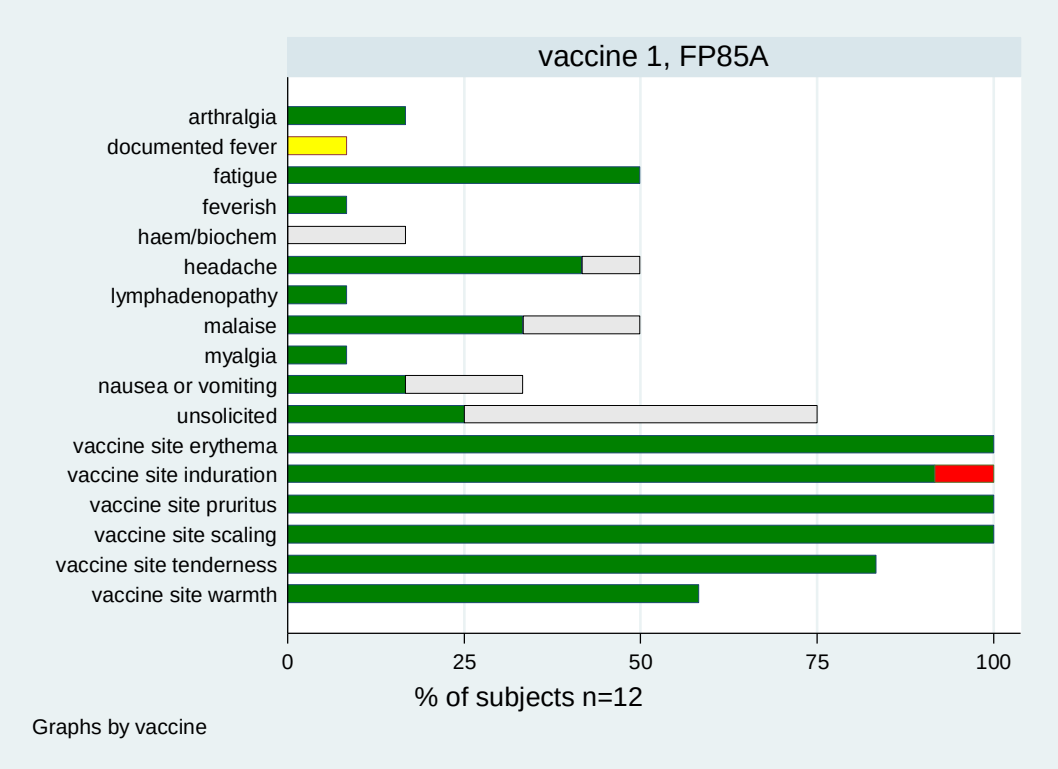
Following vaccination with FP85A, all 12 subjects developed a local reaction comprising erythema and induration, followed by scaling and pruritus, with ten subjects also reporting tenderness **(Figure 4-2)**. Induration was severe in one subject (maximum diameter 55 mm) and all other local reactions were mild.

Moderate feverish symptoms, associated with a body temperature of 37.7 ^{0}C, were recorded on the day of FP85A vaccination by one subject. All other vaccine-related systemic AEs were mild, with the most common systemic AEs being headache, malaise and fatigue **(Figure 4-2)**. One subject developed iron deficiency anaemia (haemoglobin 12.2 g/dL at trial screening; 10.9 g/dL one week post-vaccination). This was considered to be due to the volume of blood taken early in the trial (230 ml) and an underlying predisposition to iron deficiency (vegan diet low in iron). The subjects' primary care practitioner was informed. A second subject had transiently elevated white blood cells (14.5x10^{9} cells/litre [L]) 12 weeks after FP85A vaccination.

Unsolicited AEs are listed in **Table 4-2**.

One SAE occurred in one subject eleven months after vaccination with FP85A. This SAE consisted of a day case hospital admission for knee arthroscopy following an injury sustained six months after vaccination and was classified as not related to FP85A vaccination.

Figure 4-2 Percentage of subjects with each local and systemic AE in Group one (regime BF)



Coloured bars=possibly, probably or definitely related to FP85A vaccination; green=mild; yellow=moderate; red=severe; grey bars=not related to FP85A vaccination.

Table 4-2 Unsolicited AEs in Group one (regime BF)

The number (%) of subjects with each unsolicited AE after FP85A vaccination in Group one are listed.

	AE	FP85A (regime BF) n=12
Related	dizziness	1 (8 %)
Not related	abdominal pain	1 (8 %)
	back pain	1 (8 %)
	coryzal symptoms	5 (42 %)
	diarrhoea[a]	2 (17 %)
	dysmenorrhea[a]	2 (17 %)
	ear infection	1 (8 %)
	endoscopy[a] (oesophagogastroduodenoscopy)	1 (8 %)
	facial laceration	1 (8 %)
	foot pain	1 (8 %)
	hip pain	1 (8 %)
	knee pain	1 (8 %)
	migraine[a]	1 (8 %)
	mouth ulcer	1 (8 %)
	pytiriasis rosea	1 (8 %)
	seasonal rhinitis	1 (8 %)
	shingles	1 (8 %)
	skin infection	1 (8 %)
	sore throat[a]	2 (17 %)
	toe / foot infection[a]	2 (17 %)
	toothache	1 (8 %)
	vasovagal symptoms	1 (8 %)

[a]moderate severity in one subject; all other unsolicited AEs were mild in severity

Safety of sequential MVA85A and FP85A vaccinations (Group two, regime BMF)

All subjects developed vaccine site erythema and induration following vaccination with MVA85A and most reported tenderness, pruritus and scaling **(Figures 4-3 and 4-4)**. One subject had moderate erythema, induration and scaling. All other subjects had mild local reactions.

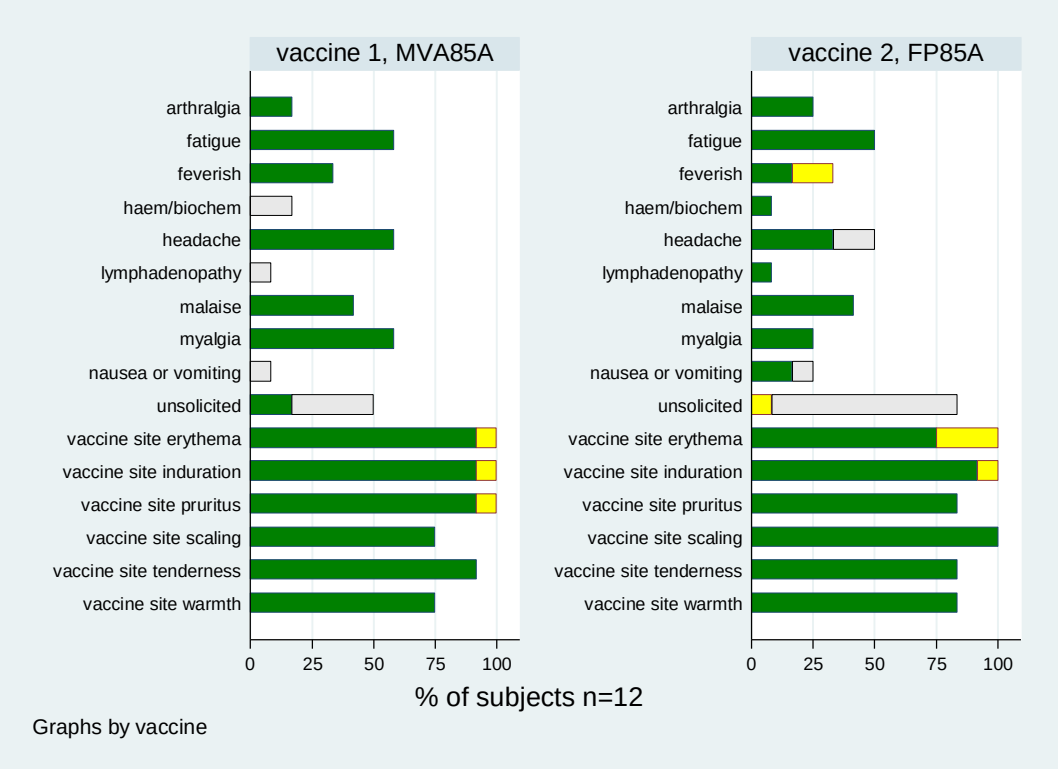
All vaccine-related systemic AEs after MVA85A vaccination were mild and there were no SAEs. Two subjects had transient hypokalaemia (2.9 millimoles [mmol]/L), associated with a transiently raised bilirubin (30 micromoles [µmol]/L) in one, but not classified as vaccine-related, due to a concurrent history of recent excess alcohol intake.

Following FP85A vaccination at week four, all subjects developed vaccine site erythema, induration and scaling and most reported tenderness, pruritus and warmth **(Figure 4-3)**. For three subjects the maximum measured diameter of erythema and/or induration was in the moderate range **(Figure 4-4)**. All other local reactions were mild. Peak (day one and two) diameters of erythema, but not induration, recorded in subject diary cards showed a trend towards being larger after the second vaccination (FP85A) compared to the first vaccination (MVA85A) **(Figure 4-4)**.

Symptoms of feverishness (in the absence of a documented fever) were reported by two subjects, one of whom described a rigor. All other systemic AEs after FP85A vaccination were mild and there were no SAEs. One subject had transient hypokalaemia (2.9 mmol/L) and bilirubinaemia (30 µmol/L) one week after FP85A vaccination.

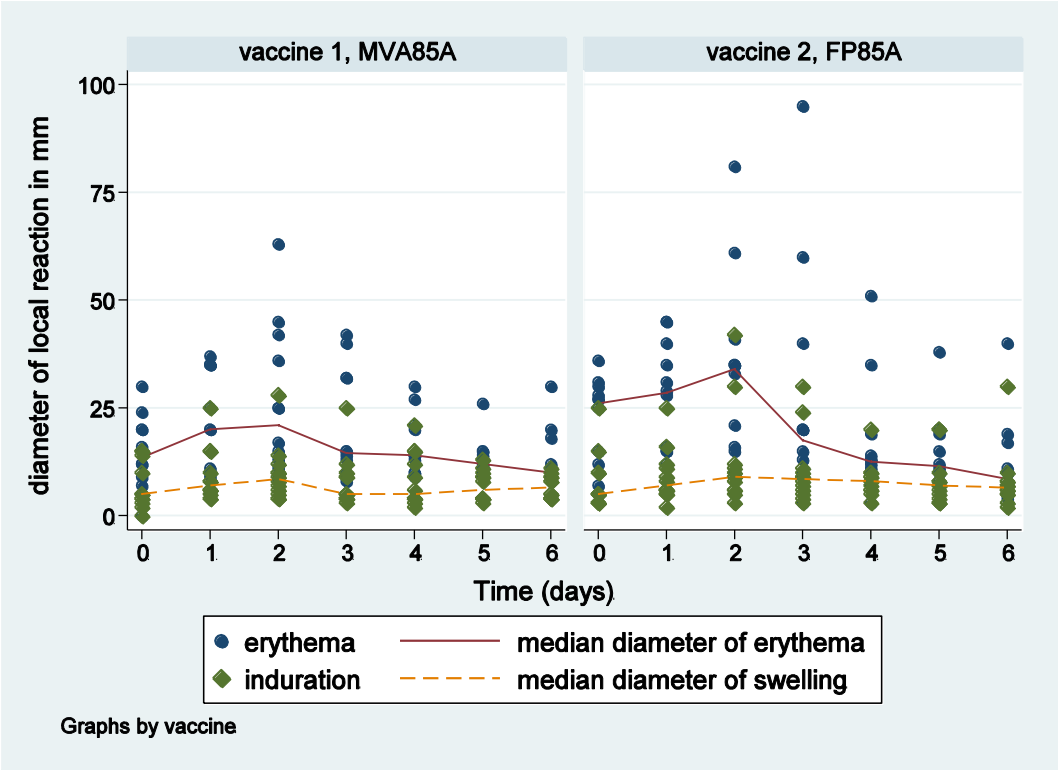
Unsolicited AEs reported by subjects in Group two are listed in **Table 4-3.**

Figure 4-3 Percentage of subjects with each local and systemic AE in Group two (regime BMF)



Left panel=MVA85A vaccination; right panel=subsequent FP85A vaccination (interval four weeks). Coloured bars=possibly, probably or definitely related to MVA85A/FP85A vaccination; green=mild; yellow=moderate; red=severe; grey bars=not related to MVA85A/FP85A vaccination.

Figure 4-4 Diameters of local reactions following MVA85A/FP85A vaccinations in Group two (regime BMF): A comparison between the first and second vaccinations



Diameter in mm (IQR)	MVA85A	FP85A	Median difference (IQR)	p value (WSR)
erythema (n=12)	20 (11 to 35)	30 (16 to 38)	-10 (-18 to 1)	0.08
induration (n=12)	8 (6 to 11)	9 (6 to 12)	0 (-4 to 3)	0.77

Vaccine 1, MVA85A=measurements recorded after MVA85A vaccination; vaccine 2, FP85A=measurements recorded after subsequent FP85A vaccination (interval four weeks). Dots=individual measurements of diameters of erythema/induration recorded in subject diary cards. Table=paired comparison of the peak diameters of erythema/induration (days one and two) after MVA85A vaccination compared to those after FP85A vaccination.

Table 4-3 Unsolicited AEs in Group two (regime BMF)

The numbers (%) of subjects with each unsolicited AE after MVA85A/FP85A vaccinations in Group two are listed.

	AE	MVA85A n=12	FP85A n=12
Related	coryzal symptoms	2 (17 %)	0
	reflux	1 (8 %)	0
	rigor[a]	0	1 (8 %)
	sinusitis	1 (8 %)	0
	sore throat	1 (8 %)	0
Not related	abdominal pain	0	1 (8 %)
	allergic symptoms	0	0
	coryzal symptoms	2 (17 %)	3 (25 %)
	depression	0	1 (8 %)
	diarrhoea	0	1 (8 %)
	dysmenorrhoea	0	1 (8 %)
	finger injury[a]	0	1 (8 %)
	foot pain	1 (8 %)	0
	hangover	0	1 (8 %)
	haemarrhoids	1 (8 %)	0
	impetigo	0	1 (8 %)
	indigestion	0	0
	laser eye surgery	1 (8 %)	0
	neck pain	0	0
	seasonal rhinitis	0	2 (17 %)
	sore throat	0	2 (17 %)
	varicose vein surgery	0	1 (8 %)

[a]moderate severity in one subject; all other unsolicited AEs were mild in severity

Safety of sequential FP85A and MVA85A vaccinations (Group three, regime BFM)

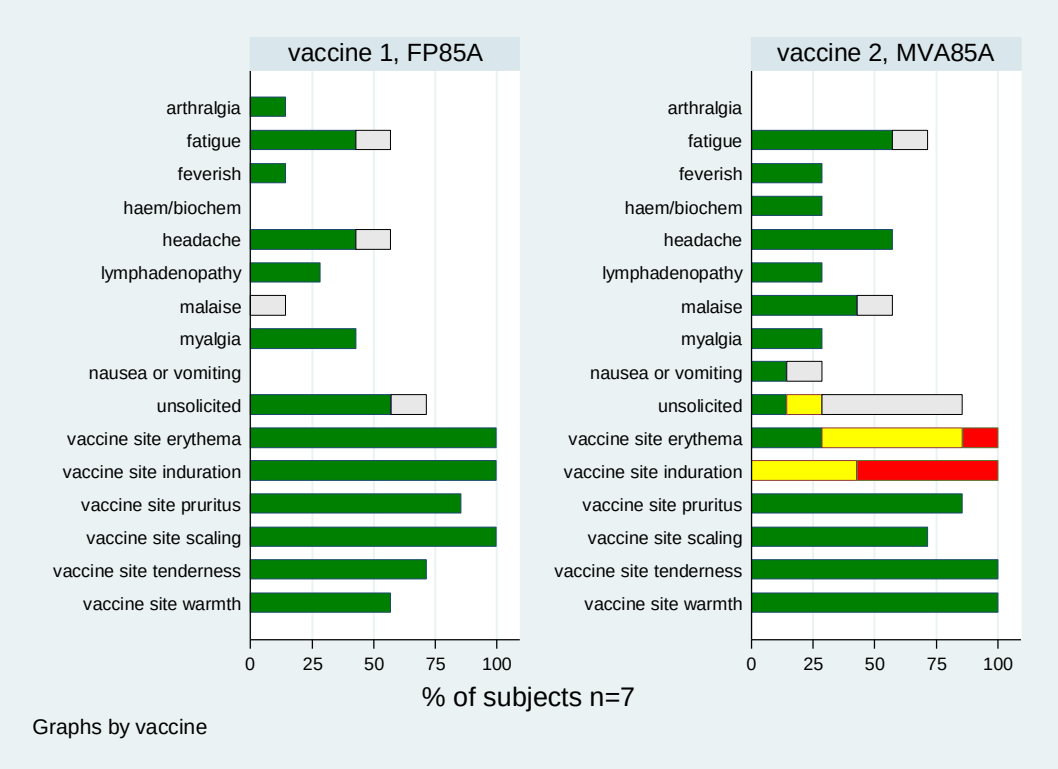
All subjects developed a local reaction (erythema, induration and scaling) and most reported vaccine site tenderness, pruritus and warmth following vaccination with FP85A **(Figure 4-5)**. All local and vaccine-related systemic AEs after FP85A vaccination were mild and there were no SAEs.

Following MVA85A vaccination at week four, all subjects developed vaccine site erythema, induration, tenderness and warmth **(Figure 4-5)**. Most subjects also reported vaccine site scaling and pruritus. The maximum measured diameters of erythema and/or induration were in the severe range in four subjects and in the moderate range in the other three **(Figure 4-6)**. All episodes of vaccine-site tenderness, warmth and pruritus were mild. Peak (day one and two) diameters of erythema, but not induration, recorded in subject diary cards were significantly larger after the second vaccination (MVA85A) compared to the first vaccination (FP85A) **(Figure 4-6)**.

There was one episode of moderate sleep disturbance four days after MVA85A vaccination. All other vaccine-related systemic AEs after MVA85A vaccination in Group three were mild and there were no SAEs. Two subjects had transiently elevated ALT (65 and 56 international units (IU)/L) one week after MVA85A vaccination and one subject had transient hypokalaemia (2.9 mmol/L) eight weeks after MVA85A vaccination.

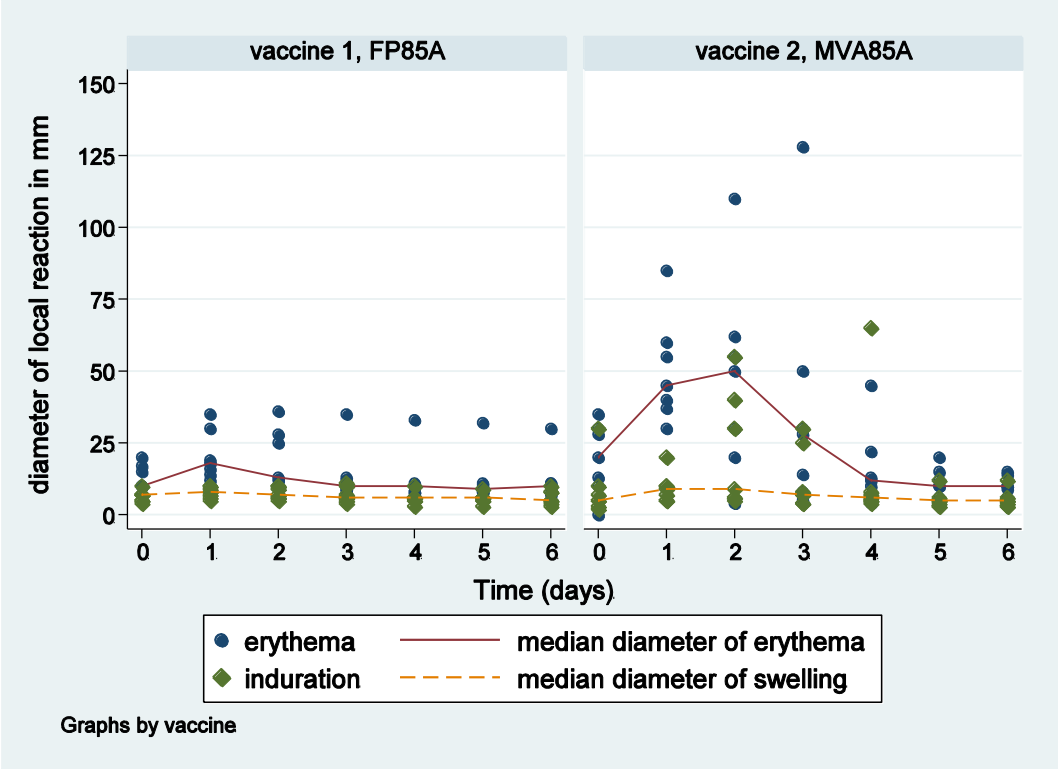
Unsolicited AEs reported by subjects in Group two are listed in **Table 4-4.**

Figure 4-5 Percentage of subjects with each local and systemic AE in Group three (regime BFM)



Left panel=FP85A vaccination; right panel=subsequent MVA85A vaccination (interval four weeks). Coloured bars=possibly, probably or definitely related to FP85A/MVA85A vaccination; green=mild; yellow=moderate; red=severe; grey bars=not related to FP85A/MVA85A vaccination.

Figure 4-6 Diameters of local reactions following FP85A/MVA85A vaccinations in Group three (regime BFM): A comparison between the first and second vaccinations



Diameter in mm (IQR)	FP85A	MVA85A	Median difference (IQR)	p value (WSR)
erythema (n=7)	17 (12 to 28)	48 (30 to 60)	-26 (-38 to 18)	0.001
induration (n=7)	8 (6 to 10)	9 (6 to 20)	-1 (-11 to 0)	0.17

Left panel=FP85A vaccination; right panel=subsequent MVA85A vaccination (interval four weeks). Dots=individual measurements of diameters of erythema/induration recorded in subject diary cards. Table=paired comparison between the two vaccinations of peak diameters (days one and two) of erythema/induration.

Table 4-4 Unsolicited AEs in Group three (regime BFM)

The numbers (%) of subjects with each unsolicited AE after FP85A/MVA85A vaccinations in Group three are listed.

	AE	FP85A n=7	MVA85A n=7
Related	coryzal symptoms	1 (14 %)	1 (14 %)
	indigestion	1 (14 %)	0
	insomnia[a]	2 (29 %)	2 (29 %)
	sore throat	0	1 (14 %)
Not related	allergic symptoms	1 (14 %)	1 (14 %)
	back pain	0	2 (29 %)
	coryzal symptoms	0	2 (29 %)
	diarrhoea[a]	0	1 (14 %)
	indigestion	0	3 (43 %)
	facial laceration	1 (14 %)	0
	neck pain	1 (14 %)	0
	shingles	0	1 (14 %)
	sinusitis	1 (14 %)	0
	toe / foot infection	1 (14 %)	0
	toothache	1 (14 %)	3 (43 %)

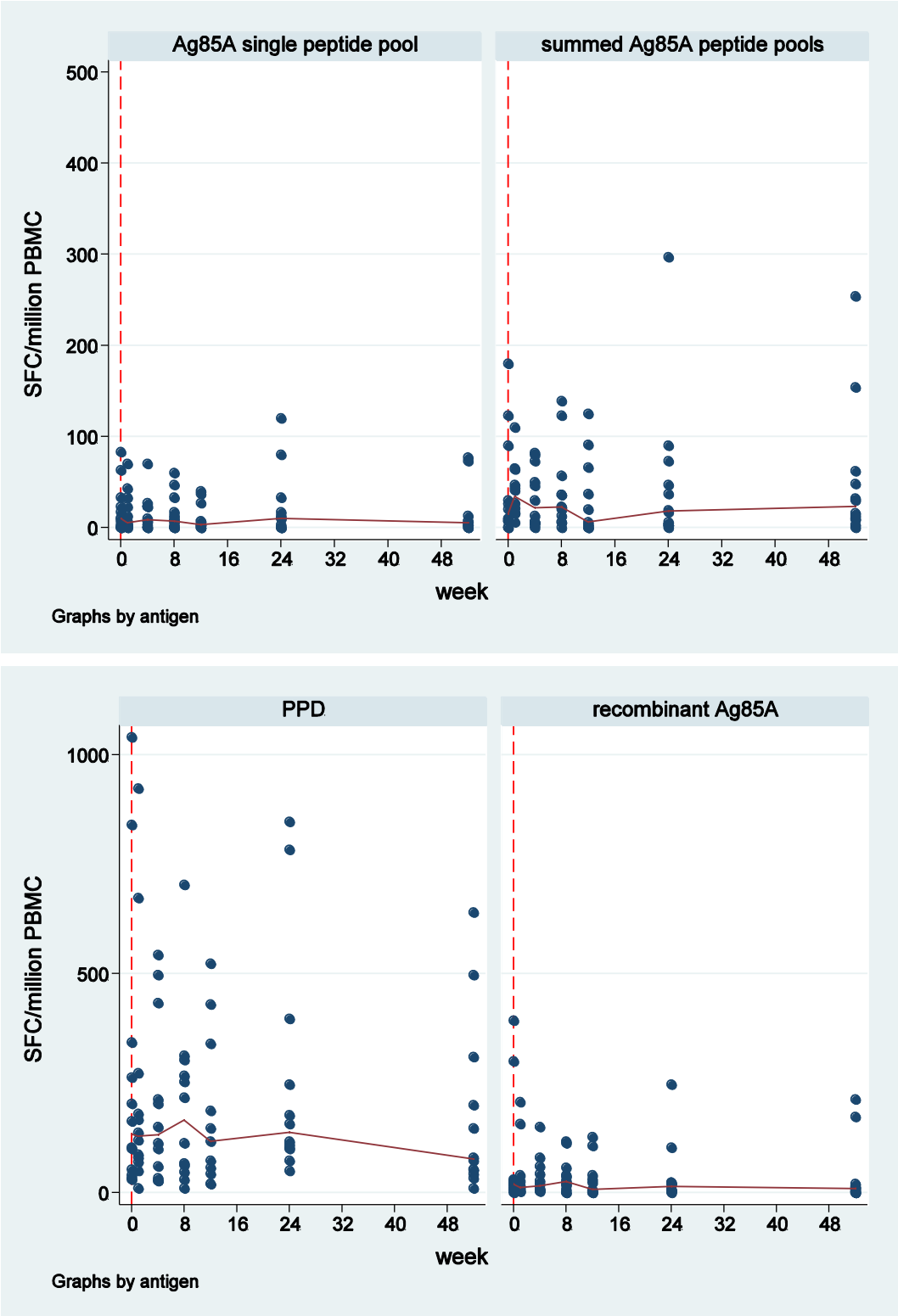
[a]moderate severity in one subject; all other unsolicited AEs were mild in severity

IFNγ ELISpot responses following FP85A vaccination in Group one (regime BF)

The kinetics and magnitude of the antigen-specific T cell responses to stimulation with Ag85A peptides, PPD and recombinant Ag85A following FP85A vaccination were assessed by IFNγ ELISpot assay **(Figure 4-7)**.

There were no increases in responses to any of the stimulating antigens after FP85A vaccination compared to pre-vaccination **(Table 4-5).**

Figure 4-7 IFNγ ELISpot responses to Ag85A and PPD in Group one (regime BF)



Dots=individual data points; red lines=connected medians; dashed x lines=FP85A vaccinations.

Table 4-5 Paired analysis comparing post-vaccination IFNγ ELISpot responses with pre-vaccination responses for FP85A vaccination in Group one (BF)

Stimulating antigen	Median pre-vaccination response (IQR)	Median post-vaccination response (IQR)[a]	Median difference (IQR)	p value (WSR)
Single pool of Ag85A peptides	10 (2 to 28)	5 (3 to 28)	-2 (-14 to 3)	0.30
Ag85A peptide pools - summed	15 (7 to 60)	34 (17 to 56)	8 (-23 to 20)	0.88
PPD	133 (47 to 303)	129 (75 to 227)	-32 (-140 to 54)	0.27
Recombinant Ag85A	19 (9 to 29)	12 (3 to 34)	-7 (-21 to -2)	0.09

[a]Interval one week

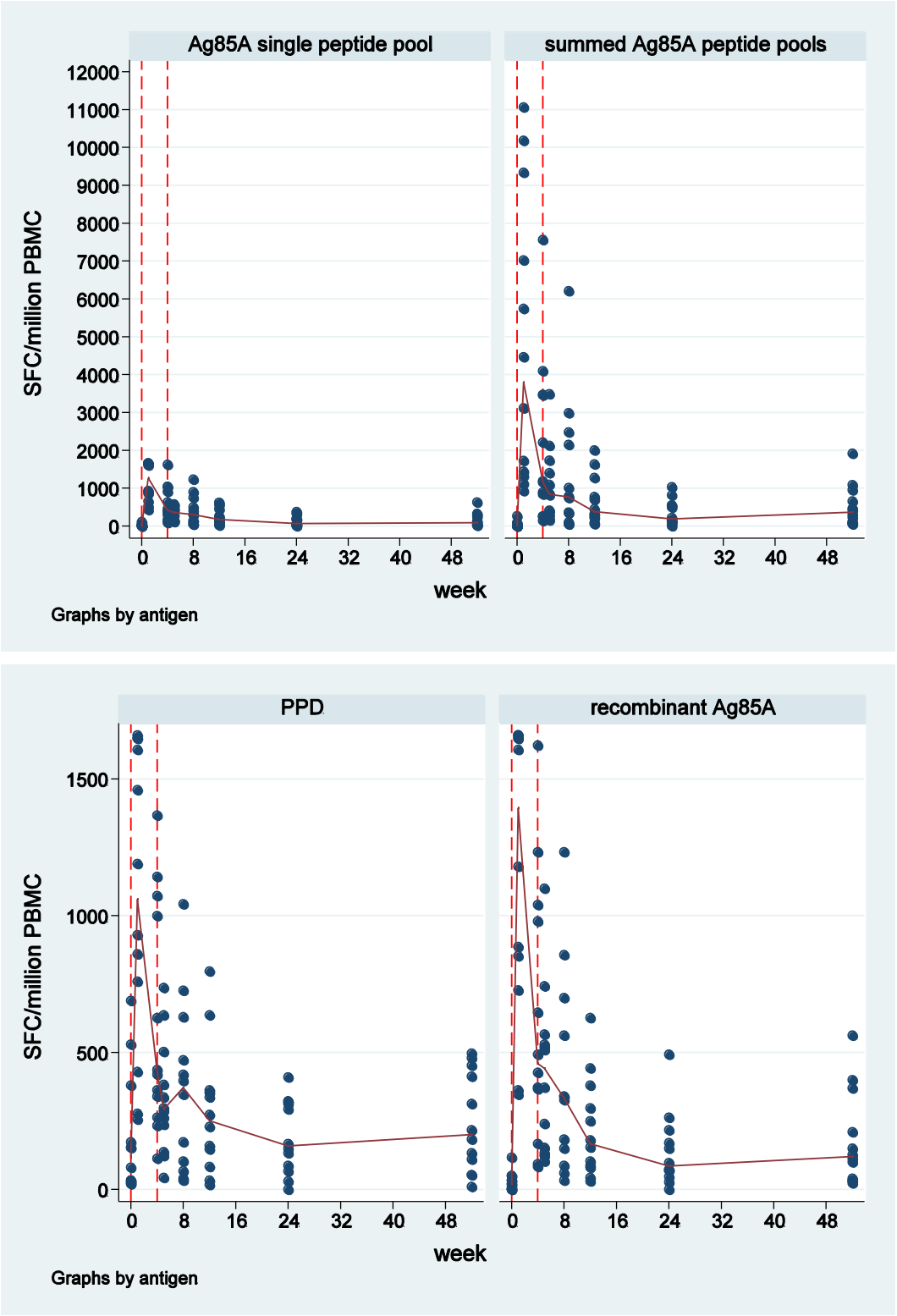
IFNγ ELISpot responses following MVA85A and FP85A vaccinations in Group two (regime BMF)

The kinetics and magnitude of the antigen-specific T cell responses to stimulation with Ag85A peptides, PPD and recombinant Ag85A following MVA85A and FP85A vaccination were assessed by IFNγ ELISpot assay **(Figure 4-8)**.

After MVA85A vaccination, expansions in IFNγ-secreting T cells were observed following stimulation with Ag85A peptides, PPD or recombinant Ag85A protein **(Table 4-6).** Responses remained significantly greater than baseline 52 weeks after enrolment for Ag85A single peptide pool (p=0.002) and summed peptide pools (p=0.003).

Following the peak in IFNγ ELISpot responses at week one, the responses declined towards a plateau and were not further boosted by subsequent FP85A vaccination at week four.

Figure 4-8 IFNγ ELISpot responses to Ag85A and PPD in Group two (regime BMF)



Dots=individual data points; red lines=connected medians; dashed x lines=vaccinations (MVA85A vaccinations=week 0; FP85A vaccinations=week 4).

Table 4-6 Paired analysis comparing post-vaccination responses with pre-vaccination responses for MVA85A and FP85A vaccinations in Group two (regime BMF)

Stimulating antigen	Vaccine	Median pre-vaccination response (IQR)	Median post-vaccination response (IQR)[a]	Median difference (IQR)	p value (WSR)
Single pool of Ag85A peptides	MVA85A (week 0)	7 (2 to 35)	1270 (760 to 1655)	1234 (733-1624)	0.002
	FP85A (week 4)	415 (228 to 974)	357 (147 to 512)	-192 (-464 to -3)	0.02
Ag85A peptide pools - summed	MVA85A (week 0)	23 (2 to 57)	3792 (1385 to 8181)	3767 (1352 to 8128)	0.002
	FP85A (week 4)	1169 (552 to 3466)	841 (1218 to 353)	-348 (-1476 to 15)	0.04
PPD	MVA85A (week 0)	117 (30 to 277)	1060 (595 to 1627)	904 (267 to 1352)	0.002
	FP85A (week 4)	429 (302 to 1037)	292 (187 to 443)	-176 (-635 to -8)	0.03
Recombinant Ag85A	MVA85A (week 0)	20 (2 to 42)	1394 (791 to 1655)	1340 (783 to 1612)	0.002
	FP85A (week 4)	460 (267 to 1010)	442 (143 to 549)	-99 (-405 to 40)	0.10

[a]Interval one week

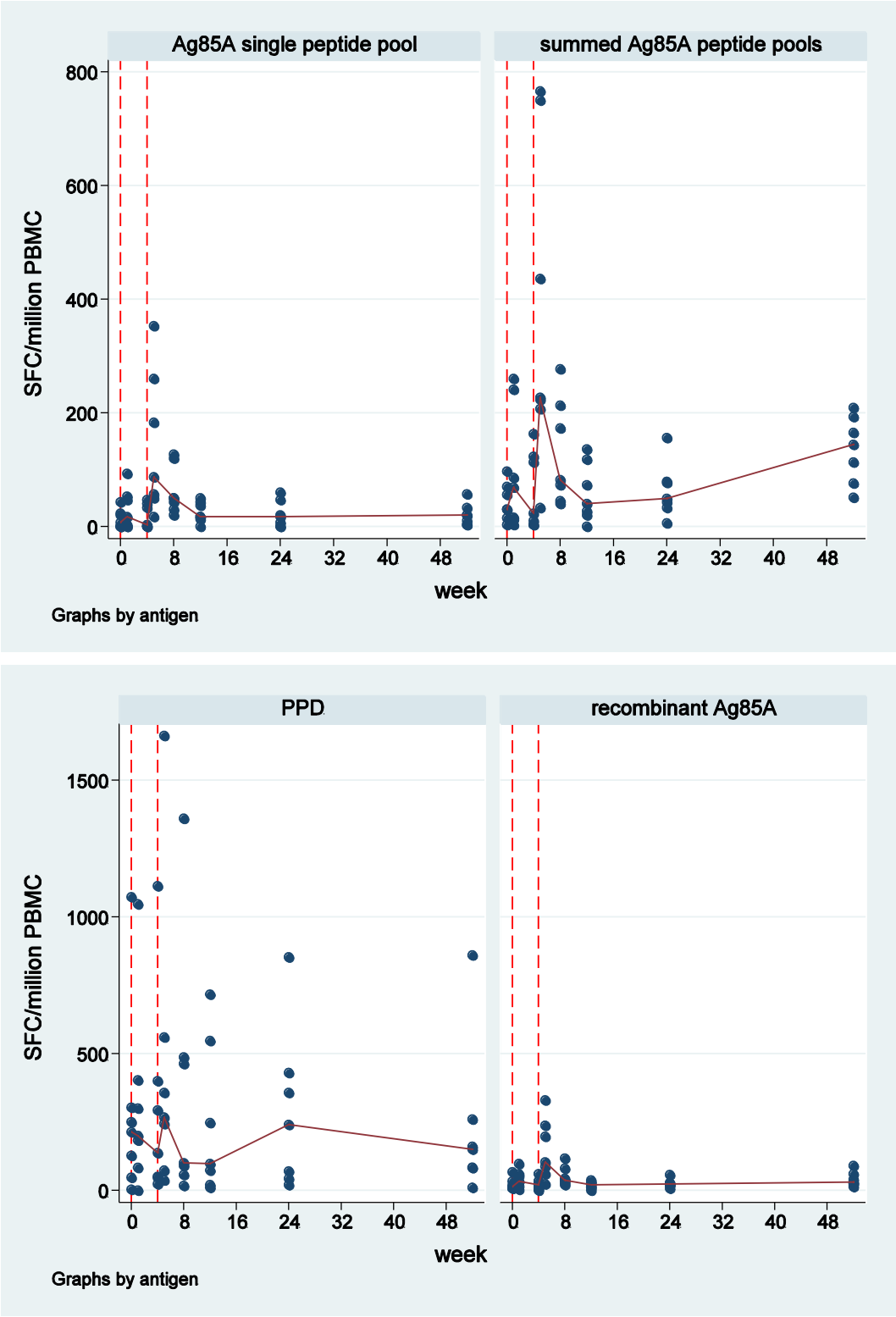
IFNγ ELISpot responses following FP85A and MVA85A vaccinations in Group three (regime BFM)

The kinetics and magnitude of the antigen-specific T cell responses to stimulation with Ag85A peptides, PPD and recombinant Ag85A following FP85A and MVA85A vaccination were assessed by IFNγ ELISpot assay **(Figure 4-9)**.

Following FP85A vaccination, there was no expansion in IFNγ-secreting T cells observed following stimulation with Ag85A peptides, PPD or recombinant Ag85A protein **(Table 4-9).**

Following MVA85A vaccination at week four, IFNγ ELISpot responses to Ag85A peptides and recombinant Ag85A protein were modestly, but significantly, elevated. There was no increase in IFNγ ELISpot responses to PPD stimulation. Summed responses to Ag85A peptide pools were maintained above baseline until week 52 ($p=0.02$) but responses to a single peptide pool were not ($p=0.07$).

Figure 4-9 IFNγ ELISpot responses to Ag85A and PPD in Group three (regime BFM)



Dots=individual data points; red lines=connected medians; red dashed lines=vaccinations (FP85A vaccinations=week 0; MVA85A vaccinations=week 4).

Table 4-7 Paired analysis comparing post-vaccination responses with pre-vaccination responses for FP85A and MVA85A vaccinations in Group three (regime BFM)

Stimulating antigen	Vaccine	Median pre-vaccination response (IQR)	Median post-vaccination response (IQR)[a]	Median difference (IQR)	p value (WSR)
Single pool of Ag85A peptides	FP85A (week 0)	7 (0 to 23)	17 (0 to 53)	10 (0 to 24)	0.07
	MVA85A (week 4)	3 (0 to 40)	87 (50 to 260)	54 (17 to 260)	0.02
Ag85A peptide pools - summed	FP5A (week 0)	30 (15 to 70)	69 (13 to 241)	10 (-12 to 171)	0.40
	MVA85A (week 4)	23 (3 to 123)	227 (207 to 750)	224 (60 to 627)	0.02
PPD	FP85A (week 0)	213 (50 to 357)	200 (83 to 403)	36 (-13 to 56)	0.18
	MVA85A (week 4)	137 (47 to 400)	267 (73 to 560)	106 (14 to 267)	0.06
Recombinant Ag85A	FP85A (week 0)	13 (7 to 33)	33 (17 to 60)	20 (-4 to 27)	0.09
	MVA85A (week 4)	20 (7 to 33)	103 (60 to 237)	96 (27 to 227)	0.02

[a]Interval one week

Comparison of IFNγ ELISpot responses to Ag85A peptides between Group two (regime BMF) and Group three (regime BFM)

There was a significant difference between median IFNγ ELISpot responses to Ag85A single peptide pool one week after MVA85A vaccination in Group two compared to Group three **(Table 4-8)**. The AUCs of the responses in each group were therefore compared and were found to be significantly higher in Group two compared to Group three **(Table 4-8)**.

Table 4-8 Comparison of responses to Ag85A single peptide pool following MVA85A vaccination between Groups two and three

	Group 2 (regime BMF) n=12 Median (IQR)	Group 3 (regime BFM) n=7 Median (IQR)	Differences in medians (95 % CI)	p value (MWU)
Post-MVA85A vaccination response, median (IQR)[a]	1270 (760 to 1655)	87 (50 to 260)	1085 (557 to 1573)	0.0004
AUC	8560 (3760 to 14114)	1110 (675 to 1235)	7816 (3100 to 18437)	0.0004

[a]Interval one week: Group 2=week 1 IFNγ ELISpot results; Group 3=week 5 IFNγ ELISpot results.

Discussion

This was the first clinical trial evaluating the safety and immunogenicity of candidate TB vaccine, FP85A, in humans.

Safety

The main purpose of Group one, FP85A vaccination (regime BF), was to determine the safety of this vaccine for the first time in humans. Most AEs were mild and there were no SAEs. The frequency and nature of local and systemic AEs were consistent with those reported in clinical trials of FP9-vectored malaria vaccines (235, 265). Also, as previously observed with other recombinant FP9 and MVA vaccines, the AE profile of FP85A vaccination was similar to that of MVA85A vaccination (235, 265).

The safety profile of MVA85A vaccination was consistent with previous experience with the same vaccine dose in healthy adult subjects in the UK (117, 194). One unexpected finding was that the maximum measured diameter of local reaction after MVA85A vaccination was in the moderate or severe range for all subjects in Group three (regime BFM), when MVA85A vaccination was preceded by FP85A vaccination with an interval of four weeks.

In Groups two and three (regimes BMF and BFM), peak diameters of erythema in diary card records were larger after the second vaccination compared to the first. This finding was out of keeping with the usual local reaction profile after poxvirus vaccines at the conservative dose of $5x10^7$ pfu used in this trial (235, 265). Previous experience with sequential heterologous poxvirus has not revealed an increased severity of local reactions after the second poxvirus (235, 265). However, vaccine-site symptoms of pruritus, scaling and tenderness were mild in all cases after the second vaccinations in Groups two and three, so there was no evidence that a larger diameter of reaction worsened vaccine tolerability.

Immunogenicity

FP85A vaccination did not induce any detectable IFNγ ELISpot responses to Ag85A peptides, PPD or recombinant Ag85A protein. In Group two (regime BMF), MVA85A vaccination induced strong Ag85A-specific cellular immunity and PPD responses, which peaked at week one and declined towards a plateau, with responses maintained above pre-vaccination levels until week 52. This was in keeping with previous trials of MVA85A vaccination (117, 194). Recombinant FP9-vectored vaccines do induce weaker immune responses than recombinant MVA vaccines, but MVA85A induces unusually high immune responses compared to other recombinant MVA vaccines (117, 236, 264,

266-268). Given the strong immune responses to MVA85A vaccination, at least modest antigen-specific immune responses following vaccination with FP85A were expected. FP85A vaccination at week four had no impact on the decline of the curve and did not further boost the immune responses to MVA85A. Recombinant FP9 vaccines have been more immunogenic as priming than boosting vaccinations when in heterologous regimes with MVA85A (236). However, the MVA85A-prime, FP85A-boost schedule in Group two (regime BMF) was included in this trial due to the protective efficacy of this regime in guinea pigs (190).

In Group three (regime BFM), as in Group one (regime BF), there were no detectable responses to FP85A vaccination. MVA85A vaccination four weeks after FP85A vaccination induced a lower magnitude of responses compared to MVA85A vaccination in Group two (regime BMF). Although the responses to MVA85A did transiently increase above pre-vaccination levels, these were only maintained until week eight, not week 52 as would be expected (117, 194). FP85A vaccination appeared to be inhibiting the immunogenicity of subsequent MVA85A vaccination. In malaria vaccine clinical trials with a number of different antigen inserts, an increased IFNγ response compared to baseline was seen in FP9-MVA regimes with a similar interval between vaccinations with different viral vectors (236, 264, 266).

A number of explanations for the lack of immunogenicity of FP85A can be considered. Identity PCR and sequencing assays had confirmed the presence of the 85A insert within the FP9 vector and no wild type FP9 was present. The clinical grade FP85A vaccine passed annual murine potency assays involving evaluation for antigen-specific cellular immune responses. The antigen insert was therefore both present within the recombinant vector and recognisable by the adaptive immune system. In the clinical trial, FP85A induced local and systemic reactions typical of poxviruses, providing additional evidence that the viral vector was immunologically active. Positive and negative controls excluded technical problems with the assays and results were reproduced using frozen samples. However, immune responses to FP85A vaccination in murine potency assays were weaker compared than those to MVA85A vaccination. The FP85A vaccine failed a murine potency assay three years following manufacture whilst the MVA85A vaccine passed all murine potency assays for up to eight years following its manufacture (McShane H, personal communication). The reasons for the differences in murine potency between the candidate vaccines FP85A and MVA85A are not clear. Subsequent exploratory studies using cryopreserved PBMC taken from subjects in this clinical trial have suggested that FP9-specific IgG antibodies with cross-reactivity for MVA85A may have inhibited the immune responses to MVA85A vaccination (227).

Limitations

Only one male was recruited into Group one (regime BF). This was due to a concurrent Phase one clinical trial recruiting male subjects only which was being conducted on the same trial site and was despite an enhanced recruitment drive (269). First-in-human regulations were not required for the FP85A vaccine due to previous experience with the vector in humans. Since the FP85A vaccine had not previously been administered to humans, the first-in-human clinical trial recommendations were incorporated into the trial planning wherever possible. This included conducting the clinical trial in a well-established clinical trial centre, but gave rise to the problem of more than one trial recruiting from the same limited pool of volunteers from the local area.

The sample size in Group three (regime BFM) was smaller than planned, with only seven of 12 subjects (58 %) recruited. This was due to the failure of the FP85A vaccine to pass the potency tests required to extend the expiry date. With the planned sample size of 12 subjects, stronger conclusions regarding the effect of sequential FP85A and MVA85A vaccinations on the diameter of local reactions could have been drawn. Similarly, only the trends in immunogenicity could be described for Group three, although the magnitude of immune responses observed for all seven subjects were lower than that consistently observed after vaccination with MVA85A.

In order to compare immunogenicity between the vaccination regimes in Groups two and three (regimes BMF and BFM) and the usual regime, BM, ideally a control group would have been recruited in which BCG-vaccinated subjects received a single vaccination with MVA85A and a second placebo immunisation. However, this clinical trial was the first time FP85A vaccination had been evaluated in human subjects and was principally to establish the safety of FP85A vaccination alone and in sequential regimes with MVA85A vaccination. A secondary objective was to observe if either of the sequential regimes resulted in strong immune responses, warranting further evaluation.

Randomising subjects into Groups two and three (regimes BMF and BFM) would have balanced the numbers recruited between each group and strengthened the immunogenicity comparison. However, the early termination of the trial was unforeseen and the sequential trial design was selected for simplicity.

Future work

The evidence obtained in this clinical trial does not support the further clinical evaluation of FP85A as a candidate TB vaccine, either alone, or in heterologous prime boost regimes with MVA85A. No detectable immune responses were detected after

FP85A vaccination and there was no evidence that preceding or subsequent FP85A vaccinations enhanced the immune responses following MVA85A vaccinations. However, stored samples and cryopreserved PBMC from this trial could be evaluated in the future for the presence of any novel biomarkers of protection against TB which may be identified in the course of TB vaccine efficacy trials.

Animal models could be used to evaluate further the anti-vector immune responses induced by FP85A and their cross-reactivity for MVA85A in comparison with other recombinant FP9 and MVA vaccines.

Adenoviruses are alternative vectors for use in prime-boost regimes with MVA85A. Phase I clinical trials of MVA85A in prime-boost regimes with candidate vaccine AERAS-402 (Ad35) and a new simian adenovirus-vectored TB vaccine candidate (ChAdOx1 85A) are underway (trial IDs NCT01683773 and NCT01829490) (131).

Conclusions

FP85A vaccination was safe when administered alone and in prime-boost regimes with MVA85A. Local reactions were larger after the FP85A vaccination in the BFM regime but were not associated with an increase in frequency or severity of vaccine-site symptoms or systemic AEs.

There were no detectable IFNγ ELISpot responses to Ag85A or PPD following FP85A vaccination in any of the three groups. Furthermore, FP85A priming vaccinations were associated with a modest magnitude of responses to subsequent MV8A5A vaccination, which were only sustained for eight weeks. Cross-reactive anti-vector immunity may be responsible for this phenomenon, which has not been previously reported for other recombinant FP9 or MVA candidate vaccines in prime-boost regimes.

Chapter 5 : Review of the safety of MVA85A vaccination in healthy adults in the UK

Introduction

The candidate TB vaccine, MVA85A, has been evaluated in a series of Phase I clinical trials in healthy adults in the UK (117, 192-194, 227). As Phase I trials have small group sizes (typically 12 subjects), only common AEs are detected by individual trials. Now that over 100 healthy adult subjects in the UK have received this vaccine, there is an opportunity to perform a detailed further evaluation of the cumulative safety data of MVA85A vaccination. A summary of MVA85A's safety profile would be useful for clinical trial protocols; volunteer information sheets; funding applications and collaborators. It would also enable comparisons with licensed vaccines and other candidate vaccines. BCG is the only vaccine currently licensed for immunisation against TB and is administered intradermally. Yellow fever vaccine is a live viral vaccine, which is widely used for immunisation of adults and is administered subcutaneously or intradermally.

This was a retrospective study to evaluate the safety of MVA85A vaccination in a series of Phase I clinical trials in healthy adults.

Aims

1. Evaluate the frequency and severity of local and systemic AEs after MVA85A vaccination for subjects grouped by vaccine regime / dose / previous BCG vaccination status / LTBI status.
2. Combine safety data for groups with similar AE profiles:
    a. evaluate the frequency and severity of local and systemic AEs ;
    b. analyse timings (onset and duration) of AEs with respect to MVA85A vaccination;
    c. describe all unsolicited AEs;
    d. compare MVA85A AE profile with the licensed vaccines against Yellow Fever and TB.
3. Analyse the relationships between objective measures of local and systemic AEs and immune responses to MVA85A vaccination.

Results

Subjects

The clinical trial described in **Chapter 4** and all previously published clinical trials of MVA85A vaccination in healthy adults in the UK were included in the study. Data were grouped according to prior mycobacterial exposure; vaccine regime and vaccine dose **(Table 5-1).**

Groups M and MM were healthy adults with negative pre-vaccination TST results and no prior history of BCG vaccination. Subjects were immunised with a single MVA85A vaccination (Group M) or two sequential doses of MVA85A vaccine, with an interval of three weeks (Group MM) (117). Subjects in Groups BM (lower dose/middle dose/upper dose) were healthy, previously BCG-vaccinated, adults who received a single MVA85A vaccination (117, 192, 194). Subjects in Group M (LTBI) were otherwise healthy adults with confirmed LTBI who received a single MVA85A vaccination (193). Subjects in Group BFM were healthy, previously BCG-vaccinated adults, who received sequential FP85A and MVA85A vaccinations **(Chapter 4)**.

Safety data from all subjects who had received an MVA85A vaccination were included in the analysis **(Figure 5-1)**. There were fewer females than males in Group M (LTBI), but more females than males in all other groups **(Table 5-2).** Subject ages were similar between groups.

Table 5-1 Clinical trials of MVA85A vaccination in healthy adults in the UK, grouped according to mycobacterial exposure, vaccination regime and MVA85A vaccine dose

Subject Group	Prior mycobacterial exposure	Vaccines administered (interval in weeks)	MVA85A Dose (pfu)	Trial ID and citation
M	naive	MVA85A	$5.0x10^7$	NCT00423566 (117)
MM[a]	naive	MVA85A-MVA85A (3)	$5.0x10^7$	NCT00423566 (117)
BM (lower dose)	BCG vaccine	MVA85A	$1.0x10^7$	NCT00465465 (192)
BM (middle dose)	BCG vaccine	MVA85A	$5.0x10^7$	NCT00427453 (194) NCT00427830 (117) NCT00653770 (227)
BM (upper dose)	BCG vaccine	MVA85A	$1.0x10^8$	NCT00465465 (192)
M (LTBI)	LTBI	MVA85A	$5.0x10^7$	NCT00456183 (193)
BFM	BCG vaccine	FP85A-MVA85A (4)	$5.0x10^7$	NCT00653770 (227)

[a]Of the 14 subjects in group M, 11 subjects received a second MVA85A vaccination within the same clinical trial after an interval of three weeks (group MM).

Figure 5-1 Flow of MVA85A-vaccinated subjects included in this analysis

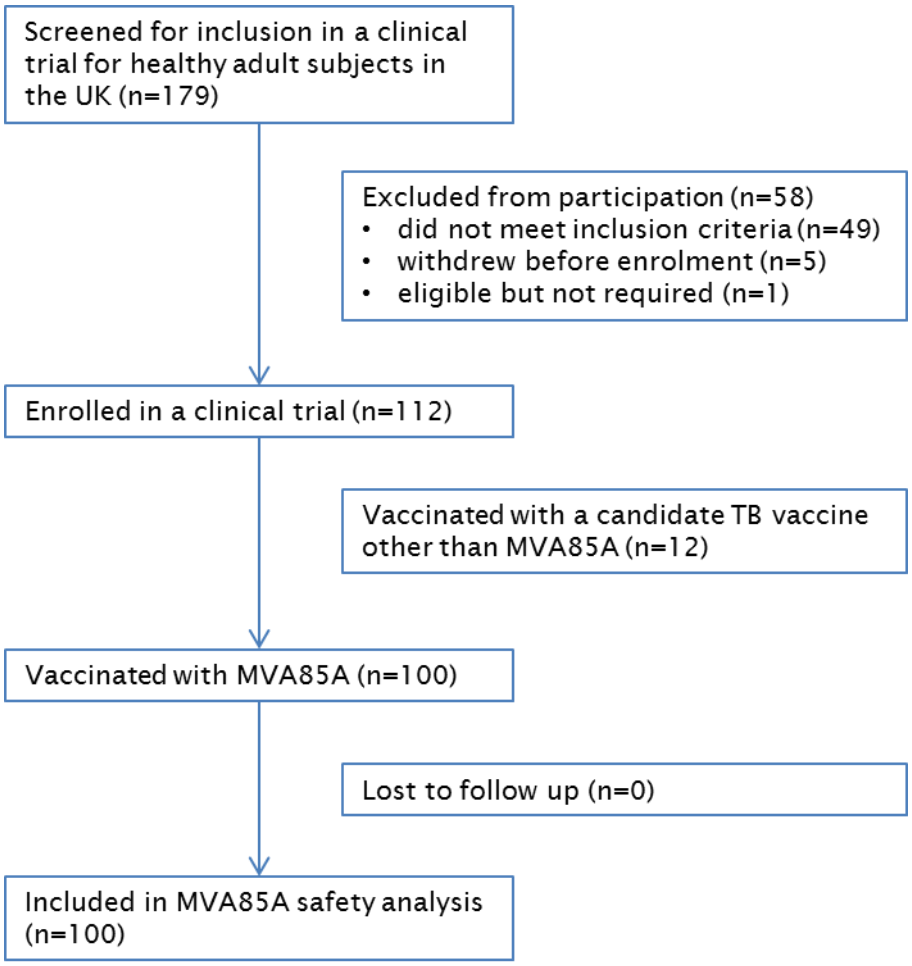


Table 5-2 Demographics of subjects vaccinated with MVA85A in the UK

Group	N[a]	Males	Age in years median (IQR)	Prior BCG vaccination
M	14	5 (36 %)	29 (22 to 31)	0
MM	11	5 (45 %)	31 (21 to 35)	0
BM lower dose	12	4 (33 %)	26 (22 to 32)	12 (100 %)
BM middle dose	43	17 (40 %)	26 (24 to 35)	43 (100 %)
BM upper dose	12	4 (33 %)	25 (22 to 28)	12 (100 %)
M (LTBI)	12	10 (83 %)	31 (26 to 44)	10 (83 %)
BFM	7	3 (43 %)	30 (27 to 47)	7 (100 %)

[a]100 subjects received 111 doses of MVA85A vaccine: 11 of the 14 subjects in Group M received a second vaccination with MVA85A (Group MM).

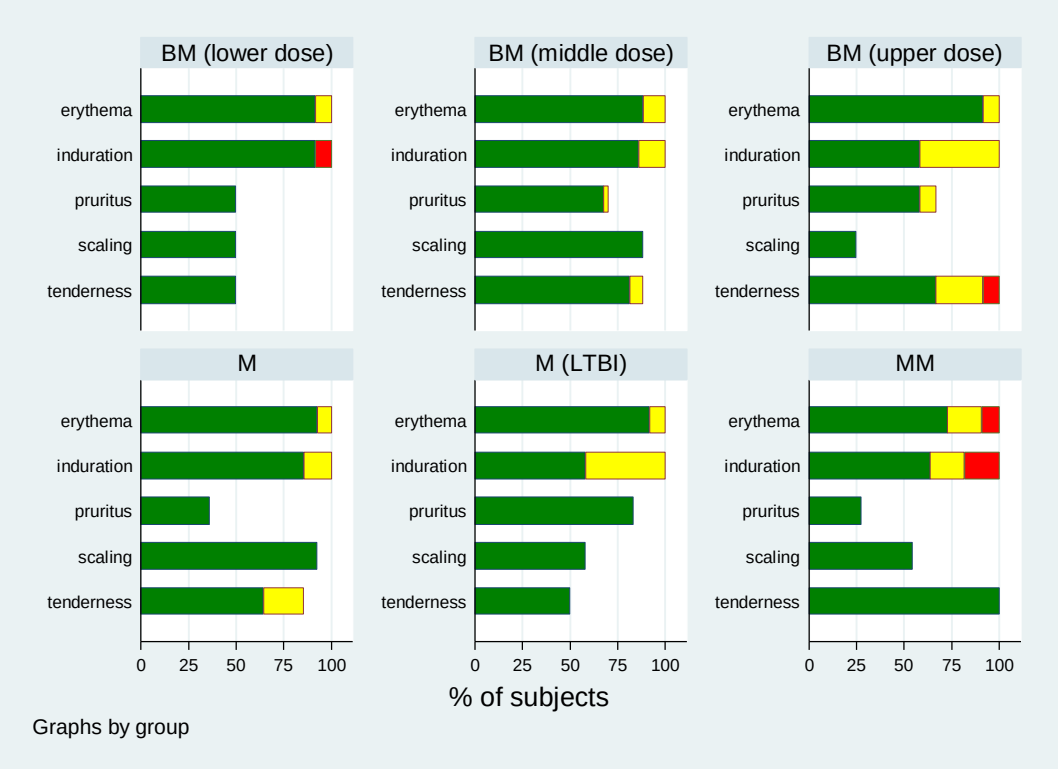
Local reactions

For the middle dose of MVA85A vaccine ($5x10^7$ pfu) the profiles of vaccine-site AEs were similar between groups M, BM and M (LTBI), with respect to local AE frequency and severity **(Figure 5-2).**

Severe erythema or induration were reported for three subjects in Group MM and five subjects in Group BFM but no subjects in the other groups had severe local reactions **(Figures 4-5 and 5-2)**. Severity classifications of erythema and induration were based on measurements of the diameters. After a second vaccination with MVA85A (Group MM), peak diameters of erythema and induration were larger compared to the peak diameters recorded after the first vaccination in the same subjects (Group M) **(Figure 5-3)**. As described earlier, peak (days one and two) diameters of local erythema, but not induration, recorded in subject diary cards were larger after the second compared to the first vaccinations in Group BFM **(Figure 4-6).**

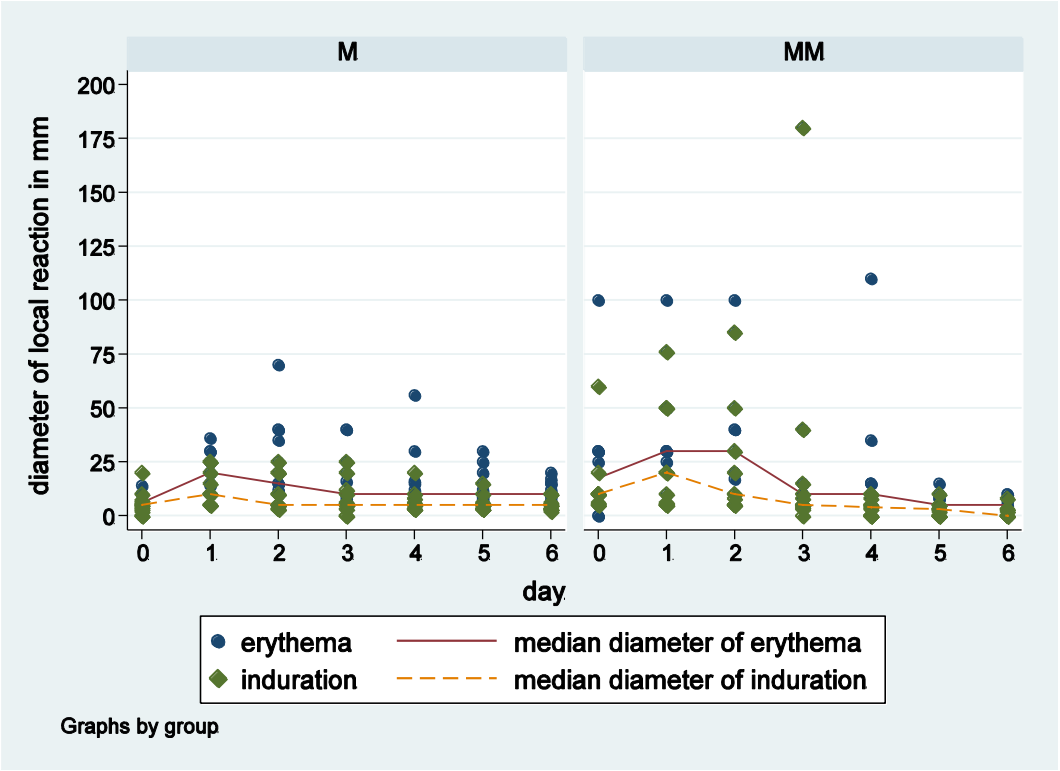
The frequencies of local AEs in the lower and upper dose groups were similar, except injection site tenderness was more frequent in the upper dose group than the lower dose group. There was one severe local AE, based on the maximum measured diameter of induration, in Group BM (lower dose) and one severe local AE, tenderness, in Group BM (upper dose). Peak (days one and two) diameters of erythema and induration from subject diary cards were similar for Group BM (middle dose) and Group BM (upper dose) **(Figure 5-4)**.

Figure 5-2 Percentages of subjects with each local AE after MVA85A vaccination



BM=MVA85A vaccination in previously BCG-vaccinated subjects: BM (lower dose)=1x10^7 pfu (n=12); BM(middle dose)=5x10^7 pfu (n=43);BM (upper dose)=1x10^8 pfu (n=12). M=single MVA85A vaccination (n=14); MM=two sequential MVA85A vaccinations (n=11). M (LTBI)=MVA85A vaccination in subjects with LTBI (n=12). Coloured bars=possibly, probably or definitely related to MVA85A vaccination; green=mild, yellow=moderate, red=severe.

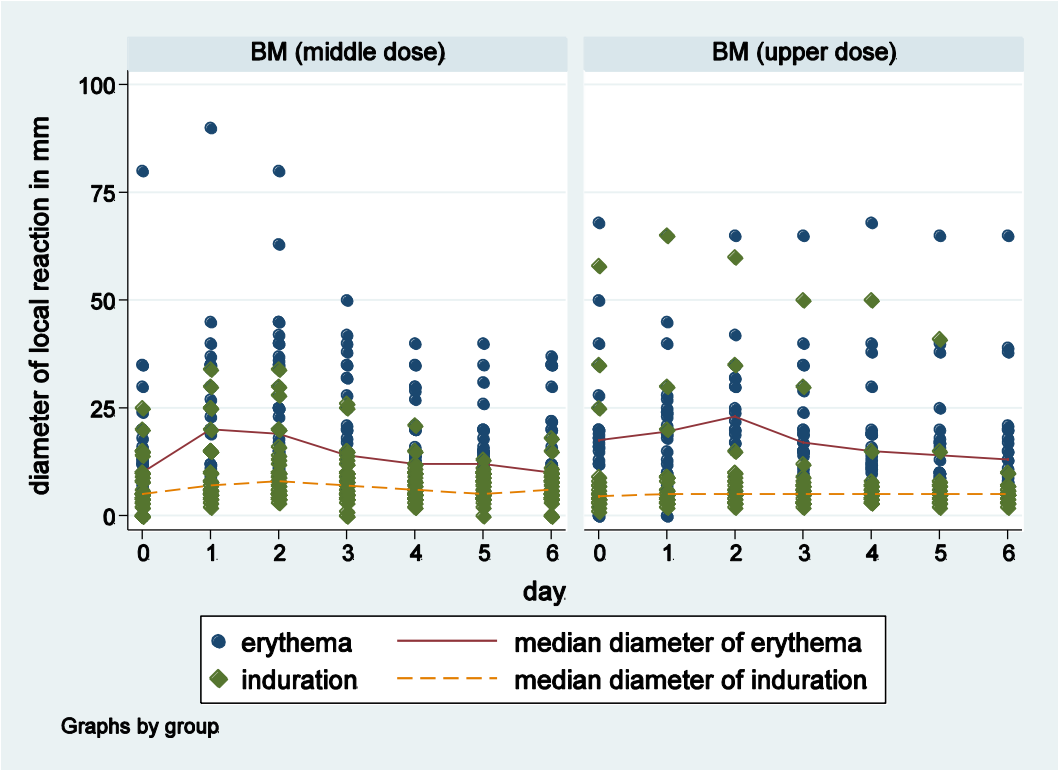
Figure 5-3 Diameters of local reactions following MVA85A vaccination: A paired analysis comparing single and sequential vaccinations with MVA85A



Diameter in mm (IQR)	MM	M	Median difference (IQR)	p value (WSR)
erythema (n=11)	30 (20 to 50)	18 (14 to 25)	10 (0 to 35)	0.01
induration (n=10)	15 (5 to 40)	10 (5 to 20)	8 (-4 to 23)	0.008

M=measurements recorded after a single MVA85A vaccination; MM=measurements recorded after second of two sequential MVA85A vaccinations (interval three weeks). Dots=individual measurements of diameters of erythema/induration recorded in subject diary cards. Table=paired comparison of the peak diameters of erythema/induration (days one and two) after the first MVA85A vaccination compared to those after the second MVA85A vaccination.

Figure 5-4 Diameters of local reactions following MVA85A vaccination: A comparison between the middle and upper doses of MVA85A vaccine



Diameter in mm (IQR)	BM (upper dose) (n=12)	BM (middle dose) (n=42)	Difference in medians (95 % CI)	p value (MWU)
erythema	21 (15 to 30)	19 (10 to 30)	3 (-1 to 7)	0.25
induration	5 (4 to 9)	8 (5 to 12)	-1 (-3 to 0)	0.03

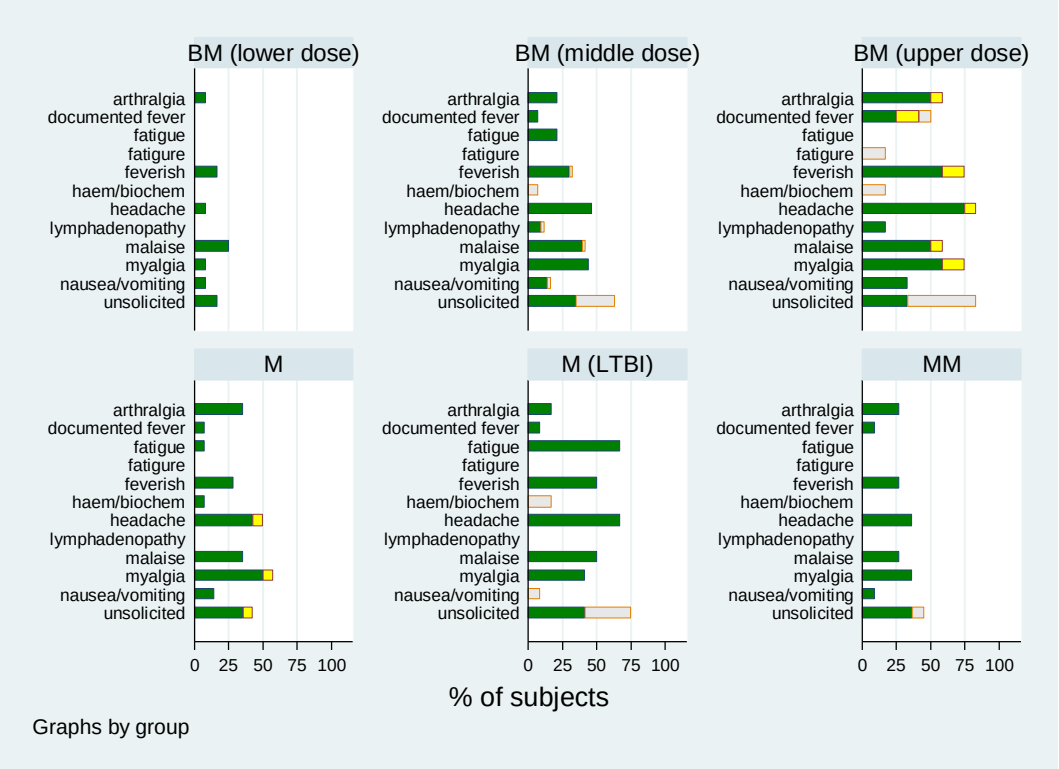
BM=MVA85A vaccination in previously BCG-vaccinated subjects: middle dose=$5x10^7$ pfu; upper dose=$1x10^8$ pfu. Dots=individual measurements of diameters of erythema/induration recorded in subject diary cards. Table=comparison of the peak diameters of erythema/induration (days one and two) between Group BM (upper dose) and Group BM (middle dose).

Systemic AEs

The frequencies and severities of systemic AEs were similar between the middle dose groups **(Figure 5-5).** In Group MM, 64 % of subjects reported any systemic AE, compared to 93 % in Group M; 86 % in Group BM (middle dose); 92 % in Group M (LTBI); and 86 % in Group BFM.

The profile of systemic AEs was similar between Group BM (lower dose) and Group BM (upper dose). As previously described, the frequencies of vaccine-related systemic AE increased with dose and five (42 %) subjects in Group BM (lower dose) reported any systemic AE, compared to twelve (100 %) in Group BM (upper dose) (192). Similarly, there were no documented fevers in Group BM (lower dose), whilst four (33 %) subjects in Group BM (upper dose) had documented fevers.

Figure 5-5 Percentages of subjects with each systemic AE after MVA85A vaccination



BM=MVA85A vaccination in previously BCG-vaccinated subjects: BM (lower dose)=1x10^7 pfu (n=12); BM(middle dose)=5x10^7 pfu (n=43); BM (upper dose)=1x10^8 pfu (n=12). M=single MVA85A vaccination (n=14); MM=two sequential MVA85A vaccinations (n=11). M (LTBI)=MVA85A vaccination in subjects with LTBI (n=12). Coloured bars=possibly, probably or definitely related to MVA85A vaccination; green=mild, yellow=moderate, red=severe; grey bars=not related to MVA85A vaccination.

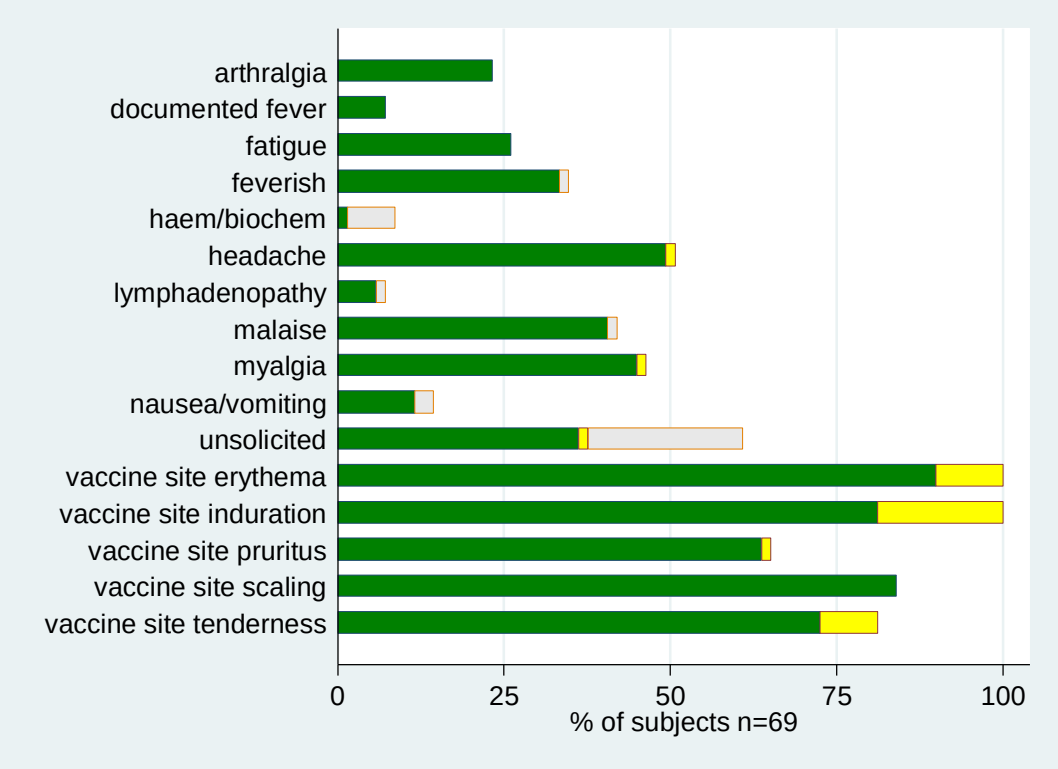
Combined analysis of AEs after MVA85A vaccination (middle dose)

All 69 subjects in Groups M, BM (middle dose) and M (LTBI) developed injection site erythema and induration, and most subjects also reported local scaling, tenderness and pruritus **(Figure 5-6).** Of all 330 local AEs, 62 (92 %) were mild, 27 (8 %) were moderate and none were severe. Injection site erythema, induration and warmth developed on the day of vaccination, followed by pain, pruritus and scaling **(Table 5-3)**. Pain, pruritus and warmth were present for no more than one week. Scaling, induration and erythema were present for averages of one, two and 15 weeks respectively. Unsolicited local AEs reported were injection site ooze in three subjects; rash in two subjects; scar in one subject; limitation in one subject and localised muscular pain in two subjects.

The most commonly reported systemic AEs which were classified as possibly, probably or definitely related to vaccination were headache, myalgia, malaise, feverish symptoms, fatigue and arthralgia **(Figure 5-6)**. Vaccine-related systemic AEs reported less frequently were nausea, documented fever and axillary lymphadenopathy. There were three moderate vaccine-related AEs (vasovagal faint, headache and muscle aches), all reported by one subject in Group M. MVA85A vaccine-related systemic AEs developed a median of one day after vaccination and resolved after a median of one day **(Table 5-3)**.

Of all 293 systemic AEs, 281 (96 %) were mild; nine (3 %) were moderate and three (1 %) were severe. The three AEs classified as severe were all SAEs, but none were classified as vaccine-related. These were a fractured ankle nine days after vaccination requiring hospitalisation in Group BM (middle dose); pregnancy diagnosed six weeks after vaccination in Group M; and a drug overdose eleven months after vaccination in Group M (LTBI). Unsolicited AEs are listed in **Table 5-4,** of which coryzal symptoms were the most commonly reported.

Figure 5-6 Combined summary of percentages of subjects with each solicited AE after MVA85A vaccination (middle dose)



Combined analysis including subjects from Group M (n=14), Group BM (middle dose) (n=43) and Group M (LTBI) (n=12). Coloured bars=possibly, probably or definitely related to MVA85A vaccination; green=mild; yellow=moderate; red=severe; grey bars=not related to MVA85A vaccination.

Table 5-3 AE onset and duration

The combined median days of onset and duration of solicited AEs for subjects in Group M, Group BM (middle dose) and Group M (LTBI) are displayed (n=69).

	AE	Median day of onset (IQR)	Median duration, days (IQR)
Local:	erythema	0 (0)	106 (37 to 168)
	pain	1 (0 to 1)	3 (2 to 6)
	pruritus	4 (2 to 7)	2 (1 to 5)
	scaling	7 (4 to 7)	8 (4 to 14)
	induration	0 (0)	15 (8 to 31)
	warmth	0 (0 to 1)	4 (1 to 6)
Systemic :	arthralgia	1 (0)	2 (1 to 2)
	documented fever	1 (0)	1 (0)
	fatigue	1 (0 to 1)	2 (1 to 3)
	feverish symptoms	1 (0 to 2)	2 (1 to 2)
	headache	1 (1 to 4)	1 (1 to 2)
	malaise	1 (0 to 2)	1 (1 to 2)
	myalgia	1 (0 to 2)	2 (1 to 2)

Table 5-4 Unsolicited systemic AEs

The combined number (%) of subjects with each unsolicited AE after vaccination with $5x10^7$ pfu MVA85A in Group M, Group BM (middle dose) and Group M (LTBI) are listed (n=69).

AE	Related[a]	Not related
abdominal pain	0	1 (0.3 %)
allergic rhinitis	1 (0.3 %)	0
anorexia	1 (0.3 %)	1 (0.3 %)
axillary pain	1 (0.3 %)	0
back pain	1 (0.3 %)	0
bone fracture	0	1 (0.3 %)
coryzal symptoms	9 (3.1 %)	17 (25 %)
deliberate self-harm	0	1 (0.3 %)
dental pain / infection	1 (0.3 %)	0
dermatofibroma excision	0	1 (0.3 %)
dizziness	1 (0.3 %)	0
eczema	1 (0.3 %)	0
haemarrhoid pain	0	1 (0.3 %)
hangover	0	1 (0.3 %)
insect bite / sting	0	1 (0.3 %)
insomnia	1 (0.3 %)	1 (0.3 %)
laser eye surgery	0 (0.0 %)	1 (0.3 %)
muscular pain	3 (1.0 %)	3 (1.0 %)
pregnancy	0	1 (0.3 %)
reflux / indigestion	2 (0.7 %)	0
sinusitis	1 (0.3 %)	0
skin infection	0	2 (0.7 %)
skin injury	0	2 (0.7 %)
skin rash	3 (1.0 %)	0
vasovagal symptoms	2 (0.6 %)	0
weight loss	0	1 (0.3 %)

[a] Related AEs=All AEs classed as possibly, probably or definitely related to vaccination

AEs after BCG and Yellow Fever vaccines: literature review

No systematic reviews or meta-analysis that included an evaluation of the reactogenicity of BCG or Yellow Fever vaccines in healthy adult subjects were identified. The search for clinical trials of intradermal BCG vaccination in healthy adults in Europe and North America yielded 211 manuscripts, of which seven met the eligibility criteria for inclusion (142, 202, 270-272). The search for Yellow Fever vaccine clinical trials in healthy adults in Europe and North America yielded 54 manuscripts, of which four met the eligibility criteria for inclusion (273-276). The published AEs after BCG and Yellow Fever vaccines are summarised in **Table 5-5.**

BCG vaccination was associated with injection site erythema, induration, pain and ulceration. Systemic AEs (headache, lymphadenopathy, and myalgia) were reported in one single arm study in 14 subjects (202).

Yellow Fever vaccine was administered intradermally to healthy adults in one RCT and subcutaneously in four RCTs. Injection site erythema, induration and pain were more frequent after intradermal than subcutaneous injections in one RCT comparing the two routes (276). Systemic AEs after Yellow Fever vaccination were headache, myalgia, fatigue, malaise, gastrointestinal events, fever and arthralgia.

Table 5-5 Frequencies of AEs after BCG and Yellow Fever vaccines

The percentages (total numbers) of subjects with each AE are displayed.

Vaccine	**BCG**	**BCG**[a]	**BCG**	**BCG**[b]	**BCG**[b]	**YF**[b]	**YF**[b]	**YF**[a]	**YF**[b]	**YF**[a]
Route[c]	**id**	**id**	**id**	**id**	**id**	**id**	**sc**	**sc**	**sc**	**sc**
Citation	(202)	(142)	(271)	(270)	(272)	(276)	(276)	(274)	(275)	(273)
N	14	17	20	48	29	77	78	659	76	106
Local erythema	100 (14)	88 (15)			97 (28)	82 (63)	32 (25)	30 (198)	18 (14)	5 (5)
Local pain	100 (14)	65 (11)			90 (26)	8 (6)	19 (15)	42 (274)	24 (18)	9 (10)
Local pruritus	64 (9)									
Local induration	100 (14)	88 (15)				68 (52)	12 (9)	21 (139)	16 (12)	0
Local ulcer		100 (17)	100 (20)	98 (47)	83 (24)					
Axillary LN[d]	14 (2)			few					5 (4)	
Arthralgia	29 (4)								9 (7)	1 (1)
Asthenia/ fatigue								30 (197)	20 (15)	8 (8)
Fever[e]	0			0		5 (4)	8 (10)	16 (102)	8 (6)	0
Feverish[f]	14 (2)									
GI event[g]								5 (32)	13 (10)	5 (5)
Headache	36 (5)							32 (210)	39 (30)	12 (13)
Malaise								19 (123)		
Myalgia	36 (5)					16 (12)	22 (27)	26 (171)	10 (8)	8 (9)
Nausea or vomiting	7 (1)							3 (21)		

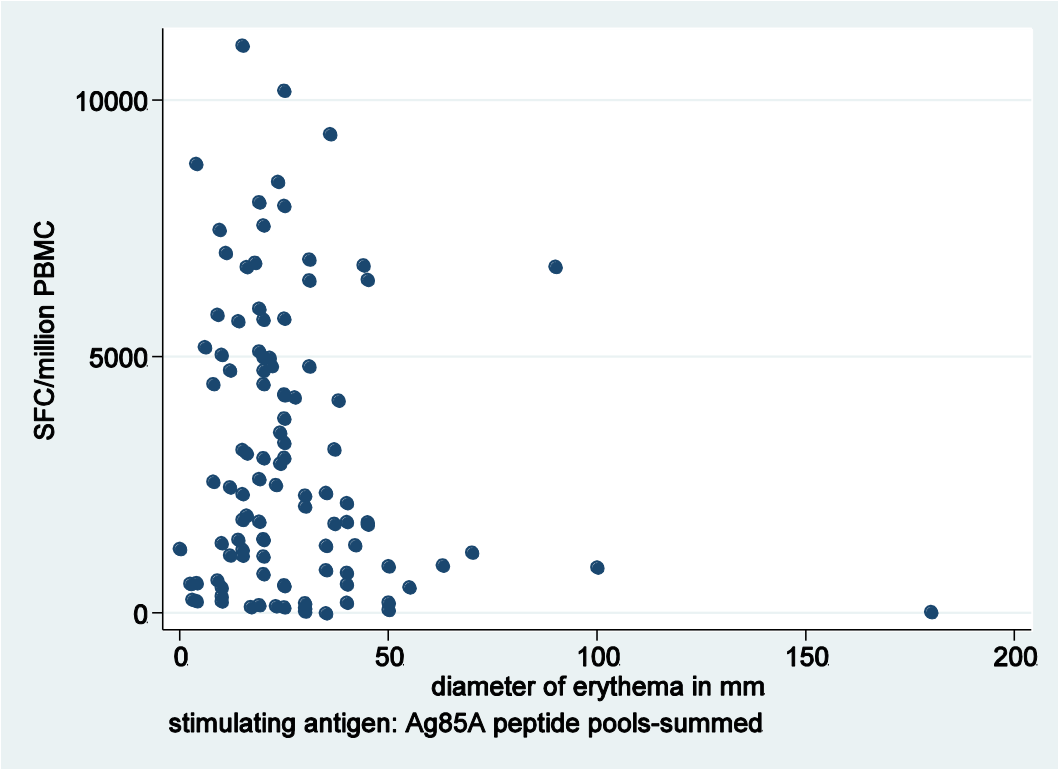
[a]double blind RCT; [b]RCT; [c]id=intradermal; sc=subcutaneous; [d]LN=lymph nodes; [e]Fever=documented fever; [f]Feverish=symptoms in the absence of documented fever; [g] GI=gastrointestinal

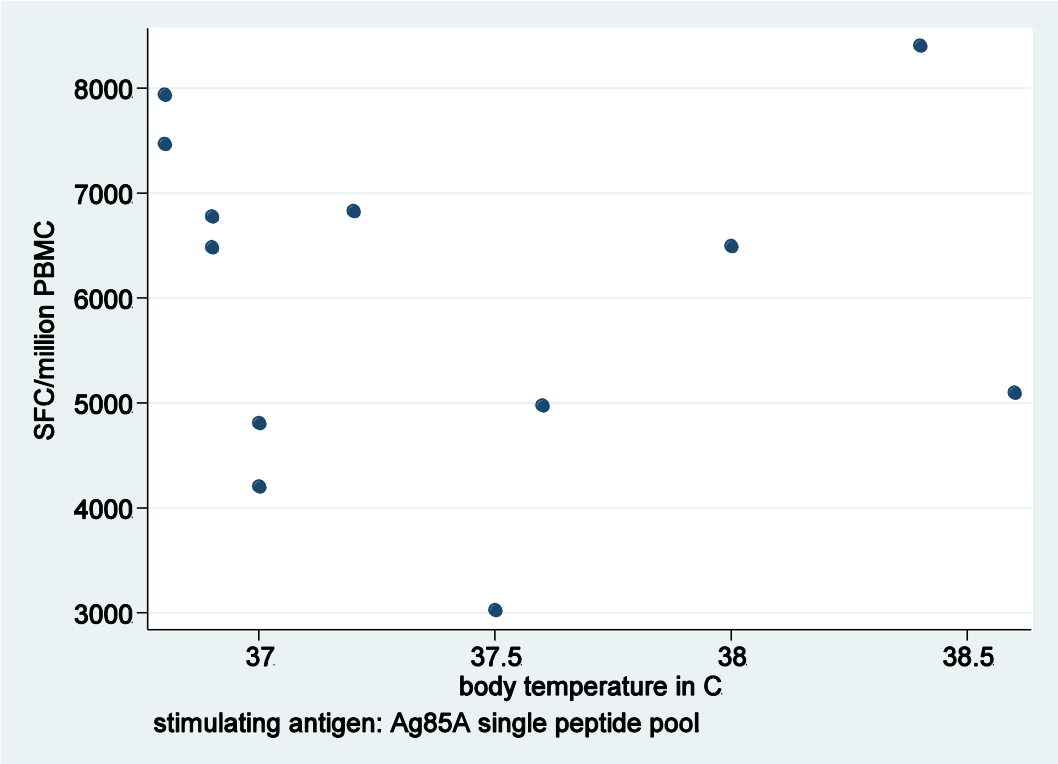
Relationships between IFNγ ELISpot responses and diameters of erythema or recorded body temperatures

Peak IFNγ ELISpot responses to summed pools of Ag85A peptides after MVA85A vaccination in six clinical trials were plotted against peak diameter of erythema for the same subjects (117, 192-194, 227). Diameters of erythema were obtained from subject diary cards and peaked on day one or two (median 23mm; IQR 15 to 35). The first investigator measurements of local reaction diameters were one week post-vaccination in the earlier trials. IFNγ ELISpot responses to Ag85A peaked one week after vaccination (median 2309 SFC/million PBMC; IQR 827 to 4980). There was no correlation between peak IFNγ ELISpot responses to Ag85A and peak diameter of erythema (Spearman rho = -0.21, p=0.51) **(Figure 5-7).**

Peak IFNγ ELISpot responses to an Ag85A single peptide pool in Group BM (upper dose) were plotted against peak body temperatures for the same subjects. Body temperatures were recorded by subjects in their post-vaccination diary cards. Peak documented body temperature was greater than 38.0 ˚C in three subjects. This group was selected because it was the only group with more than one subject with documented fever. IFNγ ELISpot responses to Ag85A were detected in all 12 subjects and peaked one week after vaccination (median 6493 SFC/million PBMC; IQR 4938 to 6992) (192). There was no correlation between peak IFNγ ELISpot responses to Ag85A and peak documented body temperature (Spearman rho=-0.14, p=0.17) **(Figure 5-7)**.

Figure 5-7 Peak IFNγ ELISpot responses and peak diameters of erythema / maximum recorded body temperatures





Upper panel=combined data from Groups M, MM, M (LTBI), BM (lower dose), BM (middle dose) and BM (upper dose); n=104. Lower panel=data from Group BM (upper dose); n=12. Peak IFNγ ELISpot responses to Ag85A=one week post-vaccination.

Discussion

A combined retrospective safety evaluation of the candidate TB vaccine, MVA85A, from a series of small, non-placebo controlled, clinical trials in adults in the UK has been presented. The AE profile after the middle dose of MVA85A vaccination was similar between mycobacterially naïve, previously BCG-vaccinated subjects and subjects with LTBI. All subjects developed local erythema and induration, which were associated with tenderness, pruritus and scaling in over two thirds of subjects one to two days after vaccination. Erythema resolved over three to six months, but this term describes any reddening or pinkness at the injection site. Warmth and pain, markers of the acute inflammatory process, were present for a median of three to four days. Of all local AEs, 92 % were mild and none were severe. The majority of subjects also reported brief and mild viral symptoms one day after MVA85A vaccination, with documented fever in 3 % of subjects. There were no severe systemic vaccine-related AEs.

As previously described, there was a lower frequency of pain in Group BM (lower dose) compared to Group BM (upper dose) and systemic AEs were more frequent in Group BM (upper dose) (192). A dose effect was also seen with an objective measure of a systemic AE. Documented fever was reported by 33 % of subjects in Group BM (upper dose) compared to 3 % in the combined middle dose groups and none in Group BM (lower dose). Whilst there was a statistically significant difference between the diameters of induration in Group BM (middle dose) and Group BM (upper dose), the median difference in diameter was one mm, which was not considered clinically meaningful. The peak (day one and two) diameters of erythema and induration were larger after a second MVA85A vaccination, compared to the first MVA85A vaccination, but local reactions were similar in size from day three. This finding was consistent with comparisons between the first and second poxvirus vaccinations in the BMF and BFM regimes described in **Chapter 4.** However, larger local reactions after a second poxvirus vaccination have not been previously described for other recombinant FP9 and MVA vaccines and group sizes were small, meaning the data were susceptible to being skewed by a small number of measurements. In addition the frequency and severity of other local and systemic AEs were similar between the first and second vaccinations.

AE profiles were compared to published data of two licensed vaccines, the existing TB vaccine, live attenuated *M.bovis* BCG and a live viral vaccine against Yellow Fever. These vaccines were selected since they have similarities to MVA85A in terms of incorporating mycobacterial antigens (BCG) or a live viral vector (Yellow Fever) and are widely administered, including to healthy adults in temperate countries. The transient local inflammation after MVA85A vaccination compares favourably to the injection site

ulceration associated with BCG vaccination. Although most trials did not report any systemic AEs after BCG vaccination, it is a live mycobacterial vaccine with a risk of more serious systemic (disseminated) BCG infection. Disseminated BCG infection is unusual in immunocompetent individuals, but the risk is much higher in the immunocompromised, and HIV infection in infants is now a contraindication to vaccination with BCG (20). Intradermal Yellow Fever vaccination induces vaccine-site erythema, induration and tenderness in most subjects, but local reactions are less frequent when the vaccine is delivered by subcutaneous injection (the usual route) (273-276). Comparable to MVA85A, Yellow Fever vaccination induces a transient mild viral syndrome, with documented fever reported in some subjects.

The frequency of local reactions seen after immunisation with MVA85A, particularly erythema and induration, reflects the intradermal route of administration as well as the vaccine itself. Local reactions are more frequent after intradermal compared to intramuscular or subcutaneous injections (265, 276-280). Safety and immunogenicity of different routes of vaccination with MVA85A are currently being evaluated. The systemic AEs reported after MVA85A vaccination are more comparable to those reported after other live viral vectored vaccines than the existing TB vaccine, BCG, suggesting the vector determines AEs to a greater extent than the insert (182, 235, 278, 281-284). This is intuitive, since the viral vector comprises the vast majority of the antigenic stimulus, and MVA is chosen as a vector for antigen delivery precisely because of the powerful innate immune response induced by the vector. Several candidate protein-adjuvant TB vaccines are also being developed (M72+AS01, H4/AERAS-404+IC31, H56/AERAS-456+IC31) and the leading candidate malaria vaccine, RTS,S is adjuvanted with AS01B or AS02A (153, 169, 170). Current data suggest that using adjuvants will, like live viral vectors, induce local and systemic reactions and that the safety profile of MVA85A is similar to that of candidate protein adjuvant vaccines (156, 163, 285, 286).

No correlations were found between peak immune responses (one week post-vaccination) as measured by IFNγ ELISpot assay and objective measures of local reactions (diameters of erythema) or systemic AEs (documented fever). The immune assays were evaluating the adaptive cellular immune response seven days post-vaccination. As both the onset of systemic AEs and the peak diameter of erythema and induration occur within the first 24 to 48 hours after vaccination, the innate immune response can be speculated to be largely responsible for the AE profile of MVA85A. If the effect of sequential poxvirus vaccination on increasing local reaction size is real, this may indicate that the adaptive immune response is also involved.

Limitations

Vaccination placebo control groups had not been included in any of the studies evaluated. The aims of these early studies were to identify any safety concerns associated with pre-vaccination mycobacterial exposure and to rapidly facilitate placebo-controlled efficacy clinical trials in adults in TB-endemic regions, currently the only means for testing TB vaccine efficacy in humans. In view of the lack of control groups, MVA85A safety was compared with published data of the safety of a licensed TB vaccine, BCG and of a licensed live viral vaccine against Yellow Fever.

Without a placebo control group, the frequencies of non-specific systemic AEs that would be reported by the same population in the same conditions in the absence of MVA85A vaccination are not known. RCTs of other vaccines have reported high rates of headache (19 %), tiredness (18 %) and any systemic AE (50 %) in the placebo groups (277, 280). Interestingly, the only clinical trial reporting any systemic AEs after BCG vaccination was performed by the MVA85A research group, using the same methods as the MVA85A clinical trials, with a seven day subject diary card and soliciting the same AEs (202). In this trial, headache, arthralgia, myalgia and feverishness were reported by up to one third of subjects.

Future Work

In order to compare the tolerability and acceptability of the leading approaches for new vaccine candidates, a head to head trial would be required. This would also be informative for the development of new vaccines against other organisms.

Standardizing safety reporting during clinical trials would facilitate comparisons between investigational vaccines. One such measure would be adopting the ICH Medical Dictionary for Regulatory Activities (MedDRA) or Brighton Collaboration definitions for describing AEs (224, 287). Secondly, the WHO has recently published updated guidelines for classifying causality of SAEs following licensed immunisations, based on a working group report on vaccine pharmacovigilance, which could be adapted for use in future clinical trials (288, 289).

A study comparing the effect of active (solicited AEs) and passive (non-solicited AEs) safety data collection on AE reporting and AE frequency is proposed to test the hypothesis that clinical trials which collect more active AE data are likely to report a greater frequency of AEs.

Pathways that have been shown to play important roles in sensing MVA and coordinating the innate response include TLR2-TLR6-MyD88, MDA-5-IPS-1 and NALP3 (290, 291). Work analysing the innate immune response after MVA85A vaccination will

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

Conclusions

The candidate TB vaccine, MVA85A, has been safely administered to over 100 healthy adult subjects in the UK, inducing strong cellular immune response. Comparisons with published safety data for other vaccines suggest that MVA85A has comparable reactogenicity to the intradermal live viral Yellow Fever vaccine. Local and systemic AEs after MVA85A vaccination were not affected by increasing mycobacterial exposure and did not correlate with the adaptive cellular immune response to MVA85A.

Chapter 6 : Determining the validity of hospital laboratory reference intervals for healthy young adults participating in early clinical trials of candidate vaccines

Introduction

The accurate interpretation of biochemical and haematological blood analyses are an important aspect of clinical trials of preventive vaccines, both for trial inclusion decisions and post-intervention safety evaluation. RIs are typically derived from historical literature values, laboratory instrument manufacturers or appropriate statistical analysis of routine clinical laboratory patient data, all of which can introduce sampling bias (229, 292). Blood results are affected by individual and laboratory factors, such as differences in age, gender, ethnicity, laboratory methods and laboratory instruments (293-295). Whilst the gold standard is to minimize laboratory and population differences by computing institutional RIs, there are inherent problems with obtaining a sufficient sample size for such analyses, so validation of externally obtained RIs is recommended (228). In clinical trials of preventive vaccines, abnormalities in blood results from healthy young adults are usually defined by comparison with all age institutional or published RIs (296-299). There is a need to validate the RIs used in clinical trials to ensure they are applicable to the trial population.

This was a retrospective study to evaluate the applicability of institutional RIs for young healthy adult subjects using a dataset of blood results from vaccine clinical trial programmes at the Jenner Institute, Oxford, UK.

Aims

1. Calculate the central 95 % ranges of each analyte within the study population and compare these with the institutional RIs.
2. Analyse the within-person variation of each analyte within the study population and compare these with published data.
3. Conduct a within-person paired analysis of pre- and post-intervention results for each analyte, where the intervention was the candidate TB vaccine, MVA85A.
4. Review the literature to suggest explanations for any observed discrepancies between the study population and the institutional RIs.

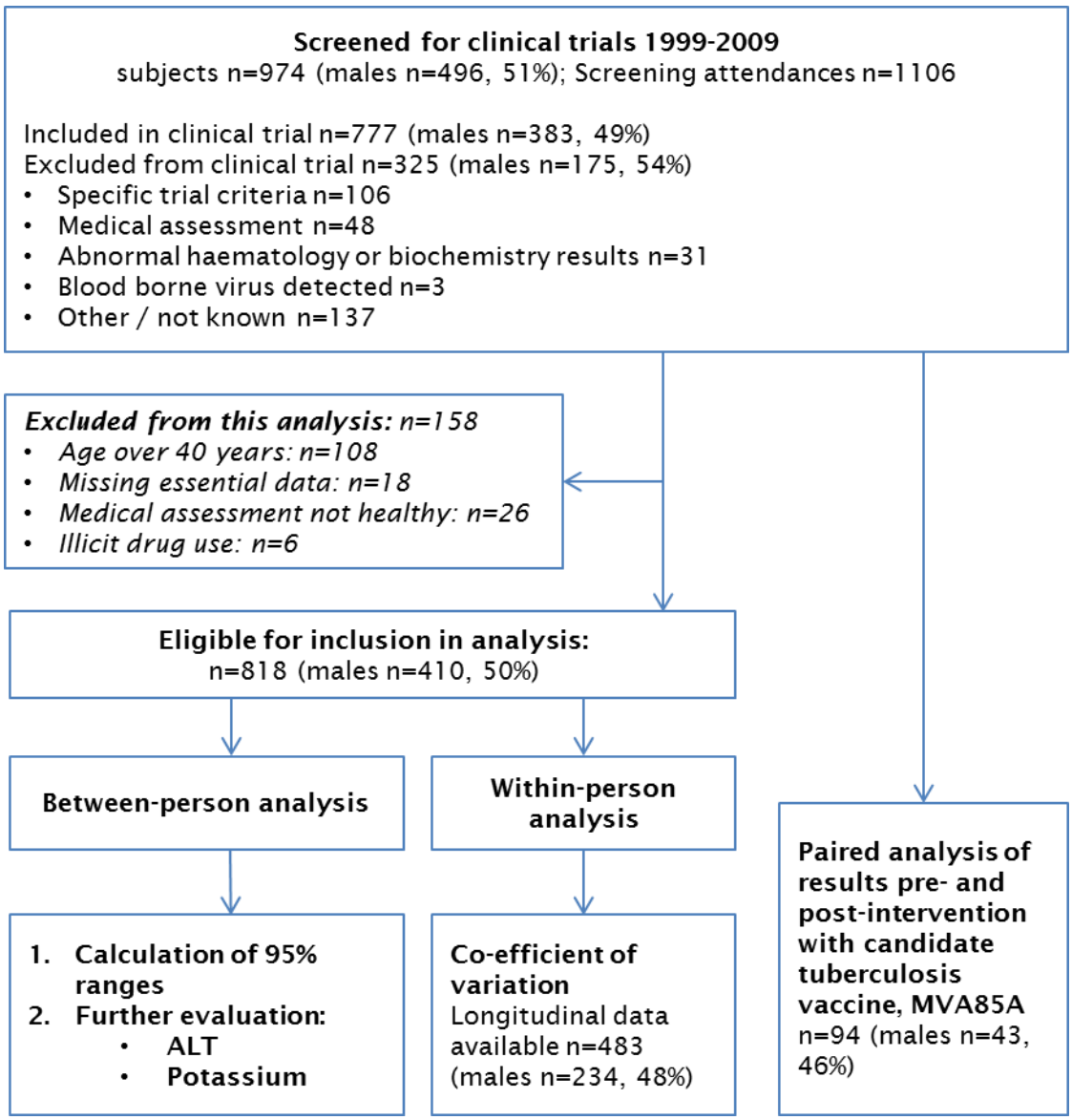
5. Develop recommendations for the interpretation of blood results obtained in young healthy adults participating in clinical trials in Oxford, UK.

Results

Clinical trials, subjects and sample results

Between 1999 and 2009, 38 clinical trials of candidate malaria, TB, influenza and hepatitis C vaccines in healthy adult subjects were conducted (trial ID of an unpublished trial: NCT00548444) (117, 192-194, 202, 227, 234, 235, 264, 265, 267, 283, 300-313). In total, 1106 screening appointments were attended by 974 subjects, of which 70 % were enrolled in a clinical trial **(Figure 6-1)**. Subjects aged 18 to 60 years were screened for inclusion in clinical trials, with a median female age of 25.3 years (IQR 9.1). The median age of male subjects was 26.3 years (IQR 9.7). Ethnicity data was not routinely collected for all trials, but in a subset of 115 subjects participating in TB vaccine clinical trials, 80 to 100 % of subjects were born in Europe (192, 194, 227, 300).

Figure 6-1 Sample selection for analysis



The flow of subject data which was included in each of the four analyses is shown. Where the same subject participated in more than one clinical trial, only the results from the first screening attendance were included in the between-person analyses.

Between-person variation

The number of subjects aged 40 years and under who were included in the between-person analyses was 818, of which 410 (50 %) were male **(Figure 6-1)**. Female subjects' median age was 24 years (IQR 7) and the median age of males analysed was 25 years (IQR 7).

Fewer than 20 data points from samples run on the Bayer Axon were within each analyte and gender subgroup, which was insufficient for between-instrument comparisons. Samples run on the Bayer Axon were therefore excluded from further analysis. Results obtained by the Abbott Aeroset and Siemens ADVIA 2400 analysers were compared using the Kolmogorov-Smirnov test for differences in distribution of results **(Table 6-1)**. Where a statistically significant difference in distributions was identified ($p<0.05$), the data were examined visually **(Appendix)**. No differences were determined to be clinically significant and results from the two analysers were combined for subsequent analyses.

Analyte and gender subgroups were partitioned into equally sized age tertiles and evaluated by Kruskal Wallis **(Table 6-2)**. A significant difference between age tertiles was detected for ALT results in males. For subsequent analyses, ALT results were subgrouped into two age ranges (18 to 24 years and 25 to 35 years) to allow a sufficient sample size for RI calculations (greater than 120 subjects per subgroup). There were no differences between age tertiles for the other analyte and gender subgroups and all results for subjects aged 40 years and under were combined for subsequent analyses.

Table 6-1 Biochemistry results partitioned by analyser

Analyser:	Abbott Aeroset		Siemens Advia 2400		
	N	Median (IQR)	N	Median (IQR)	p value[a]
Albumin g/L					
females	201	45 (4)	178	46 (4)	0.001
males	199	46 (4)	176	48 (3)	0.13
ALP IU/L					
females	199	126 (48)	175	130 (57)	0.30
males	198	156 (51)	176	161 (57)	0.47
ALT IU/L					
females	117	16 (8)	178	16 (7)	0.41
males	121	21 (14)	175	23 (13)	0.14
Bilirubin µmol/L					
females	199	9 (4)	178	9 (4)	0.03
males	199	11 (7)	175	12 (6)	0.03
Creatinine µmol/L					
females	202	79 (10)	178	81 (15)	0.12
males	197	92 (13)	177	94 (14)	0.41
Potassium mmol/L					
females	202	3.7 (0.4)	176	3.6 (0.4)	0.37
males	199	3.7 (0.5)	177	3.6 (0.5)	0.01
Sodium mmol/L					
females	202	139 (3)	178	139 (3)	1.00
males	199	140 (2)	177	141 (3)	0.03
Urea mmol/L					
females	169	3.9 (1.4)	177	4.2 (1.1)	0.01
males	180	5.0 (1.5)	174	4.9 (1.4)	0.98

[a]Kolmogorov-Smirnov

Table 6-2 Age-partitioned results

Analyte	Gender	N, median (range)			p value[a]
		Age tertile 1	Age tertile 2	Age tertile 3	
Albumin	female	127, 21 (18-22)	126, 24 (22-27)	126, 31 (27-39)	1.00
	male	125, 21 (18-23)	125, 25 (23-27)	125, 31 (27-39)	0.16
ALP	female	125, 21 (18-22)	125, 24 (22-27)	124, 31 (27-39)	0.39
	male	125, 21 (18-23)	125, 25 (23-27)	124, 31 (27-39)	0.10
ALT	female	99, 21 (18-22)	98, 24 (22-27)	98, 31 (27-39)	0.58
	male	99, 21 (18-23)	99, 25 (23-28)	98, 32 (28-39)	0.0001
Bilirubin	female	126, 21 (18-22)	126, 24 (22-27)	125, 31 (27-39)	0.82
	male	125, 21 (18-23)	125, 25 (23-27)	124, 31 (27-39)	0.21
Creatinine	female	128, 21 (18-22)	126, 24 (22-27)	126, 31 (27-39)	0.82
	male	125, 21 (18-23)	125, 25 (23-27)	124, 31 (27-39)	0.40
Potassium	female	126, 21 (18-22)	126, 24 (22-27)	126, 31 (27-39)	0.39
	male	126, 21 (18-23)	125, 25 (23-27)	125, 31 (27-39)	0.20
Sodium	female	128, 21 (18-22)	126, 24 (22-27)	126, 31 (27-39)	0.67
	male	126, 21 (18-23)	125, 25 (23-27)	125, 31 (27-39)	0.70
Urea	female	116, 21 (18-22)	115, 24 (22-27)	115, 31 (27-39)	0.29
	male	118, 21 (18-23)	118, 24 (23-27)	118, 31 (27-39)	0.87
Haemoglobin	female	126, 21 (18-22)	126, 24 (22-27)	125, 31 (27-39)	0.99
	male	125, 21 (18-23)	124, 25 (23-27)	124, 31 (27-39)	0.76
MCV	female	126, 21 (18-22)	126, 24 (22-27)	125, 31 (27-39)	0.86
	male	125, 21 (18-23)	124, 25 (23-27)	124, 31 (27-39)	0.33
Platelets	female	126, 21 (18-22)	126, 24 (22-27)	125, 31 (27-39)	0.57
	male	125, 21 (18-23)	124, 25 (23-27)	124, 31 (27-39)	0.68
White cells	female	126, 21 (18-22)	126, 26 (22-27)	125, 31 (27-39)	0.85
	male	125, 21 (18-23)	124, 25 (23-27)	124, 31 (27-39)	0.23

[a]Kruskal Wallis one way analysis of variance by ranks

The proportions of results outside the institutional RI were determined and the calculated 95 % ranges for each analyte were compared to the institutional RI **(Table 6-3)**.

The calculated 95 % range for ALT in female subjects was the same as the institutional RI. For males aged 18 to 24 years, the calculated 95 % ranges were similar to the institutional RI. For males aged 25 to 35 years, 12 % of results were above the upper limit and the calculated upper limit (71 IU/L) was more than 1.5 times greater than the institutional upper limit for ALT (45 IU/L). For male subjects, ALT results increased with increasing age in male subjects (non-parametric trend p=0.001) **(Figure 6-2)**. Repeat samples were available in 29 of the 37 (78 %) subjects 18 to 40 years with an elevated ALT. Of these subjects, 11 (38 %) had repeat samples within the institutional RI limits; 14 (48 %) subjects' repeat ALT results were mildly abnormal (up to twice the upper limit) and four (14 %) subjects' repeat ALT results were moderately abnormal (more than twice the upper limit).

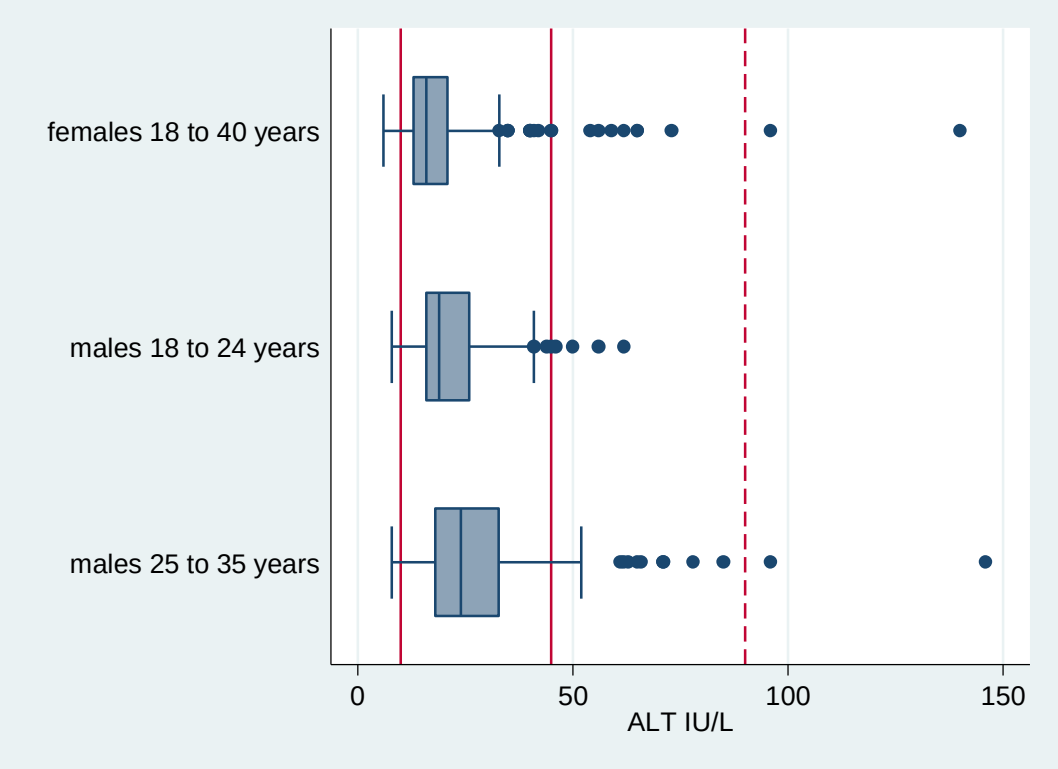
One quarter of all potassium results were below the lower limit for the institutional RI and the calculated upper and lower limits were lower than those for the institutional RI. Median monthly potassium was lower in the summer than the winter months and correlated negatively with mean maximum monthly temperature (Spearman rho=-0.76, p<0.001) **(Figure 6-3).** The frequency of below range potassium results was highest in the summer months **(Figure 6-3)**.

Table 6-3 Study population blood results and the institutional RI

Analyte	N	Median (IQR)	RI[a]	Low (%)[b]	High (%)[b]	2.5[c]	97.5[c]
Albumin g/L							
females	379	45 (4)	35-50	0	15 (4.0)	40	51
males	375	47 (4)	35-50	1 (0.3)	37 (9.9)	41	52
ALP IU/L							
females	370	129 (47)	75-250	7 (1.9)	3 (0.8)	78	216
males	374	159 (50)	95-280	6 (1.6)	2 (0.5)	99	266
ALT IU/L							
females	295	16 (8)	10-45	9 (3.0)	9 (3.0)	10	45
males (18-24)	135	19 (10)	10-45	3 (2.2)	4 (3.0)	11	46
males (25-35)	135	24 (15)	10-45	3 (2.2)	12 (12.6)	12	71
Bilirubin µmol/L							
females	377	9 (4)	3-17	4 (1.0)	19 (5.0)	5	18.0
males	374	12 (6)	3-17	1 (0.3)	58 (15.5)	6	24.5
Creatinine µmol/L							
females	380	80 (13)	70-150	49 (12.9)	0	63	101
males	374	93 (14)	70-150	6 (1.6)	0	74	115
Potassium mmol/L							
females	378	3.6 (0.5)	3.5-5.0	98 (25.9)	6 (1.6)	3	4.3
males	376	3.7 (0.4)	3.5-5.0	90 (23.9)	3 (0.8)	3.2	4.3
Sodium mmol/L							
females	380	139 (2)	135-145	2 (0.5)	2 (0.5)	136	142
males	376	140 (2)	135-145	0	4 (1.1)	137	144
Urea mmol/L							
females	346	4.1 (1.2)	2.5-6.7	9 (2.6)	6 (1.7)	2.4	5.8
males	354	4.9 (1.4)	2.5-6.7	2 (0.6)	22 (6.2)	3.2	7
Haemoglobin g/dL[de]							
females	373	13.2 (1.2)	12-15	29 (7.8)	10 (2.7)	11.5	14.8
males	377	15 (1.3)	13-17	7 (1.9)	5 (1.3)	13.2	16.5
MCV fL[f]							
females	373	89.9 (9.9)	83-105	24 (6.4)	1 (0.3)	81.7	97.6
males	377	88.5 (5.2)	83-105	21 (5.6)	0	81.2	97.1
Platelets $x10^9$/L							
females	373	261 (79)	150-400	3 (0.8)	7 (1.9)	173	375
males	377	229 (58)	150-400	18 (4.8)	2 (0.5)	154	359
White cells $x10^9$/L							
females	373	6.2 (2.2)	4.0-11.0	24 (6.4)	10 (2.7)	3.8	10.7
males	377	5.7 (2.0)	4.0-11.0	34 (9.0)	5 (1.3)	3.6	10.2

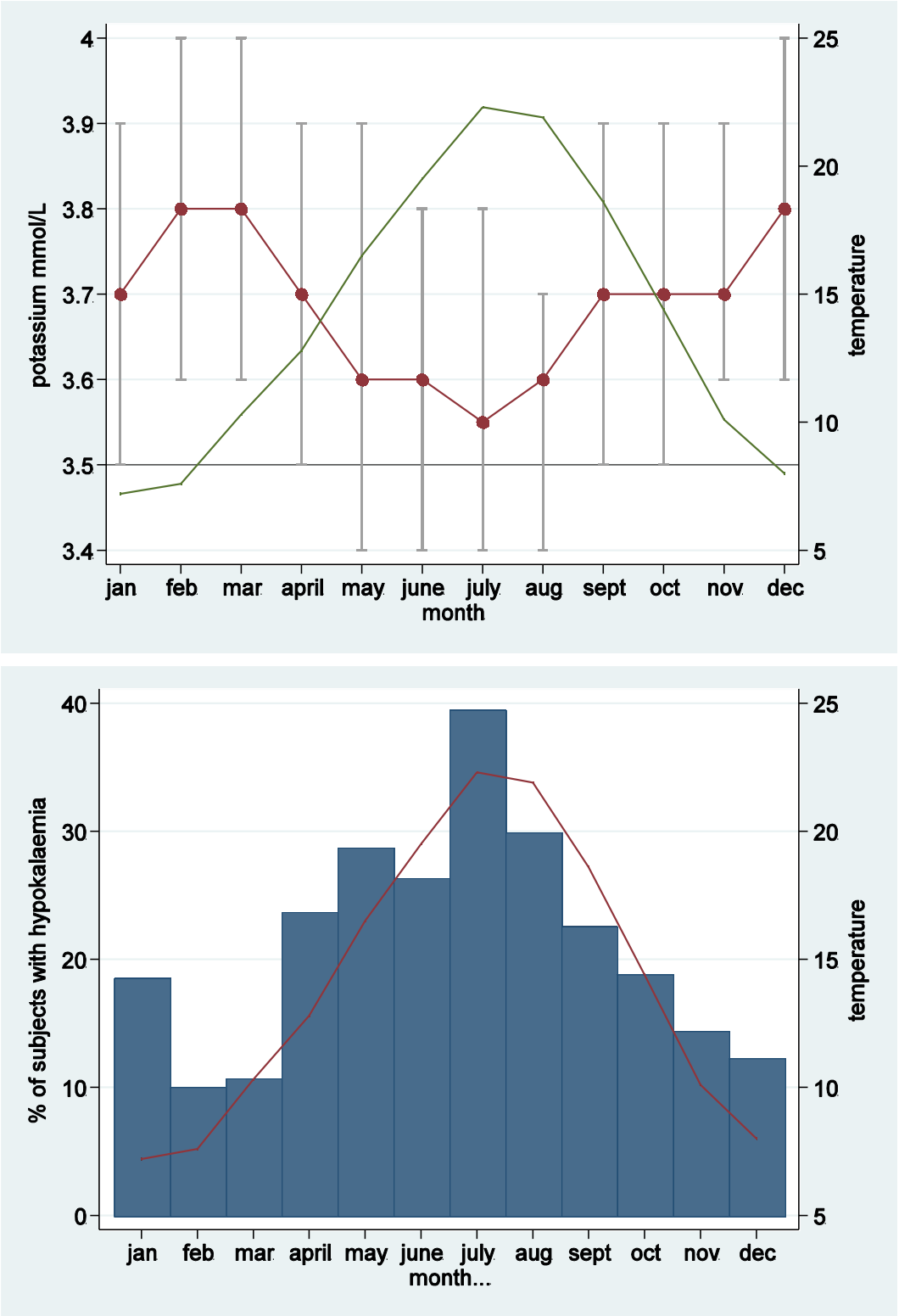
[a]Institutional RI; [b]Number of results outside the institutional RI; [c]95 % range calculated following exclusion of outliers; [d]g=grams; [e]dL=decilitres; [f]fL=femtolitres

Figure 6-2 ALT results by gender and age subgroups



ALT results for females aged 18 to 40 years, males aged 18 to 24 years and males aged 25 to 35 years; boxes contain median lines; whiskers=IQR; dots=individual outliers; solid y lines=limits of institutional RIs; dashed y line=two times the upper limit of the institutional RI.

Figure 6-3 Potassium seasonality



Upper panel: connected points=median potassium; capped bars=IQR; line=mean monthly maximum temperature in Oxford, UK, 1979-2000 (˚C); horizontal line=lower limit of institutional RI. Lower panel: vertical bars=% of potassium results below 3.5 mmol/L; line=mean monthly maximum temperature in Oxford, UK, 1979-2000 (˚C).

Within-person variation

Analytes showing the greatest within-person variability were bilirubin, ALT, white cells and urea **(Table 6-4)**. The most stable analytes with least within-person variability were sodium, MCV, haemoglobin and albumin. Trends in within-person variation were comparable to previously published figures from large datasets.

Pre-and post-intervention results for 94 subjects who participated in clinical trials of candidate TB vaccine, MVA85A, were also analysed **(Table 6-5)**. The median age of female subjects was 26 years (IQR 9) and male subjects' median age was 26 years (IQR 12). The median interval between pre-intervention screening samples and vaccination with MVA85A was 28 days (IQR 44). The median interval between vaccination and post-intervention samples was seven days (IQR 0). One post-intervention sodium result (109 mmol/L) for one subject was excluded from the analysis. This result was considered to be aberrant as there were no associated symptoms of hyponatraemia and sodium was within the normal range (139 mmol/L) on a sample taken two days later. The repeat sodium result of 139 mmol/L was included. Albumin and bilirubin results were lower post-intervention compared to pre-intervention but no results fell below the lower limit of the institutional RI. Haemoglobin and MCV were transiently reduced one week post-intervention. Haemoglobin fell below the lower limit of the institutional RI one week post-intervention in five (6 %) of subjects, all of whom were female, but in all cases was within the normal range by 12 weeks post-intervention.

Table 6-4 Within-person variation

Analyte	N	Males (%)	Within-person CV of study population	Published within-person CV (314)
Albumin	476	229 (48)	5.0 %	3.1 %
ALP	470	228 (49)	11.0 %	6.4 %
ALT	339	160 (47)	23.8 %	18.0 %
Bilirubin	479	231 (48)	28.4 %	23.8 %
Creatinine	481	231 (48)	7.8 %	6.0 %
Potassium	481	230 (48)	9.1 %	4.8 %
Sodium	449	231 (50)	1.3 %	0.7 %
Urea	339	223 (48)	16.2 %	12.3 %
Haemoglobin	483	234 (48)	4.1 %	2.8 %
MCV	483	234 (48)	2.3 %	1.3 %
Platelets	483	234 (48)	10.0 %	9.1 %
White cells	483	234 (48)	19.6 %	10.9 %

Table 6-5 Pre- and post-intervention paired analysis

Analyte	Median pre-intervention result (IQR)	Median post-intervention result (IQR)	Median difference between pre- and post-intervention results (IQR)[a]	
Albumin g/L	46 (4)	45 (4)	1 (4)	p<0.01
ALP IU/L	138 (57)	139 (60)	3 (21)	p=0.45
ALT IU/L	20 (10)	18 (10)	1 (8)	p=0.70
Bilirubin µmol/L	10 (4)	10 (6)	1 (5)	p=0.01
Creatinine µmol/L	88 (16)	88 (22)	0 (10)	p=0.47
Potassium mmol/L	3.7 (0.5)	3.7 (0.3)	0 (0.4)	p=0.75
Sodium mmol/L	139 (2)	139 (2.5)	0 (3)	p=0.44
Urea mmol/L	4.3 (1.2)	4.4 (1.4)	-0.1 (1.2)	p=0.39
Haemoglobin g/dL	14.2 (2.3)	13.8 (2.2)	0.4 (0.8)	p<0.01
MCV fl	89.1 (5.4)	89.2 (5.4)	0.3 (2.2)	p=0.01
Platelets $x10^9$/L	260 (81)	256 (85)	-4 (38)	p=0.09
White cells $x10^9$/L	5.9 (2.2)	5.7 (2.1)	0.1 (-1.6)	p=0.18

[a]WSR test

Practical applications of biochemistry and haematology results analyses for interpreting blood results in clinical trials

Background information for each analyte, with relevance for young, asymptomatic adult subjects, is summarised in **Table 6-6**. For example, this information has been applied to the interpretation of ALT results within this population, by revealing the importance of investigating even mildly raised ALT results in asymptomatic young adults. An algorithm for the management of elevated ALT results has been proposed **(Figure 6-4).**

Table 6-7 summarises the institutional RI (Oxford Radcliffe Hospitals NHS Trust) and provides a cautionary range (where relevant) and expected variation from baseline for each analyte. The cautionary range was obtained by comparing the calculated 95 % ranges from this study with the institutional RI. For example, the upper limit of the calculated 95 % range of potassium is 4.3 mmol/L but the upper limit of the institutional RI is 5.0 mmol/L. The cautionary range for potassium which has been suggested is 4.4 to 5.0 mmol/L. This aims to highlight that potassium results within this range should, for this population, be interpreted with caution, rather than being assumed to be 'normal'. Within-person analyses have been used to indicate the expected variation from baseline for each analyte, in order that results which fluctuate more than expected are interpreted with caution, even if within the 'normal range'. For example, the within-person CV for potassium was 9.1 %, corresponding to fluctuations of up to 0.5 mmol/L.

Table 6-6 Summary of background information with relevance for young, healthy adults

Analyte	Considerations
Albumin	Albumin levels decrease with age (315). Young, healthy adults are likely to have higher albumin levels due to good nutritional status and liver synthetic capacity (315).
ALP	ALP levels are stable in young adults, following cessation of growth and bone turnover and increase in the fourth decade (229, 316).
ALT	Body mass index has the strongest independent association with ALT in healthy populations, but increased ALT levels are also associated with male gender and age, peaking at 55 years of age (294, 317, 318). Non-alcoholic fatty liver disease (NAFLD) is an important cause of persistently elevated ALT levels in asymptomatic adults and is estimated to have a prevalence of 20 to 30 % in countries such as the UK (319). ALT levels at the upper end of the normal range are associated with an increased mortality from liver disease (320).
Bilirubin	The most likely cause of persistently elevated bilirubin in asymptomatic individuals with normal unconjugated bilirubin levels and otherwise normal liver function tests is Gilbert's syndrome, which has a prevalence of 5 to 10 % (321).
Creatinine	Creatinine production is determined by muscle mass and dietary intake and is affected by gender and ethnicity, with high inter-person variability, so a single RI is not entirely appropriate for a population (322). Estimates of Glomerular Filtration Rate (eGFR) may take age; gender; ethnicity; and body mass into account, but have usually been developed for patients with chronic kidney disease and may not be applicable to an asymptomatic population (322, 323).
Potassium	Potassium has a widely used reference range of 3.5 to 5.0 mmol/L (298, 299). Ambient temperature is an important cause of spurious hypo- and hyperkalaemia when samples are stored prior to analysis (324, 325).
Sodium	Sodium has a narrow homeostatic range and shows very little inter-person variation (293). Exercise-associated hyponatraemia occurs after excessive exercise, such as marathons.
Urea	Between-person urea levels are highly variable and are related to protein intake, being higher in males than females and increase with age (326). Within-person variations are associated with dehydration; alcohol intake; and concomitant medication.
Haemoglobin	Haemoglobin RIs are consistently lower in females than males (298, 299).
MCV	Causes of raised MCV include excessive alcohol intake and macrocytic anaemia. Iron deficiency and thalassaemia should be considered for low MCV values.
Platelets	Platelet counts are lower in African and Afro-Caribbeans than Caucasians (295). The most widely used lower limit is $150x10^9$ cells/L (327). Blood smear to exclude pseudothrombocytopenia is indicated for results below the lower limit (327).
White cells	White cell counts are lower in African and Afro-Caribbeans than Caucasians and are higher in women than men (295).

Figure 6-4 Algorithm for management of elevated ALT in the clinical trial setting

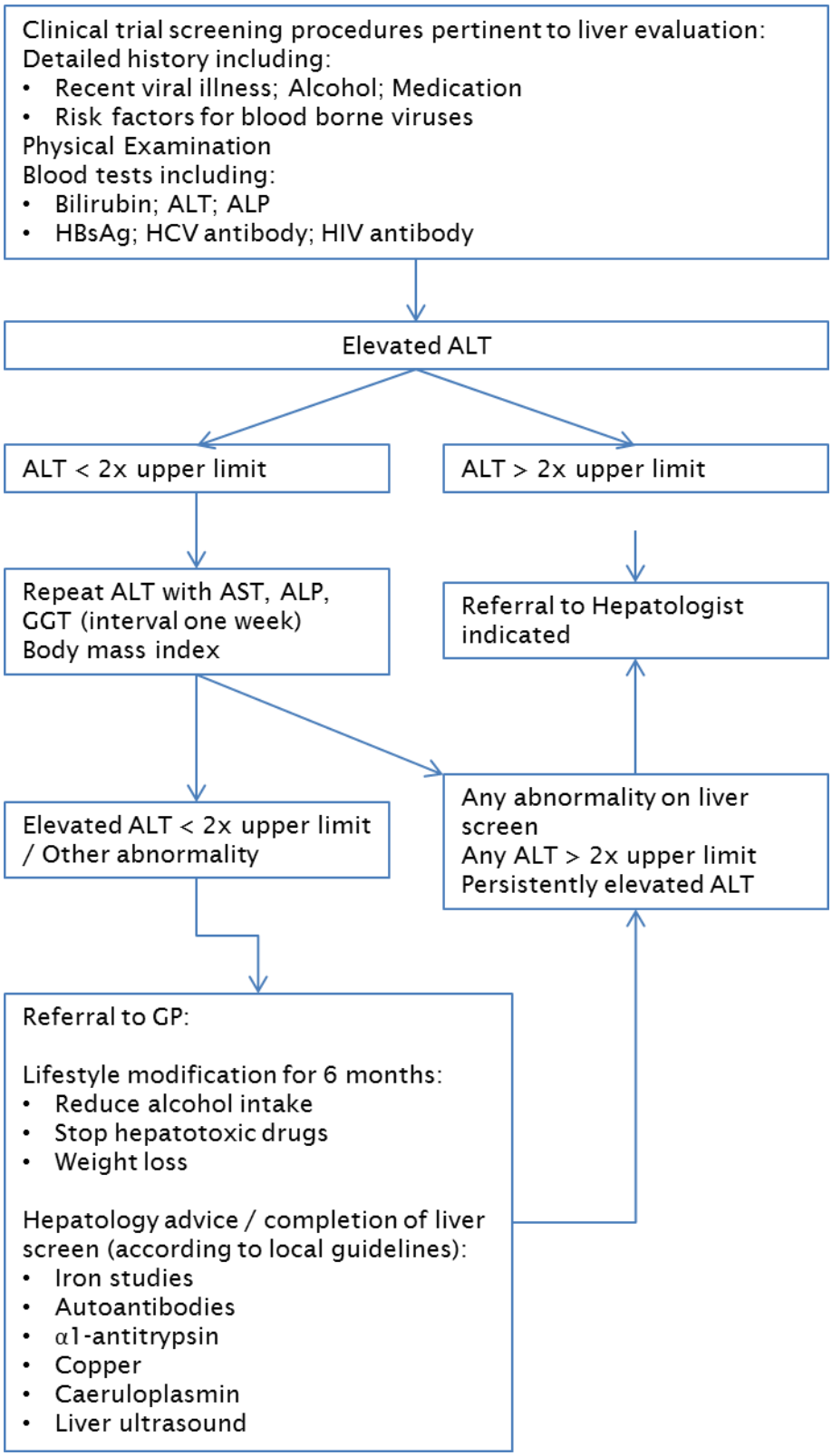


Table 6-7 Cautionary ranges and expected within-person variation

Analyte	Institutional RI	Cautionary range (if applicable)[a]	Expected variation from baseline[b]
Albumin g/L			5 %
females	35-50	35-39	2-3
males	35-50	35-40	2-3
ALP IU/L			11 %
females	75-250	217-250	8-28
males	95-280	267-280	10-31
ALT IU/L			24 %
females and males	10-45		2-11
Bilirubin µmol/L			28 %
females and males	3.0-17.0		1-5
Creatinine µmol/L			8 %
females	70-150	102-150	6-12
males	70-150	116-150	6-12
Potassium mmol/L			9 %
females and males	3.5-5.0	4.4-5.0	≤0.5
Sodium mmol/L			1 %
females and males	135-145		1-2
Urea mmol/L			16 %
females	2.5-6.7	5.8-6.7	≤1
males	2.5-6.7		≤1
Haemoglobin g/dL			4 %
females	12.0-15.0		<1
males	13.0-17.0		<1
MCV fL			2 %
females and males	83-105	98-105	2
Platelets $x10^9$/L			10 %
females	150-400	150-172 and 376-400	15-40
males	150-400	360-400	15-40
White cells $x10^9$/L			20 %
females	4.0-11.0		1-2
males	4.0-11.0		1-2

[a]Cautionary ranges based upon discrepancies between the institutional RIs and the calculated 95 % ranges; [b]expected variations from baseline based upon the calculated within person CVs.

Discussion

This was a retrospective study to evaluate the validity of institutional RIs for commonly tested biochemical and haematological analytes for a clinical trial population of asymptomatic young adults, all of whom had a medical assessment. Subjects with pre-existing medical problems, alcohol excess or infection with blood borne viruses were excluded from analysis. There were over 300 subjects per group, which was above the minimum recommended subgroup size (120 subjects) for non-parametric 95 % range calculations (228). Paired samples for the same individuals were available and all samples were processed in the same laboratory.

The proportions of results in the study population which were outside the ranges of the institutional RI were determined. The institutional RIs were two sided, defining the upper and lower limits of 95 % of results. No analyte had both 2.5 % of results above the upper limits and 2.5 % of results below the lower limits, as would be expected. For analytes such as albumin and ALP, this may reflect differences between the all adult reference populations, often patients, from which institutional RIs were derived and the population of young, asymptomatic adults volunteering to participate in vaccine clinical trials at the Jenner Institute, Oxford.

One in four potassium results in the study were below the lower limit of the institutional RI for potassium. Potassium levels in *ex vivo* blood samples are not stable when stored prior to analysis unless centrifuged at the point of care (328). Seasonal pseudohypokalaemia has been described and is hypothesised to be caused by *ex vivo* sodium-potassium ATPase-mediated increased uptake of potassium by metabolically active cells in warm conditions (324, 329). Pseudohypokalaemia, due to storage of the samples at environmental temperature (centrally heated building in winter; no air conditioning in summer) prior to transport for analysis may account for the high incidence of low potassium levels observed in this study. Centrifuges have been installed in primary care practices, on the basis that correct results can only be ensured by centrifugation of samples in gel separator tubes (329). In Scotland, source centrifugation of samples taken in primary care facilities successfully reduced the frequency of pseudohyperkalaemia (330).

Discrepancies between the study population and the institutional RIs were further evaluated by using a well-recognised method for defining RIs, which is the basis for current guidelines (228-231). The central 95 % of results for each analyte, subgrouped by gender, were calculated following removal of outliers. This method aims to maximise sensitivity and specificity by obtaining information from the extremes of the sample, whilst avoiding outliers adversely influencing the analysis. The importance of

the calculated 95 % ranges is that there is a danger that a clinically significant result which falls outside the calculated range for this healthy adult population, but within the limits of the institutional RI for all adults, may be clinically significant, but would be overlooked if referring only to the institutional RIs.

The calculated 95 % range for ALT in males aged 25 to 40 years was higher than the institutional RI, reflecting increased levels in this subgroup, but adherence to the institutional RI for ALT is essential. Minimally elevated ALT levels are of clinical significance and a number of groups have advocated reducing current upper normal limits **(Table 6-6)** (294, 331). Current recommendations are for investigation of persistently elevated ALT of any degree above standard upper limits in asymptomatic individuals (332). A clinical algorithm for the management of elevated ALT results in clinical trial populations has therefore been proposed **(Figure 6-4).**

Understanding the expected degree of within-person variation for an analyte and the factors associated with variations is of particular importance for the interpretation of sequential results. Consistent with previous published data, bilirubin and ALT showed the greatest within-person variation. Transient fluctuations are common and may be caused by recent alcohol consumption, viral illnesses, concomitant medications and exercise patterns (333). For these analytes, transient fluctuations need to be distinguished from persistent elevations, by repeat testing and possible causes of transient fluctuations documented, by relevant history taking. It is intuitive to expect that analytes which vary greatly are likely to be the most frequently reported as abnormal in post-vaccination safety evaluation. For example, in the Phase I clinical trial described in **Chapter 4**, elevated ALT post-vaccination was detected in one of 31 subjects (227). The paired analysis of 94 pre- and post-MVA85A vaccination blood results was reassuring, in showing no frequent effect of MVA85A vaccination on ALT results.

The paired pre- and post-intervention analysis identified a transient reduction in haemoglobin and MCV in females, coinciding with the time in the trials when the greatest blood volumes were drawn. When the outcome measures of a clinical trial include the evaluation of vaccination induced immune responses, sizable blood samples (60 to 80 ml) are required from each subject at regular intervals. For trials such as these, trial inclusion criteria should follow national transfusion guidelines for the selection of blood donors. For example, in the UK, the minimum haemoglobin levels for blood donors are 12.5 g/dL (females) and 13.5 g/dL (males) (334).

Limitations

Limitations were that this was a retrospective study, so various environmental factors were not controlled. Subjects were not required to fast overnight or to abstain from intense exercise; and alcohol intake was not limited prior to phlebotomy. Diet, alcohol and recent exertion may have been confounding pre-analytical factors, but most specimens were taken in the mornings to minimise the effects of diurnal variation. Phlebotomy was performed with subjects at rest and data used for between-person analyses were taken pre-intervention, so subjects were maintaining their usual lifestyle. Recreational drug use was an exclusion criterion, but subjects taking certain prescription drugs (such as the oral contraceptive pill) or over-the-counter remedies (such as analgesis for coryzal and musculoskeletal symptoms) were not excluded. After phlebotomy, times to sample analysis were not controlled. At the point of laboratory analysis, samples were not frozen, so between-run variation could not be eliminated and replicate analyses of specimens were not performed. Ethnicity and socio-economic data, anthropometric measurements, iron studies in females and detailed smoking, alcohol, recent diet, exercise and menstrual history would strengthen the study (233).

Future work

There is a recognised need to standardise safety reporting across clinical trials of vaccines and the Brighton Collaboration is developing case definitions and guidelines to this end (223). This study provides important data on which to base further work to validate RIs for young, asymptomatic adults to enable the accurate interpretation of blood results in clinical trials. The reliability of the results obtained could be confirmed by repeating these analyses in a prospective study with improved control of environmental factors and collection of ethnicity data and anthropometric measurements as detailed above.

A prospective study to evaluate the effect of centrifugation at source, with stratification of results by storage time would help optimise procedures for minimising pseudohypokalaemia.

A longitudinal study of ALT levels in asymptomatic young adults with detailed concurrent history taking and appropriate investigations would determine the incidence of persistently elevated ALT in this population and may indicate the most likely aetiologies of transiently elevated ALT levels.

Clearly, the cautionary ranges and expected variation from baseline summarised in **Table 6-7** are only valid for young, asymptomatic adults whose blood samples are analysed at the Oxford University Hospitals NHS Trust laboratories, UK. However, the

methods described are straightforward and studies conducted in young healthy adults in other clinical trial sites would determine the reproducibility of these findings.

Conclusions

This study demonstrates the benefit of evaluating haematological and biochemical results for a healthy young adult clinical trial population. Comparing calculated 95 % ranges with institutional RI can reveal important discrepancies, some of which are related to the institutional RIs encompassing all adult populations. Calculating within-person and between-person variation can aid clinical decision making and safety evaluation, for example by identifying cautionary ranges and expected variation from baseline for each analyte. The need for appropriate investigation of any persistently elevated ALT has been highlighted, following the identification of a high incidence of elevated ALT in male subjects aged 25 to 40 years. Environmental temperature and pre-analysis storage times are hypothesised to account for the high incidence of low potassium results. Key components of the clinical assessment conducting during clinical trial screening, pertinent to the interpretation of blood results, include ethnic origin; detailed alcohol, smoking, diet, exercise and medication history; recent mild illness; history of significant acute or chronic disease; height, weight and blood pressure measurements and urinalysis for blood, protein and glucose. When taking post-intervention blood tests for safety evaluation, concurrent history of recent symptoms, diet, exercise, medication and alcohol intake are recommended.

Chapter 7 : Concluding Remarks

The specific background, conclusions, limitations and future directions of individual studies have been discussed within each of the results chapters. In this concluding section, the work is summarized and relevant issues are selected for further discussion, within the context of considering the future directions for the field.

Summary of findings

This thesis describes early clinical trials and retrospective studies, conducted in the UK and focusses on the evaluation of two poxvirus-vectored subunit candidate TB vaccines, MVA85A and FP85A. The two clinical trials represent the first evaluation of MVA85A vaccination in HIV-infected adults and the first clinical trial of FP85A vaccination in humans. A single FP85A vaccination and sequential FP85A and MVA85A vaccinations were evaluated in healthy human adults. In addition the cumulative safety profile of 111 MVA85A vaccinations from a series of clinical trials in 100 healthy adult subjects was reviewed.

Local reactions at the vaccine sites, consisting of erythema and induration, were reported by all subjects in all clinical trials. Other frequently reported local reactions were scaling, pruritus and tenderness. The most frequently reported systemic AEs were headache, fatigue, arthralgia, malaise and feverishness, usually occurring within 48 hours after vaccination. There was no correlation found between objective measures of vaccine safety (local reaction diameter and body temperature) and immune responses to vaccination.

To improve the interpretation of routine biochemical and haematological blood tests which are routinely performed pre- and post-intervention in clinical trials, a retrospective analysis of pre-intervention results from almost 1000 asymptomatic adults was conducted. Calculated RIs for the study population were compared with the standard RIs issued by the hospital laboratory performing the assays. Potassium results showed seasonality, with lower potassium levels in summer compared to winter months. There was a high incidence of elevated ALT results in young males and an algorithm for the management of abnormal ALT results was proposed, in recognition that sustained elevations in ALT can be an indicator of NAFLD.

The primary immunological readout for the studies was the detection of IFNγ production by *ex vivo* Ag85A-stimulated PBMC using ELISpot assays. In HIV-infected adults, IFNγ ELISpot responses were detected after MVA85A vaccination in both groups. Responses were sustained for one year after vaccination in Group two (vaccine dose:

$1x10^8$ pfu) but not in Group one (vaccine dose: $5x10^7$ pfu). No IFNγ ELISpot responses were detected after FP85A vaccination in healthy adults. When subjects received MVA85A vaccination four weeks prior to FP85A vaccination, there was no detectable enhancement of IFNγ ELISpot responses. MVA85A vaccination of subjects who had previously received FP85A vaccination was associated with significantly reduced IFNγ ELISpot responses compared to those usually observed after MVA85A vaccination in previous clinical trials.

First-in-human clinical trials

The site for both clinical trials described in this thesis was the Jenner Institute, Oxford University, UK; where over 40 Phase I and II clinical trials of candidate vaccines have been conducted since 1999 (335). This is in line with the recommendations that first-in-human clinical trials are conducted at established clinical trial sites. The great advantage of this is the wealth of experience and improved efficiency for staff training and establishing SOPs to ensure compliance with the principles of ICH GCP and GMP and the requirements for contained use of GMOs. Other groups are also conducting clinical trials at the same site, creating further opportunities for sharing resources, training and expertise between research groups. The location, in the same building as an infectious diseases ward, on an acute hospital site, means there are opportunities for collaboration with other clinical staff as external safety monitors, for example, and access to resuscitation facilities. One disadvantage of conducting concurrent clinical trials at one site is the impact on subject recruitment, as encountered in the FP85A vaccine trial **(Chapter 4)**. The trial in HIV-infected subjects demonstrated how the use of well-established clinical trial sites can be combined with measures to increase the pool of subjects **(Chapter 3)**. Additional sites in other cities were used for subject recruitment and follow up. For vaccination, subjects travelled to the CCVTM, where the facilities, approvals, procedures and experience for the contained use of GMO products and early post-vaccine safety monitoring are in place.

Candidate TB vaccines have traditionally entered first-in-human clinical trials in affluent regions which are not endemic for TB, typically Europe or the USA. Twelve years ago, when MVA85A first entered clinical trials, there was real concern about the theoretical risk of a Koch reaction. There were also issues of infrastructure and systems for obtaining regulatory and ethical approval were less efficient compared to European countries and the USA. There is now considerable experience in conducting clinical trials of candidate TB vaccines in TB-endemic regions, particularly South Africa, with no evidence of Koch reactions in *M.tb*-exposed recipients. The first-in-human clinical trial of H56/AERAS-456+IC31®, one of the newest candidate TB vaccines, was conducted in South Africa (trial ID NCT01967134) (131, 159).

Diagnosing LTBI in clinical trial subjects

In clinical trials of candidate TB vaccines, potential subjects need to be screened for LTBI and active TB disease. In the FP85A vaccine clinical trial, both TSTs and IGRAs were performed for each subject as part of the screening process. In the clinical trial recruiting HIV-infected subjects with preserved $CD4^+$ T cell counts, IGRAs were performed. However, whilst negative test results are helpful in excluding either TB or LTBI, these tools can not differentiate LTBI from active TB disease, or indeed current LTBI from past, resolved LTBI (80, 336). Clinical and radiographic investigations were used in the described clinical trials to exclude active TB disease. This problem is augmented in areas of high TB incidence, where the rate of positive TST and IGRA results in clinical trial subjects will be much higher.

There is now considerable interest in using systems biology approaches to systematically characterize the host immune response to TB, aiming to reveal disease-specific signatures for LTBI and active TB disease respectively (337, 338). This term encompasses proteomic, metabolomic and transcriptomic techniques, each of which yield high numbers of results, which require computational modelling (336). Proteomic and metabolomic studies have identified differences between subjects with active TB disease and those with LTBI, such as a role for tryopophan in TB pathogenesis; and a link between increased IL-18 levels and risk of reactivation (336, 339, 340). One observation in metabolomic studies is that LTBI can be misclassified as active TB disease in a subset of subjects, which may reflect poor specificity; but could represent early, subclinical reactivation in these subjects (340-342). A transcriptional signature for TB was identified, but found to be similar to that of sarcoidosis (343). Potential biomarkers are microRNAs, which are also being investigated as targets for chronic lung inflammation (344, 345). It may be that technologies are combined to provide unique biomarkers for LTBI and TB.

Surrogate endpoints in clinical trials of TB vaccines

For infectious diseases against which antibody-mediated immunity is protective, such as hepatitis B; measles; mumps; and rubella, serum antibodies can be used as surrogate endpoints of vaccine-induced protection in clinical trials. Immune biomarkers which correlate with protection against TB disease are yet to be identified. In the trials described in this thesis, for consistency with previous clinical trials of MVA85A, the main immunological outcome was the detection of IFNγ-producing *ex vivo* PBMCs by ELISpot assays (117, 193, 194). Although, IFNγ production does not correlate with protection, the benefits of this assay are that it is vaccine-specific; IFNγ and $CD4^+$ T cells are essential for protective immunity against TB; and the methods have been

standardised for use with all clinical trials of MVA85A vaccine (96). Indeed, the kinetics of the frequency of IFNγ producing PBMCs in both these trials were comparable to that observed in previous clinical trials (117, 192, 193). In both clinical trials, the IFNγ ELISpot assays were performed in real time using fresh PBMCs. Sufficient sample volumes were obtained in order to cryopreserve PBMCs and serum for use in further exploratory immunology assays, as recommended by a WHO-sponsored expert panel (88). The benefit of cryopreserved samples is that cells obtained on different dates can be run simultaneously in controlled conditions. In addition, as new techniques for evaluating immunogenicity are developed, new assays can be applied to samples stored from historical clinical trials (88). Since not all candidate TB vaccines can feasibly progress to efficacy studies, harmonisation and standardisation of immunological assays and the introduction of a simple assay for use in all clinical trials would enable comparisons between different candidate TB vaccines (88).

Alongside efforts to harmonize assays, various approaches are in development, aiming to identify biomarkers which correlate with vaccine-induced protection against TB disease. Collaborative activities which would streamline efforts to identify biomarkers include central bio banks, such as the collection of biological specimens from patients with culture-positive TB by the Consortium for TB Biomarkers (CTB2); and sharing data from clinical trials and observational cohort studies (80). An ICH tripartite guideline governing the requirements for regulatory qualification of biomarkers has been developed (346).

The possibility of applying a systems approach for the evaluation of vaccine-induced immune responses was first demonstrated for a Yellow Fever vaccine (347). Early changes in gene expression after vaccination compared to baseline were examined in combination with multiplex cytokine analysis; flow cytometric analysis of cell function and phenotype; antibody titres and computational modelling (347). A similar approach has been applied to the study of MVA85A vaccine-induced innate immune responses within the first week after vaccination (348). Using a systems approach to evaluate vaccine-induced immune responses may enable the identification of early changes in gene expression, reflecting the innate immune response, which may then predict the nature of the adaptive immune response. This may also be a means to identifying immune biomarkers which correlate with protective immunity (336, 347).

Lymphocyte-based and whole blood-based *in vitro* mycobacterial growth inhibition assays (MGIAs) are promising functional assays (349-355). Improved BCG growth control after BCG vaccination has been demonstrated in whole blood samples from infants and PBMC from British schoolchildren (349, 356). Mycobacterial growth control was found to be diminished in HIV-infected children and enhanced by the introduction

of ART (357). A UK-based clinical trial evaluating whole blood-based and PBMC-based MGIAs demonstrated greater mycobacterial growth inhibition in BCG-vaccinated subjects compared to BCG-naïve subjects; and no enhancement of growth control after BCG revaccination (358). Mycobacterial growth inhibition did not correlate with PPD antigen-specific IFNγ producing T cells, measured by ELISpot (358). Promisingly, this is in keeping with the BCG's protective efficacy in the UK; the lack of evidence for improved protection after a repeat BCG vaccination and the lack of correlation between IFN-producing T cells and vaccine efficacy (18, 359). Issues which need addressing in order for MGIAs to be used in clinical trials are intra-assay variability, partly due to variations in viability of mycobacteria; inter-assay variability, due to differences in MGIA protocols; and the most ideal post-vaccination time point for assessing growth inhibition (358). Possible applications for MGIAs include comparing vaccine candidates in animal models and early clinical trials to select those most suitable for clinical efficacy testing; and the use of small volume whole blood-based assays in efficacy trials (358, 360).

A validated mycobacterial challenge model for use in humans and adults would greatly assist the selection of TB vaccine candidates for further development. Human challenge models exist for other infections for which immune correlates have not been identified, including controlled human malaria infection; influenza virus challenge and experimental dengue virus challenge (361-364). *M.tb* cannot safely or ethically be used as a challenge agent in humans. Furthermore, the use of animal *M.tb* challenge models is limited by the requirement for Category Three containment (365). Human BCG challenge models are being developed, whereby intradermal BCG vaccination could be a surrogate for *M.tb* infection (trial ID of pending clinical trial NCT01868464) (131, 303, 366, 367).

The future of TB vaccine development

Evaluating efficacy

Until surrogate endpoints are identified, the only means of evaluating the effectiveness of TB vaccines is in efficacy trials, with clinical endpoints of active TB disease. Defining clinical endpoints of active disease will be a particular challenge in these studies. Bacteriological endpoints can be used for adults and adolescents, but TB disease in infants and people infected with HIV is typically pauci-bacillary (368).

Despite the global prevalence of LTBI, TB incidence is relatively low, with the highest incidences in Swaziland (1300/100,000) and South Africa (1000/100,000) (24). Large numbers of subjects are therefore required for efficacy trials and the funding, resource and expertise requirements are considerable. The importance of partnering for efficacy

trials is recognised. For example, the MVA85A vaccine was developed by an academic institution, University of Oxford, with funding from the Wellcome Trust and other charitable bodies. For the Phase IIb efficacy trial, Oxford-Emergent Tuberculosis Consortium (OETC), a joint venture between the University of Oxford and Emergent BioSolutions, was formed and the trial was conducted in partnership with Aeras, with additional funding from the Wellcome Trust, and the EDCTP (159, 203). Since all the existing candidate TB vaccines have been developed in high income, low TB-incidence countries, field site partners in TB-endemic regions are necessary. The South African TB Vaccine Initiative (SATVI), University of Cape Town trial site in Worcester, South Africa is very well established and the Phase IIb efficacy trial of MVA85A was conducted here (203). Other advanced clinical trial sites include the Kenya Medical Research Institute/Centre for Disease Control field station, Kisumu, Kenya and the Medical Research Council (MRC) unit in The Gambia. Capacity building and field-site development in different countries and continents, particularly in India and South East Asia, is required in readiness for conducting multi-site large scale efficacy trials.

Phase IIb efficacy clinical trials of candidate TB vaccines are designed as proof-of-concept studies to select candidates for progression to Phase III pre-licensure efficacy trials (24). The aim of Phase III double blind placebo controlled RCTs will be to further evaluate vaccine safety, particular with respect to rare AEs and to determine vaccine efficacy. Although the prevalence of LTBI is high, the incidence of TB cases is small so large numbers of subjects are required for efficacy studies. Pre-licensure regulatory efficacy studies will need to be performed on an even larger scale and in multiple sites and different age cohorts. Indeed, similarly large scale trials will be necessary in order to validate new biomarkers (81).

In the first Phase IIb efficacy trial of a candidate TB vaccine, MVA85A vaccination of BCG-vaccinated infants did not enhance the protection afforded by BCG vaccination alone (203). Importantly, this trial demonstrated the feasibility of conducting efficacy trials for candidate TB vaccines. A number of factors need considering in interpreting the results. Firstly, antigen-specific IFNγ ELISpot responses after MVA85A vaccination are up to ten times lower in infants than in adults, including HIV-infected adults (195, 200, 254). Secondly, BCG is protective in infants, so considerable vaccine efficacy and very large trial sizes are required to demonstrate improved protection in this population. A proof-of-concept efficacy trial of MVA85A vaccination in adults, in whom BCG is poorly effective, may be justified.

Clinical grade recombinant poxviruses, such as MVA85A, are manufactured in liquid form, stored at -80 ºC, administered within one hour of thawing and subject to the contained use regulations for GMOs. This raises practical challenges for scale up of

vaccine manufacture and delivery. For large scale efficacy trials, vaccine stability at higher temperatures would need to be established and alternative formulations developed, such as freeze dried vaccines. For mycobacterial vaccines, the ethics of giving a vaccine candidate in place of BCG and the feasibility of performing non-inferiority efficacy studies between the candidate vaccine and BCG are further considerations. For subunit vaccines, the optimal age for boosting immunisation and the optimal interval between BCG and boosting vaccine will need to be determined (368).

New directions

The disappointing results of the Phase IIb efficacy trial of MVA85A in infants highlight the importance of ongoing early development of alternative vaccine candidates, approaches and regimes, alongside efficacy trials.

Vaccines are typically administered systemically by intramuscular or intradermal injection, but intuitively a vaccine may be most effective if delivered at the site at which host defence is required. There are data from murine studies suggesting that the location of antigen specific T cells within the lung is required for optimal protection against pulmonary TB and is best achieved by delivery of a vaccine directly to the lung mucosa (127, 172, 173). Following preclinical toxicology studies, aerosolised administration of MVA85A vaccination has been investigated in Phase I clinical trials using a new delivery device (trial IDs NCT01497769 and NCT01954563) (131, 369).

With so many candidate vaccines in clinical trials and in advanced stages of preclinical development, another approach may be combined clinical trials, allowing pooling of resources and head to head comparisons. Also, it may be that the optimal regime includes two different vaccines in combination. For example, MVA85A vaccination is being evaluated in prime-boost regimes with AERAS-402 and ChAdOx1 85A (trial IDs NCT01683773 and NCT01829490) (131, 159).

Alternative viral vectors under investigation include orally-delivered Ad4; recombinant simian adenoviruses; human parainfluenza virus and recombinant cytomegaloviruses (159). Cholera toxin has adjuvant properties via IL-17 induction and has been shown to enhance BCG and BCG/MVA85A vaccination-mediated protection against aerosolized *M.tb* challenge in mice (370).

Conclusions

A new TB vaccine is an essential component if the goal of eliminating TB disease is to be realised. At least 15 candidate TB vaccines have been evaluated in more than 50

clinical trials over the past ten years (24). This reflects both a substantial investment in TB vaccine research and difficulties with preclinical candidate selection due to the lack of validated animal models (371).

This thesis has described two early safety clinical trials involving two candidate TB vaccines. MVA85A vaccination was evaluated in HIV-infected subjects for the first time and candidate TB vaccine, FP85A, was evaluated in humans for the first time. The results support further studies of MVA85A in HIV-infected subjects, but do not support further clinical development of FP85A, since there were no detectable immune responses to vaccination. A system for electronic capture of safety data during clinical trials of candidate vaccines was devised and a retrospective review of the safety of MVA85A in healthy human subjects in the UK was conducted. The validity of institutional RIs for healthy adult subjects was evaluated to support the accurate interpretation of screening and follow up blood results in clinical trial populations recruited for early safety studies.

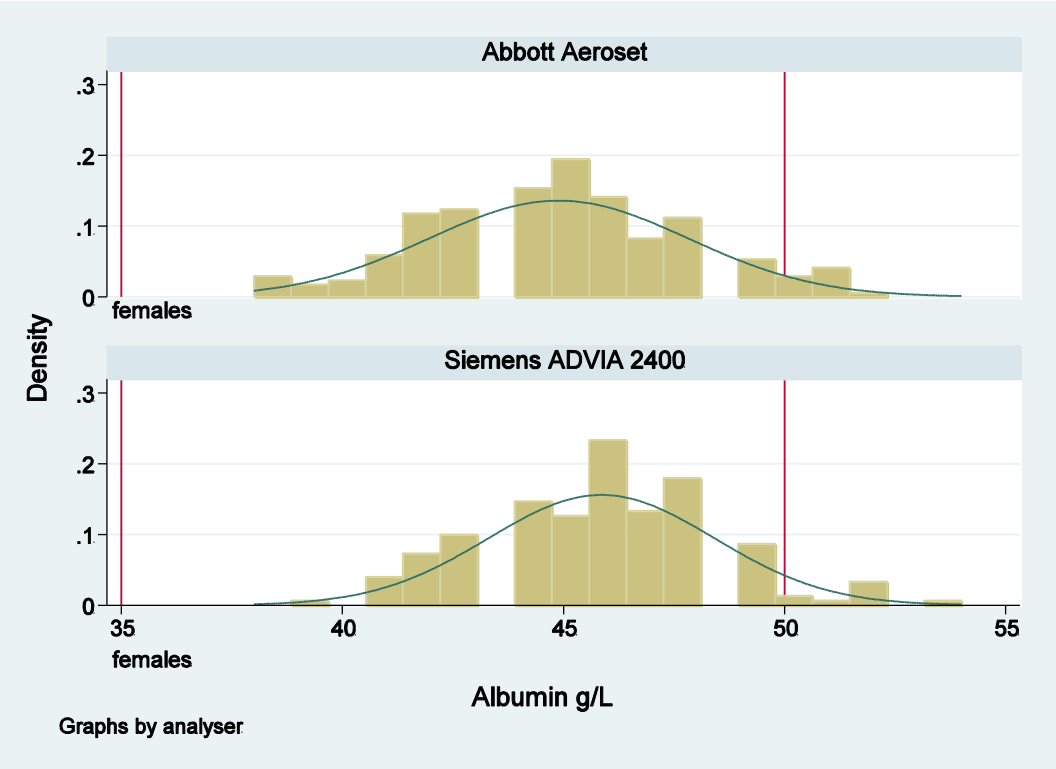
The next era of vaccine development will include further proof-of-concept efficacy trials of the most advanced candidate vaccines alongside ongoing preclinical and early clinical development of new candidate vaccines, delivery routes and carriers. Understanding the components of a protective host immune response will inform the design and evaluation of new vaccines. Validated animal models and biomarkers for LTBI and TB diagnosis and vaccine-induced immunity would greatly enhance the efficiency of vaccine selection. Much has been achieved and much is still required.

Appendix

Supplementary results data for Chapter 6, subsection "Between-person variation"

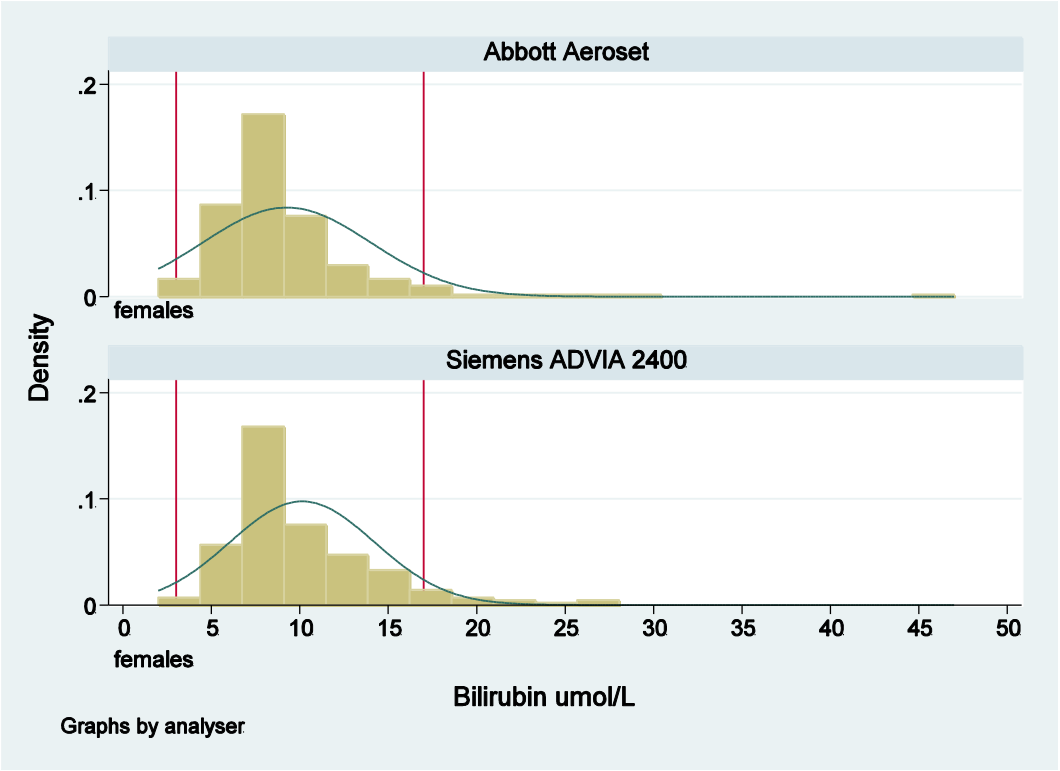
Histograms of results obtained using each analyser are displayed for those analyte gender subgroups for which a statistically significant difference in distributions (Kolmogorov-Smirnov) was identified.

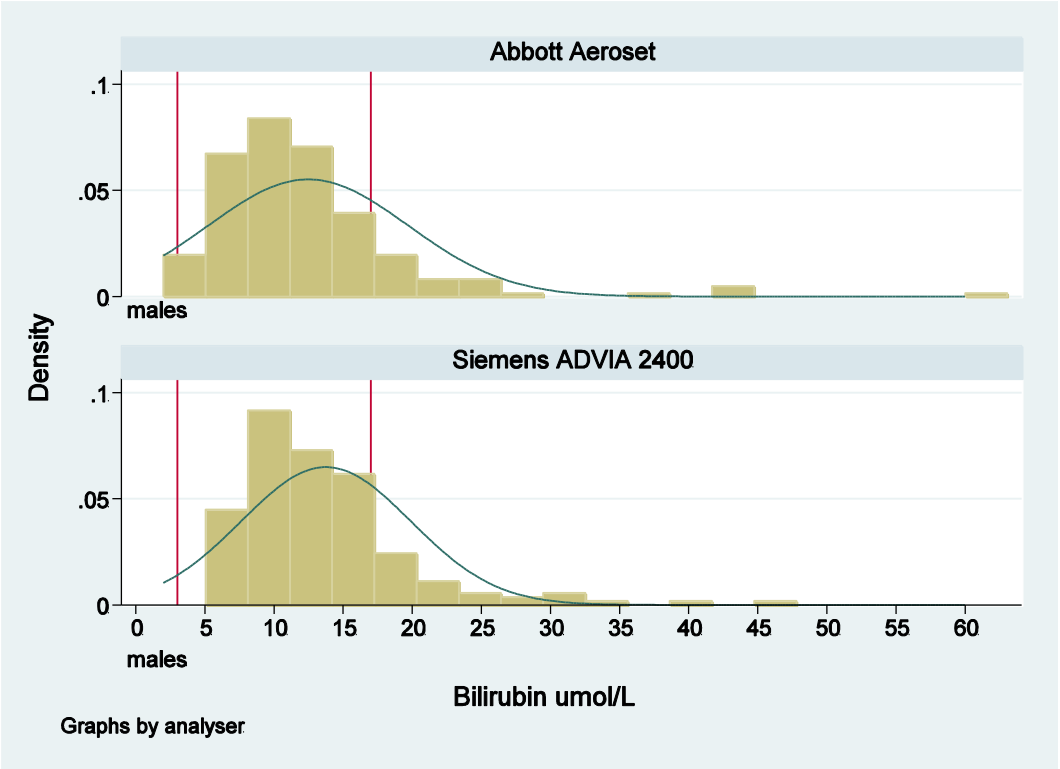
Albumin results in females by analyser



x lines=limits of institutional RI; curve=empirical normal distribution

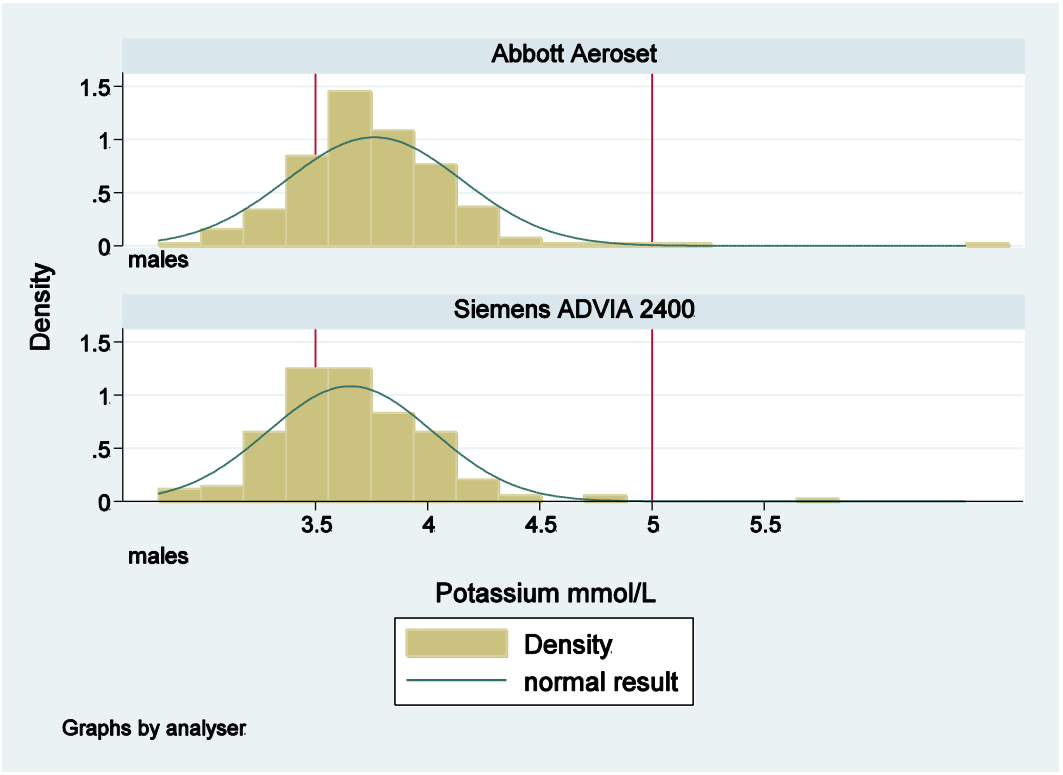
Bilirubin results in females and males by analyser

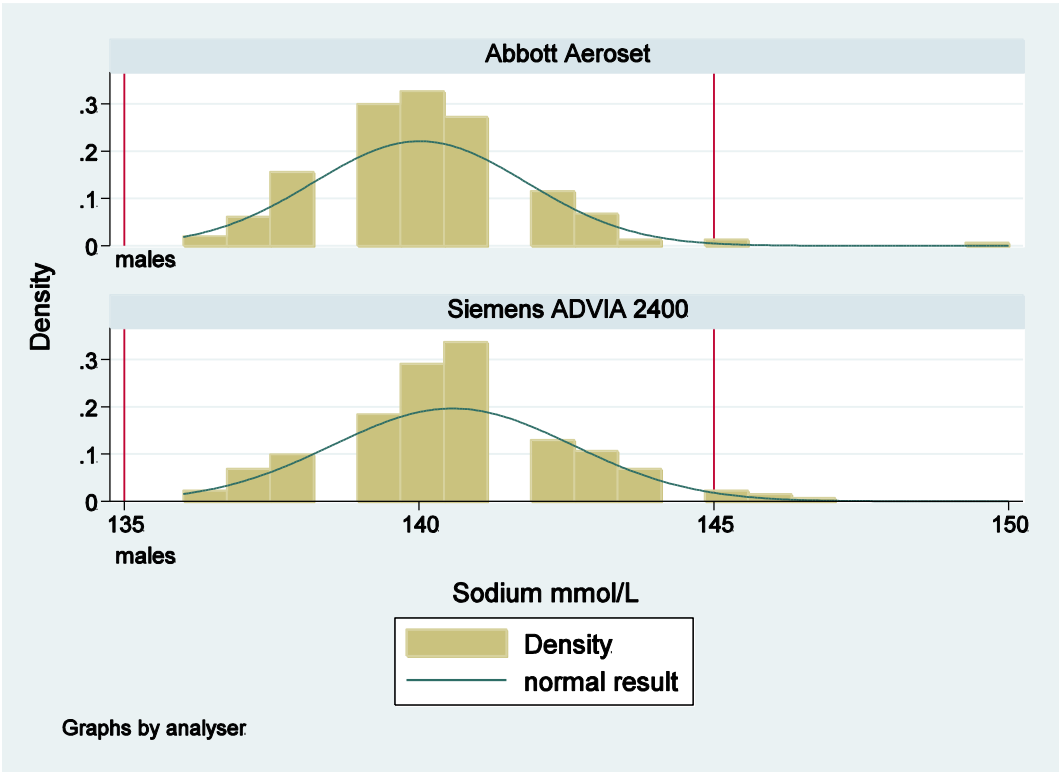




x lines=limits of institutional RI; curve=empirical normal distribution

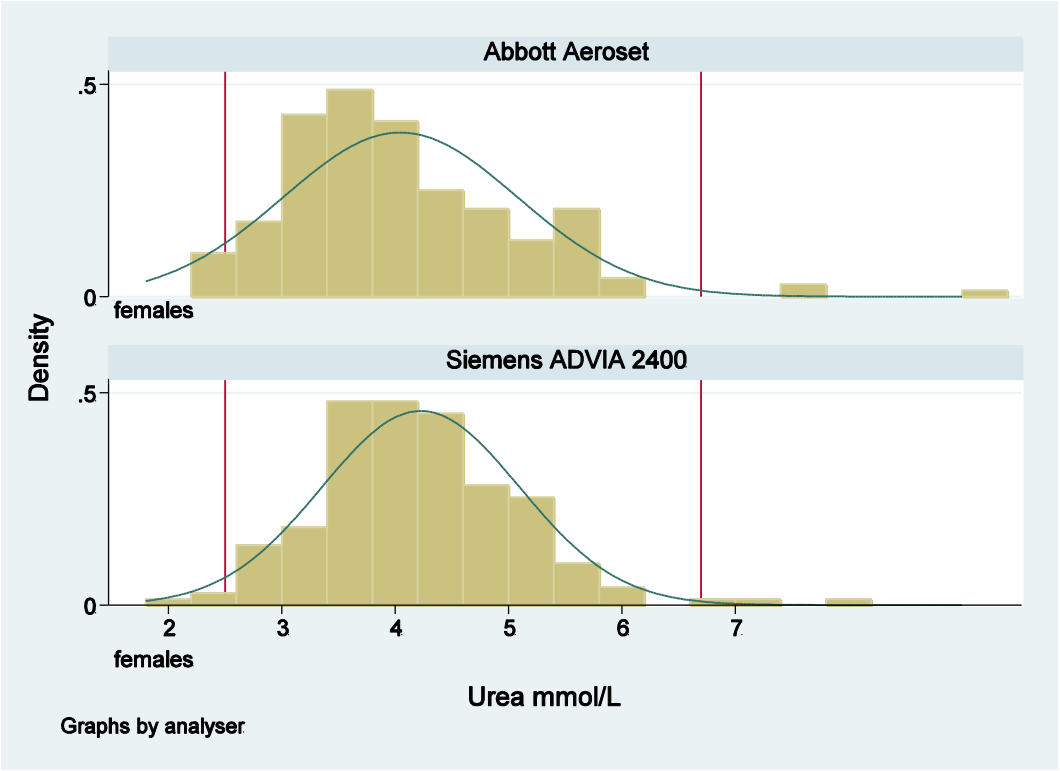
Potassium and sodium results in males by analyser





x lines=limits of institutional RI; curve=empirical normal distribution

Urea results in females by analyser



x lines=limits of institutional RI; curve=empirical normal distribution

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