BMC
Infectious Diseases

REVIEW   **Open Access**

Vaccine against tuberculosis: what's new?

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Abstract

Background: one of the World Health Organization Millennium Development Goal is to reduce tuberculosis incidence by 2015. However, more of 8.5 million tuberculosis cases have been reported in 2011, with an increase of multidrug-resistant strains. Therefore, the World Health Organization target cannot be reach without the help of a vaccine able to limit the spread of tuberculosis. Nowadays, bacille Calmette-Guérin is the only vaccine available against tuberculosis. It prevents against meningeal and disseminated tuberculosis in children, but its effectiveness against pulmonary form in adolescents and adults is argued.

Method: a systematic review was performed by searches of Pubmed, references of the relevant articles and Aeras and ClinicalTrial.gov websites.

Results: 100 articles were included in this review. Three viral vectored booster vaccines, five protein adjuvant booster vaccines, two priming vaccines and two therapeutic vaccines have been analyzed.

Conclusions: Several vaccines are in the pipeline, but further studies on basic research, clinical trial and mass vaccination campaigns are needed to achieve the TB eradication target by 2050.

Background

One of the World Health Organization (WHO) Millennium Development goals (MDGs) is to reduce tuberculosis (TB) incidence by 2015. The targets of the Stop TB Partnership, an international coalition designed to coordinate the efforts against TB, are to halve deaths and prevalence of TB by 2015, relative to 1990 levels and to reduce the global incidence of less than one per million population by 2050 [1].

However, WHO reported an estimated 8.7 million new TB cases in 2011, 0.5 million of which occurred among children, and 1.4 million deaths [2]. Among incident cases, 13% are co-infected with human immunodeficiency virus (HIV) [2]. An additional threat to TB control includes the spread of multidrug-resistant (MDR) strains. Among TB-treatment-naïve cases, 3.7% have been estimated to be MDR, percentage that reaches 20% in previously treated TB cases [2].

Considering the above mentioned data and that an active TB case will typically infect 10-15 contacts, advances in diagnostic and therapeutic strategies are not sufficient to achieve the goal of elimination of TB by 2050 [3]. Therefore, new vaccines development is urgently

needed for the control of TB. A vaccine will limit initial infection, progression of disease and reactivation of latent TB [4]. Moreover, it can also be an essential tool in tackling the spread of MDR TB.

Nowadays, bacille Calmette-Guérin (BCG) is still the only vaccine available against TB. It was firstly administered as oral vaccine to an infant in 1921 and it is still the only vaccine licensed to prevent TB. It is a live attenuated strain of *Mycobacterium bovis*, obtained by Albert Calmette and Camille Guérin through 230 *in vitro* passages over a 13 year-period [5]. Since daughter strains of BCG have been distributed around the world to produce vaccine in manufacture, genetic and antigenic differences have emerged between vaccine strains [6]. Thus, global concerns about safety and efficacy between different strains arose.

BCG is widely used in TB endemic countries, where newborns are immunized as soon as possible after birth with a single intradermal dose [7]. To date, it is estimated that BCG has been administered over 4 billion times and that 120 million children receive BCG every year globally [8].

Meta-analyses of published studies have clearly reported that BCG prevents against meningeal TB and disseminated forms in children [9,10]. However, randomized clinical trials have reported estimates of protection against

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pulmonary TB that vary from nil to 80% [6,11]. Therefore, since pulmonary TB is the most prevalent form of disease in adolescents and adults and the most significant source of TB transmission, BCG is estimated to have little impact in limiting TB spread. Several causes have been considered to explain the variable efficacy of BCG. These includ differences in BCG strains, host genetic and nutritional factors, variable virulence among *Mycobacterium tuberculosis* (*Mtb*), interference of environmental mycobacteria, switch to a type 2 immunological response in presence of helminthic infection and variation among trial methods [3,5,8,12,13]. Recent studies, evaluating interferon-γ (INF-γ) release assay (IGRA) results, suggested that BCG could not only protect against disseminated TB in children, but also against infection [14].

Several studies demonstrated that a booster dose of BCG did not improve protection aginst TB [15].

BCG is a safe vaccine in healthy infants. Loco-regional adverse reaction, including regional adenitis are usually self-limiting [12]. However, it can cause severe disseminated disease, named BCG-osis, in patients affected by some primary immunodeficiency, such as severe combined immunodeficiency and chronic granulomatous disease [16]. Different studies revealed an increased risk of disseminated BCG disease in HIV-infected children, even if asymptomatic at time of vaccination [17,18]. Thus, WHO Global Advisory Committee on Vaccine Safety recommends that BGC should not be administered in HIV-infected patients [19]. This is an important limitation, considering that countries where TB is endemic are the same where HIV spreads. Since that, a safer and more effective vaccine is urgently required. In response to this challenge, the development pipeline now includes 12 vaccine candidates [20], that can either boost BCG's initial effects (booster vaccine) or replace BCG (priming vaccine).

The aim of booster vaccines, that include viral vectored or protein adjuvant vaccines, is to enhance the immune response of BCG, whereas the priming vaccines aim at replacing BCG with a safer and long-lasting vaccine, that can be a recombinant BCG strain or a genetically attenuated *Mtb*.

A third strategy is to develop therapeutic vaccines reducing duration of TB therapy.

The aim of this review is to provide updated information on vaccine entered into clinical trials.

Methods

Data included in this review were retrieved by searches of Pubmed, references of the relevant articles and open-access website of Aeras and ClinicalTrial.gov. The search was limited to English-language studies published between 1st September, 2003 and 1st September 2013. Studies reporting data on vaccine now in clinical trials were included in this review. Article were excluded if redundant or not pertinent on the basis of titles and abstracts. Articles on pre-clinical studies of vaccine not yet in clinical trial were excluded.

Search strategy

The search strategy was: Vaccine[Title] AND (tuberculosis[Title] OR tb[Title] OR bcg[Title]) AND ("2003/09/01"[PDAT] : "2013/09/01"[PDAT]) AND (Journal Article [ptyp] AND English[lang]) NOT ("leishmania"[MeSH Terms] OR "leishmania"[All Fields]) NOT ("leprosy"[MeSH Terms] OR "leprosy"[All Fields]) NOT ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) NOT ("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields]) NOT ("vitamins"[Pharmacological Action] OR "vitamins"[MeSH Terms] OR "vitamins"[All Fields] OR "vitamin"[All Fields]) NOT ("lactoferrin"[MeSH Terms] OR "lactoferrin"[All Fields]).

Results

The search performed generated a total of 461 results. A total of 100 articles were included in the review (Additional file 1). Three viral vectored booster vaccines, five protein adjuvant booster vaccines, two priming vaccines and two therapeutic vaccines have been analyzed.

Viral-vectored booster vaccines
MVA85A

MVA85A is the most clinically advanced vaccine candidate (Table 1). It is the first new TB vaccine to enter into clinical trials in 2002 and to be tested in infants since BCG.

It is a recombinant strain of Modified Vaccinia virus Ankara expressing the *Mtb* antigen 85A (Ag85A), designed to enhance response induced by BCG [21]. The live viral vector cannot replicate in human.

Several trials reported its safety in healthy adults, *Mtb* and HIV infected patients, adolescents, children and infants[22-26]. Minor local and systemic reactions are frequently reported in the first week after immunization. No serious vaccine-related adverse events have been reported [22-26].

Vaccine is normally performed intradermally, even if a phase I trial reported a safe and higher immunogenic profile of MVA85A delivered intramuscularly in healthy adults [27].

MVA85A has shown to induce a polyfunctional CD4+ T-cells population, expressing INF-γ, interleukin-2 (IL-2), tumour necrosis factor-α (TNF-α), interleukin-17 (IL-17)

Table 1 Viral vectored booster vaccines

Vaccine Name	Composition	Primary type	Development phase
MVA85A	Modified Vaccinia virus Ankara expressing Ag85A	Booster vaccine	Phase 2b
Ad5Ag85A	Recombinant adenovirus 5 expressing Ag85A	Booster vaccine	Phase 1
Ad35	Recombinant adenovirus 35 expressing Ag85A-Ag85B-TB10.4 fusion protein	Booster vaccine	Phase 1/2

and granulocyte-macrophage colony-stimulating factor and a modest CD8^{+} T-cells response [22,25,26,28,29].

Data showed that a dose of 1 x 10^{8} plaque forming units was as safe as lower doses and induced a higher immune response in healthy previously BCG vaccinated UK adults [23].

However, a recent phase 2b trial on safety and efficacy of MVA85A was conducted in 2797 healthy South African infants previously vaccinated with BCG. The vaccine was well tolerated and immunogenic, but it was poorly protective against TB infection [22]. Authors suggested that the immunologic response induced by the vaccine could not be related to protective effect against TB infection.

The safety and immunogenicity of MVA85A priming and BCG-booster vaccination is currently being evaluated in a phase 2 trial [30].

Moreover, a phase 1 trial testing safety of MVA85A combined with a different carrier protein, IMX313, is ongoing [31].

Another poxvirus-vectored candidate vaccine, Fowlpox virus expressing the *Mtb* antigen 85A has been entered clinical trial in 2008 but it failed to induce an adequate immune-response [32].

AdAg85a

AdAg85a is a recombinant strain of replication-deficient adenoviral vector expressing the *Mtb* Ag85A [33] (Table 1).

Experimental data showed that it provided potent protection against pulmonary TB infection in mice when administered intranasally, either as priming and as booster vaccine for BCG [33,34]. Intranasal administration enhanced a better protection than intramuscular in both settings [33,34]. In guinea pigs, both intranasal and intra-muscular vaccination were protective against pulmonary TB infection. However intranasal route seemed to provide stronger protection [35].

Santuosso et al. demonstrated that intranasal AdAg85a was able to elicit robust mucosal CD4^{+} and CD8^{+} T-cells responses in the airway lumen [34,36].

A phase 1 trial evaluating safety and immunogenicity of AdAg85a administered intramuscularly in previously and not-previously BCG-vaccinated healthy adults has been terminated in July 2013 [37].

Mu et al. reported that a new intranasally bivalent adenovirus-vectored vaccine expressing Ag85A and TB10.4 antigen provided an improved protection against TB pulmonary infection in mice [38].

Ad35/Aeras-402

Ad35/Aeras 402 is a recombinant, non-replicating adenovirus, serotype 35 vaccine, which expresses a fusion protein from the *Mtb* Ag85A, antigens 85B (Ag85B) and TB10.4 [39] (Table 1).

Good safety profiles have been shown in phase 1 trials on healthy previously BCG vaccinated adults. No serious adverse events related to the vaccine have been reported. However, mild to moderate local adverse events were frequent [40,41].

Ad35/Areas 402 provided strong CD4^{+} and CD8^{+} T-cells responses in mouse, especially if intranasally administered [39]. Similarly, it was able to induce potent CD4^{+} and CD8^{+} T-cells responses in healthy adults, with important IFN-γ, TNF-α and IL-2 production if administered as booster BCG vaccine [40,41]. However, it did not elicit any IL-17 secretion [41].

Trials evaluating safety and immunogenicity of Ad35/Aeras 402 in infants and HIV-infected adults are ongoing [42,43]. Moreover, a study to asses safety and immunogenicity of Ad35/Aeras 402 followed by MVA85A started on September 2012 [44].

Protein adjuvant booster vaccines
H1/IC31

H1/IC31 is a recombinant subunit vaccine, composed by the hybrid protein of Early Secretory Antigenic Target 6 (ESAT6) and Ag85B adjuvanted with IC31, an adjuvant system composed by the cationic protein polyaminoacid KLK and oligodeoxynucleotide ODN1a [45] (Table 2).

The fusion protein Ag85B-ESAT6 has been extensively evaluated in several animal models and combined with different adjuvants [46-49].

H1/IC31 has been shown to be safe in healthy adults. No serious adverse events related to the vaccine have been reported. Local and systemic described events were mild and quickly resolving (<48 hours) [45,50,51].

H1/IC31 provided long-lasting T_H1 responses either in mycobacterially-naïve subjects as well in BCG-vaccinated or previously TB infected voluntaries. The immune response can be amplified by booster vaccinations. H1/IC31 induce a weak immune response against ESAT6; however this vaccine provided a more potent protective effect than a vaccine with Ag85B alone [45,50,51].

IGRAs measure the amount of INF-γ produced by lymphocytes after *in vitro* incubation with *Mtb* antigens, included ESAT6. Even if H1/IC31 has shown to induce

Table 2 Protein adjuvant booster vaccines

Vaccine Name	Composition	Primary type	Development phase
H1/IC31	ESAT6-Ag85B fusion protein + IC31 adjuvant	Booster vaccine	Phase 1
HyVac4	TB10.4-Ag85B fusion protein + IC31 adjuvant	Booster vaccine	Phase 1/2
ID93/GLA-SE	Rv2608-Rv3619-Rv3620-Rv1813 fusion protein + GLA-SE adjuvant	Booster vaccine	Phase 1
H56/IC31	Ag85B-ESAT6-Rv2660c fusion protein + IC31 adjuvant	Booster vaccine	Phase1/2a
M72/AS01E	Mtb39a-Mtb32a fusion protein + AS01E adjuvant	Booster vaccine	Phase 2

QuantiFERON positivity in only few subjects, further studies evaluating possible interference of the vaccine with IGRA results are needed [45,50,51].

HyVac4/Aeras 404

HyVac4/Aeras 404 is a booster vaccine developed by the same group of H1/IC31. The antigen ESAT6 was replaced by TB10.4, to avoid the interference with IGRAs and the fusion protein was combined with the adjuvant IC31 [52] (Table 2).

Dietrich *et al.* demonstrated that the fusion protein Ag85B-TB10.4 (HyVac4) combined with the adjuvant protein dimethyl dioctadecyl ammonium/monophosphoryl lipid A (DDA/MPL) was highly immunogenic and induced strong protection against TB in mice model [53].

HyVac 4/Aeras 404 is safe and protective against pulmonary TB when administered as priming or booster vaccine in guinea pigs and mice [52,54]. When used as booster vaccine for BCG in mouse model, it induced expression of IFN-γ, TNF-α and IL-2 triple positive CD4$^+$ T-cells that seemed to be correlated with protection against TB [54].

Several phase I trials on HyVac4/Aeras 404 have been terminated [55] and a phase I/II trial on its safety and immunogenicity in BCG vaccinated healthy infants is ongoing [56].

ID93/GLA-SE

ID93/GLA-SE is a protein-adjuvant vaccine, composed by ID93, a fusion protein comprising four *Mtb* antigens (Rv2608, Rv3619, Rv3620 and Rv1813), combined with the glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE) [57] (Table 2).

ID93/GLA-SE induced a significant T_H1 immune response, with multifunctional IFN-γ, TNF-α and IL-2 CD4$^+$ T-cells production in BCG-vaccinated or not-vaccinated mice and guinea pigs [57,58]. That response led a strong protection against pulmonary TB. Moreover, it was well tolerated and induced T_H1 and T_H2 CD4$^+$T-cells responses in BCG-vaccinated non-humans primates and it elicited CD4$^+$ and CD8$^+$ T-cells responses in BCG-vaccinated or TB-exposed human peripheral blood mononuclear cells [57]. In contrast, if combined with SE alone, ID93 elicited a T_h2 immune response, that did not improve protection against pulmonary TB in mouse and guinea pig models [58].

Experimental data showed that ID93/GLA-SE protected also against MDR-TB in animal models [57].

Baldwin *et al.* demonstrated that ID93 combined with an adenovirus type 5 vector induced strong CD8$^+$ T-cells responses, but it did not provide long-lived immune responses if not combined with a priming or booster ID93/GLA-SE vaccination [59]

Two clinical trials evaluating safety, tolerability and immunogenicity of ID93/GLA-SE in healthy adults, either as priming vaccine as well as booster vaccine, are ongoing [60,61].

H56/IC31

H56/IC31 is a protein-adjuvant vaccine composed by H56, a fusion protein containing Ag85B, ESAT6 and the latency-associated protein Rv2660c, combined with the adjuvant IC31 [62] (Table 2). In mouse models, H56 elicited a strong multifunctional CD4$^+$T-cells response and limited reactivation of latent TB [63].

Lin *et al.* demonstrated that H56/IC31 was safe and immunogenic in BCG-vaccinated non-human primate models. Moreover the vaccine showed excellent control of latent infection [62]. Notably, in vaccinated non-human primate anti-TNF antibodies treatment did not induce reactivation of latent TB [62].

A phase 1/2a trial on safety and immunogenicity of H56/IC31 in HIV-negative, BCG vaccinated with or without latent TB is ongoing [64].

M72/AS01E

M72/AS01E is a recombinant vaccine developed to boost BCG-induced or *Mtb*-induced immune response (Table 2).

The M72 antigen is strictly related to Mtb72F, a fusion protein comprising the Mtb39a and Mtb32a antigens [65]. A point mutation was performed in the Mtb32a antigen of M72 to improve the long-term stability of Mtb72F [66].

AS01E is an adjuvant system containing the immunostimulants MPL and *Quillaja saponaria* fraction 1 (QS21) combined with liposomes [66]. It induced humoral and T_H1 cellular responses [65].

Mtb72F, combined with the adjuvant AS02 (oil-in-water emulsion of MPL and QS21) has been tested in mice, guinea pigs, rabbits and non-human primates models, revealed good safety and immunogenicity profiles [67-69]. Moreover, Mtb72F/AS02 was rather well tolerated and

immunogenic in adults with or without previous exposure to *Mtb* or BCG [65,70].

Meanwhile, concerns for the real effectiveness of Mtb72f have emerged do to variation in Mtb32a and Mtb39a antigens sequences in different *Mtb* strains [71].

M72/AS01E presented frequent local adverse events, resolving within one week of vaccination, predominantly due to the adjuvant. No serious adverse events related to the vaccination have been reported [66,72].

M72/AS01E induced long-lasting multifunctional $CD4^+$T-cells responses in adults with or without BCG or *Mtb* contacts, that was higher than the responses elicited by the AS02-adjuvanted vaccine. As shown in MVA85A, M72/AS01E seemed not to induce robust $CD8^+$T-cells responses [66,72].

Phase 2 trials on safety and immunogenicity of M72/AS01E in adults with HIV and with TB are ongoing [73,74].

Priming vaccines
VPM1002

VPM1002 is a recombinant BCG strain that expresses membrane-perforating listeriolysin (encoded by the gene *hly*) of *Listeria monocytogenes*, lacking the urease C gene (BCG *ΔureC::hly*) and that contains a hygromycin resistance marker [75] (Table 3).

It has shown to produce a better protection against TB by stimulating type 1 and type 17 cytokines in mice compared with parental BCG (pBCG) [76]. Moreover, in a mouse model, apoptotic vescicles from BCG *ΔureC:: hly*-infected macrophages induced greater $CD4^+$ and $CD8^+$ T-cells responses than pBCG [77].

VPM1002 was safe in healthy adults, with adverse events profile comparable to BCG. No serious adverse events related to the vaccine have been reported and no human-to-human transmission has been documented [75]. VPM1002 induced robust $CD4^+$ and $CD8^+$ T-cells and antibodies responses [75].

A phase 2 trial evaluating safety and immunogenicity of VPM1002 in comparison with BCG in newborns is ongoing [78].

Notably, the recombinant BCG strain Aeras 422, that showed promising results in animal models , failed to overcome phase 1 trial, do to some reactivation of shingles in vaccinated healthy adults [79] and the development of rBCG30 strain, an overexpressing Ag85B recombinant vaccine that showed safety and immunogenicity in a phase 1 trial, did not carry on [80].

MTBVAC

MTBVAC is the first live-attenuated *Mtb* vaccine entered in phase 1 clinical trial in January 2013 [81] (Table 3).

It derives from the SO2, an attenuated strain obtained by the insertion of a kanamycin-resistance cassette in the *phoP* gene of *Mtb*. *phoP* is a transcription regulator, therefore its mutation determines lack of expression of several genes, including virulence factors, such as ESAT6 [82]. Although SO2 seemed to be immunogenic and protective against TB in animal models [82,83], it failed to satisfy the Geneva consensus requirements for progressing new vaccines into clinical trials [81].

Hence, the same research group developed a new vaccine strain, with tow stable mutations in the *phoP* and *fadD26* genes [81]. *fadD26* product is required for the synthesis of phthiocerol dimycocerosates, a component of cell envelope that protect *Mtb* from host defenses [84].

MTBVAC was safe, immunogenic and protective against TB in mouse and guinea pig models [81]. Since it was functionally comparable to SO2, it has been authorized to enter in clinical trial after these tests.

Therapeutic vaccines
RUTI®

RUTI® is a therapeutic vaccine constituted by detoxified liposomal fragments of *Mtb* [85] (Table 4). It was developed to complete latent TB treatment after a short course of antimicrobial therapy [85,86]. Experimental data showed that RUTI® is safe and able to elicit T_H1-T_H2-T_H3 responses in animal models. Moreover, $CD8^+$ T-cells and antibodies responses have been reported [85,87].

A phase 1 trial revealed that RUTI® administered subcutaneously was rather well tolerated in BCG-naïve healthy adults, except local reaction. No serious adverse events have been reported [88]. Moreover, it induced a specific cellular and humoral responses [88].

Mycobacterium vaccae

A whole inactivated *Mycobacterium vaccae* (MV) administered intradermally was firstly evaluated as a therapeutic vaccine (Table 4). Clinical trials showed conflicting results [89]. A meta-analysis on MV added TB chemotherapy in never-treated TB patients, showed that it is effective in improving both sputum conversion and X-ray imagines [90].

More recently, MV was tested as prophylactic vaccine to prevent TB, especially in HIV-infected patients

Table 3 Priming vaccines

Vaccine Name	Composition	Primary type	Development phase
VPM1002	Recombinant BCG strain	Priming vaccine	Phase 2
MTBVAC	Live-attenuated *Mycobacterium tuberculosis*	Priming vaccine	Phase 1

Table 4 Therapeutic vaccines

Vaccine Name	Composition	Primary type	Development phase
RUTI®	Detoxified liposomal fragments of *Mycobacterium tuberculosis*	Therapeutic vaccine	Phase 2
Mycobacterium vaccae	Whole inactivated *Mycobacterium vaccae*	Therapeutic vaccine	Phase 3

[91-93]. MV was well tolerated and no serious adverse events have been reported. A meta-analysis revealed that MV was able to prevent TB in high risk category and was safe and immunogenic in HIV-infected patients [94].

In a phase 3 trial, MV has shown to induce variable IFN-γ and humoral responses, according to CD4+ T-cells count, HIV viral load and previous TB treatment [95].

Conclusions

One of the WHO's MDG is to reduce TB incidence by 2015 and one of the Stop TB Partnership targets is to eradicate TB by 2050. Hence, combined strategies based on faster diagnostic tools, drugs effective against MDR TB and able to shortness duration of treatment and vaccines are essential to reach these targets.

Several vaccines against TB are in the pipeline, either as priming, booster and therapeutic vaccines. Since the three options operate at different levels (pre-exposure or post-exposure), they can be considered complementary and hopefully they can succeed in eradicating TB.

Notably, as seen for MVA85A, vaccines that resulted to be immunogenic in animal models and humans can failed to show effectiveness in late phase trials [22]. Therefore, immune mechanisms of protection against TB should be simultaneously explored.

The existence of several lines of research can mean that a main road does not exist at the present.

Finally, even if several vaccines are in the pipeline, further investments on basic research, clinical testing and mass vaccination campaigns are essential to achieve the ambitious goals of eradication.

Additional material

Additional file 1: (textfile) Articles included in the review

List of abbreviations

WHO: World Health Organization; MDG: Millennium Development Goal; TB: tuberculosis; HIV: human immunodeficiency virus; MDR: multidrug-resistant; BCG: bacille Calmette-Guérin; Mtb: *Mycobacterium tuberculosis;* INF-γ: interferon-γ; IGRA: interferon-γ release assay; Ag85A: antigen 85A; IL-2: interleukin-2; TNF-α: tumour necrosis factor-α; IL-17: interleukin-17; Ag85B: antigen 85B; ESAT6: Early Secretory Antigenic Target 6; DDA/MPL: dimethyl dioctadecyl ammonium/monophosphoryl lipid A; GLA/SE: glucopyranosyl lipid adjuvant-stable emulsion; QS21: *Quillaja saponaria* fraction 1; pBCG: parental BCG; MV: *Mycobacterium vaccae*

Competing interests

The authors declare that have no competing interests

Authors' contributions

CM, EC, LG conceived the idea, carried out the literature search, and drafted the manuscript. MdM contributed to devise and develop the idea and helped to draft and critically reviewed the manuscript.

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Published: 8 January 2014

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