

# Vaccine strategies against tuberculosis

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## Summary

The need for an improved vaccine against tuberculosis has never been more urgent. The HIV epidemic and the emergence of multi and extensively drug-resistant strains of *Mycobacterium tuberculosis* mean that global control of this pathogen remains inadequate. The existing vaccine, BCG, confers only variable protection against pulmonary disease. Exposure to environmental mycobacteria may contribute to this variability in protective efficacy. Protective immunity against *Mycobacterium tuberculosis* is dependant on a cell-mediated immune response. Boosting BCG with a subunit vaccine, and/or replacing BCG

with an improved BCG are both strategies currently being investigated. Since 2002, there have been increasing numbers of TB vaccine candidates entering into clinical trials. The first of these candidates, MVA85A, is safe and highly immunogenic in all trials to date. In addition, the cellular immune response induced is highly polyfunctional. The protective efficacy of MVA85A will be evaluated in a Phase IIb trial commencing in early 2009 in South African infants.

*Key words:* TB; vaccine; BCG; MVA85A; cellular immunity

## Introduction

There has never been a more urgent need for a new vaccine against tuberculosis (TB). It is estimated that there are currently 8.8 million new cases per annum and 1.7 million deaths throughout the world [1]. The emergence of multi- and now extensively drug-resistant strains of *Mycobacterium tuberculosis* (*M.tb*) has made control of this pathogen even more challenging. In addition, it is estimated that one third of the world (2 billion people) are latently infected with *M.tb*, and are at risk of reactivation of disease [2]. Globally, coinfection with HIV is the commonest cause of

immunosuppression, and infection with HIV increases the risk of reactivation of latent *M.tb* infection from a 10% lifetime risk to a 10% annual risk [3].

In 2006, the Global Plan to Stop TB set out some detailed and ambitious targets for global TB control [1]. This plan explicitly recognises that in addition to the tools currently available, e.g. directly observed therapy, short-course (DOTS), new tools are needed in order for these targets to be achieved. Those new tools include new drugs, new diagnostic tests and new vaccines.

## Existing vaccines – BCG

The only currently licenced vaccine against TB, bacilli Calmette-Guerin (BCG) is an attenuated strain of *M. bovis*. BCG was first used in humans in 1921, when it was administered *per os* [4]. Since that time there have been many clinical trials throughout the world to evaluate the protective efficacy of this vaccine. When administered at birth, as it is throughout the developing world, BCG confers consistent and reliable protection against disseminated disease, particularly TB meningitis, in the first 10 years of life [5]. However, the protection conferred against pulmonary disease is much more variable [6]. A meta-analysis of 14 prospective trials and 12 case-control studies determined that overall the protective effect of BCG against pulmonary disease was 50%, but

that latitude had a significant effect on this efficacy [6]. Furthermore, a recent randomised controlled trial of BCG revaccination has shown that revaccination in adolescents does not improve protective efficacy [7].

Understanding the mechanism behind the variability in efficacy conferred by BCG is important in the development of better vaccines. Several factors have been cited to explain the variability in protective efficacy conferred by BCG throughout the world. It is clear that there are genetic differences between the different strains of BCG that have been used throughout the world [8], but the immunogenicity of different strains of BCG is comparable and it is not clear that these genetic differences confer any difference in pro-

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tection [9]. Nutritional differences in the populations in which the protective efficacy of BCG has been evaluated may also be a contributory factor [10].

The best explanation for the variable protective efficacy of BCG, however, lies in variability in exposure to environmental mycobacteria. Environmental mycobacteria are ubiquitous organisms which live in the soil, and exposure to these mycobacteria is universal but increases with increasing proximity to the equator. It is postulated that these organisms interfere with BCG vaccination either by masking or by blocking. The masking hypothesis has been elegantly demonstrated by studies of BCG vaccination carried out in parallel in the UK (a population where BCG has been shown to be effective) and Malawi (a population where BCG is not effective) [11]. In these studies, at baseline, BCG naïve adolescents in the

UK had minimal anti-mycobacterial immune responses. In contrast, BCG naïve adolescents in Malawi already had high baseline responses to mycobacterial antigens. Most of the UK adolescents responded well to BCG vaccination, and there was a significant incremental rise in immune responses to mycobacterial antigens in this population. In contrast, in Malawi most responses did not significantly rise after BCG vaccination. This data fits with the masking hypothesis, whereby exposure to environmental mycobacteria induces some anti-mycobacterial immunity, and vaccination with BCG does not incrementally increase that response. Blocking is a more active process, whereby it is postulated that pre-existing immunity induced by exposure to environmental mycobacteria inhibits the replication of BCG and hence vaccine 'take'. This mechanism has been demonstrated in animals [12].

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## Protective immunity to *M.tb*

In addition to an understanding of the mechanism behind the failure of BCG, an understanding of the nature of protective immunity to *M.tb* is important in the development of new vaccines. There is considerable interindividual variability in outcome after exposure to *M.tb*. Approximately 70% of individuals exposed to *M.tb* successfully clear the infection [13]. Only approximately 30% of people go on to become infected. This early response is attributable to an effective innate immune response, although the precise nature of this rapid response is not clearly defined [14]. Of the 30% of individuals who become infected, 80–90% are able to contain the initial infection and the mycobacteria enter a state of latency [14]. Only 5–10% of adults develop primary disease. Once latently infected, individuals are at risk of reactivation of this latent infection should they become immunosuppressed for any reason.

*M.tb* is an intracellular pathogen and resides primarily inside macrophages. It is unlikely that antibodies have a major role to play in protective immunity to *M.tb*. Cellular immunity is essential.

It is clear from animal studies, and the increased susceptibility to TB disease seen in HIV infected subjects, that Class II restricted CD4<sup>+</sup> T cells are essential for protective immunity to *M.tb* [3, 15]. Class I restricted CD8<sup>+</sup> T cells probably also play a role in protective immunity, although the precise mechanism by which they work is as yet not clearly elucidated. It may be that this T cell subset is important in maintaining the latent state [16].

In addition to the importance of the different cell types, it is clear that a robust Th-1 type immune response, with secretion of interferon gamma (IFN- $\gamma$ ) from antigen specific T cells, is necessary for an effective immune response against *M.tb*. Animals and humans who have deficiencies in the IFN- $\gamma$  processing pathway are more susceptible to TB [17, 18]. However, IFN- $\gamma$  alone does not appear to be sufficient for protection. Other Th-1 cytokines such as IL-12 and TNF are also important. The importance of TNF has recently been highlighted by the reactivation of latent TB seen in patients commencing therapy with a monoclonal antibody against TNF [19].

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## New vaccines in development

The two main current approaches in developing improved prophylactic TB vaccines are either to use modified BCG or *M.tb* to replace BCG; or to use selected immunodominant antigens in a subunit booster vaccine which is administered some time after BCG vaccination. For subunit vaccines, antigen delivery systems include recombinant viral vectors and protein/adjuvant combinations. BCG is likely to be included in any new TB vaccine regimen, at least in the short to medium term, because of the protection conferred against disseminated disease in childhood [5]. The use of BCG in any new

regimen, either alone as a recombinant BCG or when combined with a subunit boost, allows the retention of these protective effects of BCG in childhood. The two strategies can be combined and a booster vaccine could be used to boost an improved BCG. The leading new prophylactic TB vaccine candidates in development are summarised in table 1.

For the rest of this review paper the focus will be on MVA85A, the vaccine candidate developed by the University of Oxford. This candidate will be used to illustrate the pathway for development of a new TB vaccine.

**Table 1**

Leading prophylactic TB vaccine candidates in development.

Replacement for BCG or booster	Vaccine name	Vaccine characteristics	Developer	Stage of development
Replacement	rBCG30	Recombinant BCG overexpressing antigen 85B	UCLA/Aeras	Entered Phase I study in US in February 2004. Not currently in clinical development
	rBCG (Aeras 403)	Recombinant BCG with endosome escape and overexpressing antigen 85A, antigen B and TB10.4	Aeras	Preclinical development
	rBCGΔUre:CHly*	BCG with endosome escape	Max Planck Institute for Infection Biology, Berlin	Preclinical development
	Pho p <sup>-</sup>	Attenuated strain of <i>M.tb</i>	University of Zaragoza	Preclinical development
Booster	Mtb72F/M72	Fusion protein of 2 antigens from <i>M.tb</i> (32 and 39kDa antigens); used with AS01/AS02 adjuvant	GlaxoSmithKline	Entered Phase I study in US in February 2004; now Phase IIa in Africa
	Hybrid 1	Fusion protein of antigen 85B and ESAT6	Statens Serum Institute	Phase I in Europe commenced in November 2005
	HyVac 4	Fusion protein of antigen 85B and TB10.4	Statens Serum Institute	Phase I in Europe commenced in December 2007
	Aeras 402	Recombinant adenovirus (AdHu35) expressing antigen 85A, antigen 85B and TB10.4	Crucell/Aeras	Phase I in US commenced in October 2006; currently in Phase IIa in Africa
	MVA85A	Recombinant MVA expressing antigen 85A	University of Oxford	Phase I in UK commenced in September 2002; currently in Phase IIa in Africa

## MVA85A

MVA85A is a recombinant strain of modified vaccinia Ankara (MVA) expressing antigen 85A from *M.tb*. MVA is a highly attenuated strain of vaccinia virus which has been passaged over 500 times through chick embryo fibroblasts. As a result, host range and cytokine genes have been deleted and it cannot replicate in human cells [20]. MVA was used at the end of the smallpox eradication campaign to vaccinate over 100,000 people in southern Germany, and was found to have an excellent safety profile [21]. Recombinant pox viruses have been demonstrated to be excellent at boosting previously primed cellular immune responses. The antigen selected for insertion into the MVA vector is antigen 85A. Antigen 85A is a highly immunodominant antigen in both preclinical and clinical studies which is present in all strains of mycobacteria sequenced to date [22]. It is protective when administered alone in small animals [23]. Importantly, the use of this antigen in a new vaccine does not interfere with the new diagnostic tests which are increasingly in clinical use [24].

MVA85A first entered into clinical development in September 2002 [25]. These early clinical studies were designed primarily to demonstrate safety. The first studies were conducted in the UK in BCG naïve, tuberculin skin test (TST) negative adults. The aim with these studies was to identify subjects who were as mycobacterially naïve as possible in this first clinical trial, before going on to evaluate this vaccine sequentially in BCG vaccinated subjects and then *M.tb* latently infected subjects. In addition, each subject group was evaluated first in the UK before being evaluated in a TB endemic country. The reason for this caution was concern within the field of TB vaccine research about the induction of a Koch phenome-

non. The Koch phenomenon describes the induction of immunopathology at the site of infection in animals infected with *M.tb*. This phenomenon has been demonstrated in preclinical models and was also seen in humans, when Robert Koch developed his 'remedy' for TB at the end of the 19<sup>th</sup> century [26, 27].

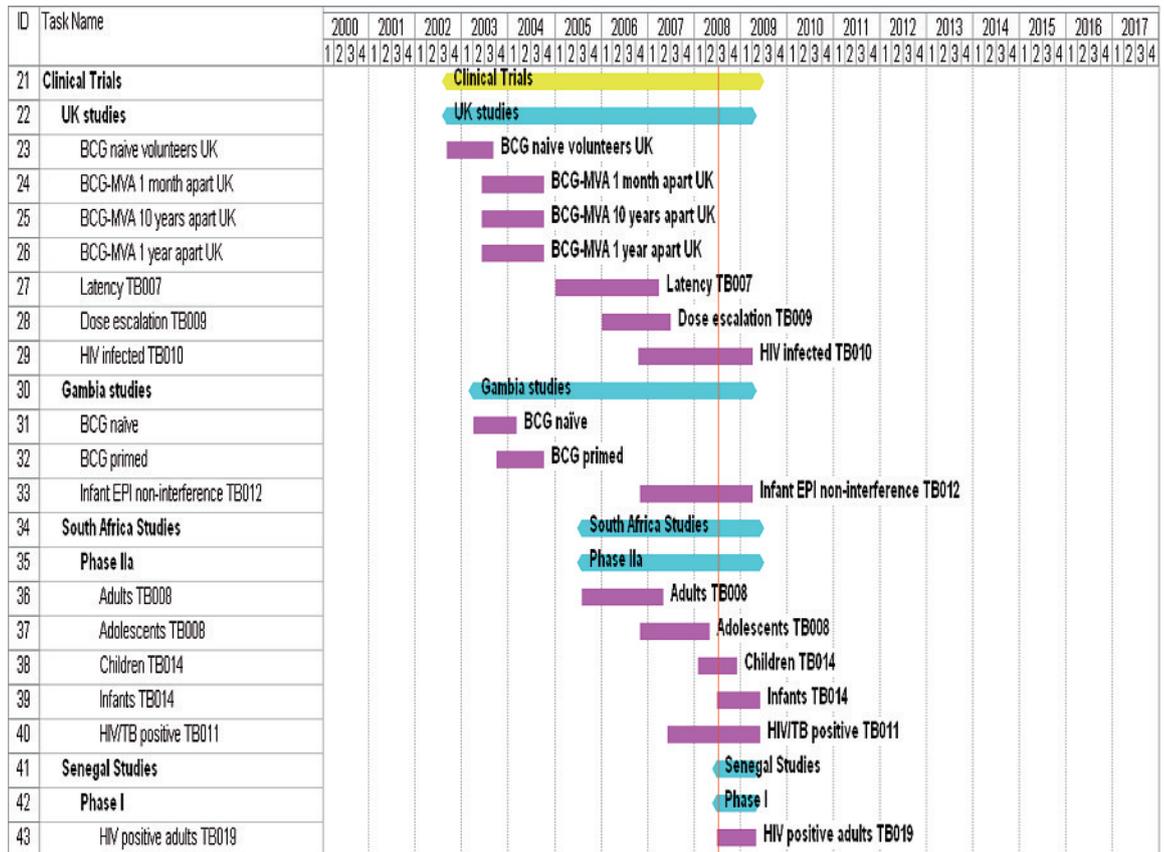
The Gantt chart in figure 1 outlines the clinical trials conducted with MVA85A since 2002. This is taken from the overall Gantt chart for the full product development of MVA85A. The outcome measures in all of these clinical trials have been safety and immunogenicity. The primary immunological readout in all of these clinical trials has been the ex-vivo IFN- $\gamma$  Elispot assay, using an 18-hour overnight incubation. In addition, a more detailed immunological analysis has been conducted using cryopreserved peripheral blood mononuclear cells, in order to fully characterise the vaccine induced immune responses.

To date in April 2008, 258 subjects have been vaccinated with MVA85A and there have been no vaccine related serious adverse events. MVA85A is administered intradermally, and mild local adverse events at the site of injection are common [28]. Mild self-limiting systemic adverse events which typically occur in the first 12–24 hours after vaccination are also common [28]. Importantly, there have been no signs of immunopathology in any of the trials to date, which include latently infected subjects in both the UK and South Africa (Sander et al., submitted; Tameris, personal communication).

The immunogenicity results from the trials conducted to date are also encouraging. When administered to BCG naïve subjects in the UK, MVA85A induces a significant antigen specific T cell response which reaches a peak one week after

**Figure 1**

Gantt chart summarising the clinical trials conducted with MVA85A since 2002.



vaccination and then declines. Three weeks after vaccination, the response is not greater than baseline and boosting with a second MVA85A vaccination at this timepoint does not amplify the response further [28]. In contrast, administering MVA85A to subjects who had previously been vaccinated with BCG (median interval between BCG and MVA85A was 18 years) resulted in significantly higher antigen specific T cell responses one week after vaccination when compared with the BCG alone or MVA85A alone group [28]. Importantly for the induction of a central memory T cell response, these responses remained significantly higher than either vaccine alone up to 24 weeks after vaccination.

A more detailed immunological analysis of the samples from the BCG-MVA85A vaccinated subjects has demonstrated that the antigen specific CD4<sup>+</sup> T cells induced by vaccination with MVA85A are highly polyfunctional [29]. One week after vaccination almost 50% of the antigen specific T cells are positive for all four of the markers measured during this study: IFN- $\gamma$ , IL2, TNF $\alpha$  and MIP-1 $\beta$ . This polyfunctional profile persists, and six months after vaccination the responses remain considerably more polyfunctional than the baseline responses. Further analysis of the phenotype of these antigen specific CD4<sup>+</sup> T cells shows them to be relatively immature, with a non-terminally differentiated phenotype. In addition, proliferation studies confirm the proliferative potential of these cells [29].

Other trials with MVA85A have been conducted to investigate the effect of different intervals between BCG and MVA85A. Vaccination with

MVA85A soon after BCG vaccination might be comparable to boosting in infancy when BCG has been given at birth. In contrast, vaccination with MVA85A many years after BCG might mimic a clinical scenario of boosting in adolescence. Studies comparing the magnitude of immune response after MVA85A vaccination soon (one month) and a long time (median 18 years) after BCG vaccination show that there is no difference in the magnitude of immune response seen in these two groups when comparing ex-vivo IFN- $\gamma$  Elispot responses [30].

When sufficient safety data had been accumulated in the early studies with MVA85A, a study was conducted in *M.tb* latently infected subjects (Sander et al., submitted). Detailed clinical monitoring, including high resolution CT scans, was performed pre- and post-vaccination in this study in order to detect any clinical or sub-clinical immunopathology. Reassuringly, the safety profile was very comparable to previous studies and there were no signs of any immunopathology. Importantly, the immunogenicity of MVA85A in this latently infected population was also very comparable to previous studies.

In 2005, a Phase IIa programme of clinical trials with MVA85A commenced in South Africa. These trials include a series of studies in progressively younger populations: adults, adolescents, children and infants. To date the safety and immunogenicity data has been similar to that seen in the UK studies [31]. Importantly, the responses in South African adults are as durable as the UK responses, with responses remaining significantly higher than baseline for at least one year after vaccination [31].

In 2006 we commenced a Phase IIa non-interference study in Gambian infants. The purpose of this study was to assess whether co-administration of MVA85A together with the routine EPI schedule vaccines would lead to immunological interference; either with the humoral immune response to the EPI vaccines or with the cellular immune response to MVA85A (Ota, personal communication).

In summary, in all of the clinical trials conducted to date MVA85A is safe and immunogenic. The populations in whom it has been evaluated include *M.tb* infected adults in the UK and South Africa; HIV infected adults in the UK and South Africa; and children and infants in South and West Africa. The immune profile induced by MVA85A is consistent with what is understood about protective immunity against *M.tb*, in that this vaccine induces high levels of highly polyfunctional CD4<sup>+</sup> T cells which proliferate and are not terminally differentiated. This vaccine also improves BCG induced protection in animal models.

The key question is, 'Does MVA85A enhance BCG induced protection in humans?' There are three main target populations most in need of an improved TB vaccine, and these are the groups in which one would wish to evaluate the protective efficacy of MVA85A: infants; adolescents; and HIV infected adults. In early 2009, a Phase IIb proof-of-concept trial evaluating MVA85A in BCG vaccinated infants is scheduled to commence in South Africa, in collaboration with the South African TB Vaccine Initiative (SATVI) and Aeras Global TB Foundation. This important trial will provide insights into the potential immunological correlates of protection and increase our understanding of the relevance of the various animal models.

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## References

- 1 Stop TB Partnership. The Global Plan to Stop TB. 2006.
- 2 WHO. Global tuberculosis control. Surveillance, planning and financing. WHO report. WHO, Geneva, Switzerland 1-247. 2005:1-247.
- 3 Corbett EL, De Cock KM. Tuberculosis in the HIV-positive patient. *Br J Hosp Med*. 1996;56(5):200-4.
- 4 Fine PC, IAM; Milstien JB, Clements CJ. Issues relating to the use of BCG in immunisation programmes. WHO, Geneva, 1999.
- 5 Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculosis meningitis and miliary tuberculosis: a meta-analysis. *Int J Epidemiol*. 1993;22(6):1154-8.
- 6 Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA*. 1994;271(9):698-702.
- 7 Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. *Lancet*. 2005;366(9493):1290-5.
- 8 Behr MA, Wilson MA, Gill WP, Salamon H, Schoolnik GK, Rane S, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. *Science*. 1999;284(5419):1520-3.
- 9 Gorak-Stolinska P, Weir RE, Floyd S, Lalor MK, Stenson S, Branson K, et al. Immunogenicity of Danish-SSI 1331 BCG vaccine in the UK: Comparison with Glaxo-Evans 1077 BCG vaccine. *Vaccine*. 2006;24(29-30):5726-33.
- 10 Epstein PR. BCG vaccination and nutrition. *Lancet*. 1990;335(8704):1536-7.
- 11 Black GF, Weir RE, Floyd S, Bliss L, Warndorff DK, Crampin AC, et al. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. *Lancet*. 2002;359(9315):1393-401.
- 12 Brandt L, Feino Cunha J, Weinreich Olsen A, Chilima B, Hirsch P, Appelberg R, et al. Failure of the *Mycobacterium bovis* BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to tuberculosis. *Infect Immun*. 2002;70(2):672-8.
- 13 Manabe YC, Bishai WR. Latent *Mycobacterium tuberculosis*-persistence, patience, and winning by waiting. *Nat Med*. 2000;6(12):1327-9.
- 14 Bhatt K, Salgame P. Host innate immune response to *Mycobacterium tuberculosis*. *J Clin Immunol*. 2007;27(4):347-62.
- 15 Caruso AM, Serbina N, Klein E, Triebold K, Bloom BR, Flynn JL. Mice deficient in CD4 T cells have only transiently diminished levels of IFN-gamma, yet succumb to tuberculosis. *J Immunol*. 1999;162(9):5407-16.
- 16 van Pinxteren LA, Cassidy JP, Smedegaard BH, Agger EM, Andersen P. Control of latent *Mycobacterium tuberculosis* infection is dependent on CD8 T cells. *Eur J Immunol*. 2000;30(12):3689-98.
- 17 Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med*. 1993;178(6):2249-54.
- 18 Dorman SE, Picard C, Lammas D, Heyne K, van Dissel JT, Baretto R, et al. Clinical features of dominant and recessive interferon gamma receptor 1 deficiencies. *Lancet*. 2004;364(9451):2113-21.
- 19 Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. *Lancet Infect Dis*. 2003;3(3):148-55.
- 20 Blanchard TJ, Alcamí A, Andrea P, Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. *J Gen Virol*. 1998;79(Pt 5):1159-67.
- 21 Mayr A, Stickl H, Müller HK, Danner K, Singer H. The smallpox vaccination strain MVA: marker, genetic structure, experience gained with the parenteral vaccination and behavior in organisms with a debilitated defence mechanism (author's transl). *Zentralbl Bakteriol [B]*. 1978;167(5-6):375-90.
- 22 D'Souza S, Rosseels V, Romano M, Tanghe A, Denis O, Jurion F, et al. Mapping of murine Th1 helper T-Cell epitopes of mycolyl transferases Ag85A, Ag85B, and Ag85C from *Mycobacterium tuberculosis*. *Infect Immun*. 2003;71(1):483-93.
- 23 Huygen K, Content J, Denis O, Montgomery DL, Yawman AM, Deck RR, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. *Nat Med*. 1996;2(8):893-8.
- 24 Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis*. 2004;4(12):761-76.
- 25 McShane H, Pathan AA, Sander CR, Goonetilleke NP, Fletcher HA, Hill AV. Boosting BCG with MVA85A: the first candidate subunit vaccine for tuberculosis in clinical trials. *Tuberculosis (Edinb)*. 2005;85(1-2):47-52.
- 26 Taylor JL, Turner OC, Basaraba RJ, Belisle JT, Huygen K, Orme IM. Pulmonary necrosis resulting from DNA vaccination against tuberculosis. *Infect Immun*. 2003;71(4):2192-8.
- 27 Koch R. Fortsetzung der Mitteilungen über ein Heilmittel gegen Tuberkulose. *Dtsch Med Wochenschr*. 1891;17:101-2.
- 28 McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nat Med*. 2004;10(11):1240-4.
- 29 Beveridge NE, Price DA, Casazza JP, Pathan AA, Sander CR, Asher TE, et al. Immunisation with BCG and recombinant MVA85A induces long-lasting, polyfunctional *Mycobacterium tuberculosis*-specific CD4<sup>+</sup> memory T lymphocyte populations. *Eur J Immunol*. 2007;37(11):3089-100.
- 30 Pathan AA, Sander CR, Fletcher HA, Poulton I, Alder NC, Beveridge NE, et al. Boosting BCG with Recombinant Modified Vaccinia Ankara Expressing Antigen 85A: Different Boosting Intervals and Implications for Efficacy Trials. *PLoS ONE*. 2007;2(10):e1052.
- 31 Hawkrigde T, Scriba TJ, Gelderbloem S, Smit E, Tameris M, Moyo S, et al. Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in healthy adults in South Africa. *J Infect Dis*. 2008;198(4):544-52.