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REVIEW ESSAY

Preventive effects of *Mycobacterium vaccae* on HIV-associated tuberculosis: A systematic review

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Keywords
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Abstract

Objective To evaluate the effectiveness and safety of *Mycobacterium vaccae* (*M.vaccae*, MV) for prevention of HIV-associated tuberculosis (TB).

Methods MEDLINE, Embase, Biosis, the Cochrane Central Register of Controlled Trials, SCI, CBM, VIP, and CNKI were searched for relevant randomized controlled trials (RCTs) and non-randomized controlled trials (NRCTs). The GRADE approach was used for quality assessment. Data were analyzed using RevMan 5.0 software. Results were described or pooled using relative risks (RRs) for binary outcomes with 95% confidence intervals (CIs).

Results Seven studies were included, and the methodological quality assessment found there was a risk of methodological bias. The evidence quality of the critical endpoint was moderate. The incidence of definite TB was (33/1006 vs. 52/1007, P = 0.03). The levels of IFN-γ response to *M. vaccae* sonicate (MVS) in MV recipients increased compared to baseline or control groups after three or five doses. MV recipients' level of lymphocyte proliferation assays (LPAs) in response to MVS was higher than that of the saline group after a five-dose series (RR = 2.49, 95% CI 1.40 to 4.41, P = 0.002). Compared to hepatitis B vaccine (HBV) recipients, LPAs to MVS were not significantly different in the MV group vs. saline group, or vs. HBV, after a three-dose series (RR and 95% CI were 0.20 (−0.03 to 0.44) and 1.13 (0.26 to 4.91), respectively). Changes in CD4+ cell count and HIV viral load after immunization were not statistically significant. MV immunization had no systematic adverse effects.

Conclusion The current evidence indicates that MV is safe and well-tolerated. It appears to prevent HIV-infected patients with CD4+ $\geq$ 200/mm^3 from contracting TB by enhancing their immunogenicity. Yet, because of the relatively low quality of the available evidence, well-designed and -conducted RCTs are needed.

The estimates of the global burden of disease caused by TB in 2009 are as follows (1): 9.4 million incident cases (range, 8.9 million–9.9 million), 14 million prevalent cases (range, 12 million–16 million), and 0.38 million deaths among HIV-positive people. An estimated 11%–13% of incident cases were HIV-positive. While, at the end of 2009, about 740,000 AIDS and HIV-infected patients were reported to be living (2) and the incidence of tuberculosis (TB) patients detected in HIV-positive people is 1.5 per 100,000 people in China (1). Since HIV-infected people are at greater risk of developing TB than are HIV-negative people, and TB infection is a major cause of morbidity and mortality among HIV-infected people, preventing TB/HIV co-infection has become a challenge in China.

As a live vaccine, BCG is not appropriate for people with symptomatic AIDS, as they may become infected by the vaccine (3). Its preventive effect also decreases with age (4, 5). In contrast, inactivated *Mycobacterium vaccae* (MV) is

an investigational vaccine prepared from an environmental mycobacterium that expresses antigens common to many mycobacteria (6). It was the only immunization agent recommended by the World Health Organization (WHO) (7) in the Tuberculosis Strategic Development Plan of the 1990s (7). *M. vaccae* can enhance cellular immune function through induction of Treg (regulatory T lymphocytes), as well as produce an immune response against *M. tuberculosis,* enhance $CD4^+$ counts, and boost the BCG-primed effect. Several animal experiments and clinical trials (8, 9) have shown that MV can produce an immune response against *M. tuberculosis* by changing type 2 T-helper (Th2) lymphocytes to type 1 T-helper (Th1) lymphocytes.

Our previous systematic review (10) discussed the preventive effect of MV for high risk people based on phase I and II trials (n = 145). In this paper, we update that systematic review with newer studies and give recommendations to inform clinical applications of, and further research on, MV.

Materials and methods

Study selection

Participants

AIDS patients or HIV-infected people without TB.

Intervention

MV inoculated to prevent TB (MV group) compared with either an uninoculated group (blank group) or a group injected with placebo (normal saline or HBV vaccine, CV group).

Study design

Because the number of published RCTs of interest was limited, all clinical trials (RCTs and NRCTs) were considered for inclusion.

Outcome measures

The endpoint: incidence of tuberculosis = (the number of TB-infected patients at endpoint/the number of intention-to-treat patients)*100%.

The surrogates: (1) IFN-γ response to *M. vaccae* sonicate (MVS) was considered valid if SI (stimulation index) ≥ 3 (SI = the mean antigen-stimulated counts per minute (cpm)/the mean non-antigen control cpm). (2) Lymphocyte proliferation assays (LPAs) were defined as positive if proliferation after MVS was greater than or equal to two times proliferation in un-stimulated assays (or if SI ≥ 3). (3) Antibody levels varied among trials. (4) PPD conversion: a reaction on the purified protein derivative (PPD) test was defined as a change of $\geq$5 mm in diameter, and conversion was a change of $\geq$10 mm or from $<$5 mm to $\geq$5 mm.

Safety indicator: $CD4^+$ lymphocyte count and HIV viral load were used as safety indicators. Adverse effects were described.

Search strategies

Using the key words "tuberculosis," "HIV," "AIDS," "Mycobacterium vaccae," and "M. vaccae," We searched MEDLINE (1950 to December 2010), Embase (1966 to December 2010), the Cochrane Central Register of Controlled Trials (Issue 4, 2010), SCI (1995 to December 2010), the Chinese Biomedical Literature Database (CBM, 1978 to December 2010), the China Academic Journals Full-Text Database (CNKI, 1979 to December 2010), and the Chinese Scientific Journals Database (VIP, 1989 to December 2010).

Data extraction and quality assessment

Two reviewers (Qunfei Chen and Xiangyu He) independently selected literature, extracted data, and assessed quality, and differences were resolved by discussion with a third reviewer (Ling Li). The following criteria, based on the Cochrane Handbook for Systematic Reviews of Interventions 5.0, were applied to assess the quality of included studies: (1) Was the allocation sequence adequately generated? (2) Was allocation adequately concealed? (3) Was knowledge of the allocated interventions adequately prevented during the study? (4) Were incomplete outcome data adequately addressed? (5) Were reports of the study free of suggestion of selective outcome reporting? (6) Was the study apparently free of other problems that could put it at a high risk of bias? (We evaluate the comparability of baseline of the included trials in this review). The reviewers used 'Y,' 'U,' and 'N' to answer these questions. The answer 'Yes' (Y) indicated a low risk of bias, 'Unclear' (U) indicated an unclear risk of bias, and 'No' (N) indicated a high risk of bias.

Data analysis

Statistical analysis was carried out by Revman5.0 software. A heterogeneity test was applied to the included studies, and P values of less than 0.05 were considered to indicate statistical significance. A fixed effect model was applied in groups without heterogeneity; otherwise, a random effect model was used. Risk ratio (RR) and its 95% confidence interval (95% CI) were used for binary variables. Results were described for data that could not be combined and for safety evaluation.

Results

Study description

The initial search yielded 45 potential articles. We reviewed the full text of 15 of the articles, of which 7 (11–17) met the

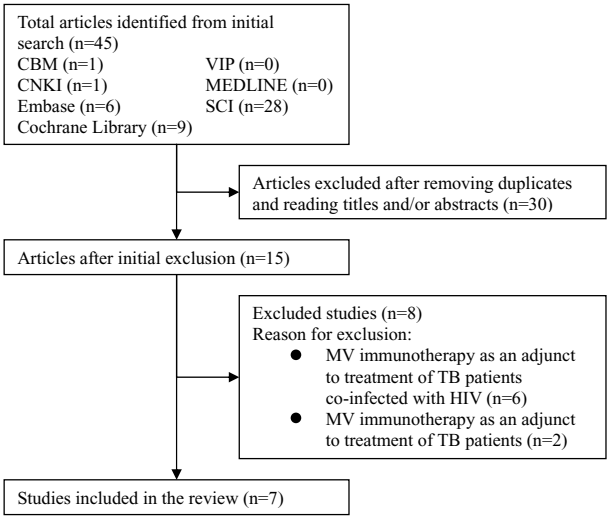

Figure 1 Study selection process.

inclusion criteria (Figure 1). These seven articles together described six RCTs, since two studies (16, 17) targeted the same group of patients but with different indicators. The six trials were conducted in the United States, Zambia, Finland, and Tanzania, and involved a total of 2158 subjects. Except for one (13) that recruited children, the studies' participants were all 18 years or older. The vaccine was administered as a 0.1 ml intradermal injection for all adults (11, 12, 14–17) (0.05 ml for children) (13). Two studies (11, 15) applied three doses at 0 weeks (dose 1), 2 months (dose 2) and 4 months (dose 3). The other five studies (12–14, 16, 17) administered an additional two doses after 4 months. Two (13, 15) studies used hepatitis B vaccine (HBV) as the control, while four studies (12, 14, 16, 17) used normal saline, and one study (11) used a before-and-after comparison (Table 1).

Quality assessment

Methodological quality (Table 2)

Sequence generation and allocation concealment: none of the studies described their sequence generation process or methods of allocation concealment.

Blinding: Three studies (13, 15–17) conducted blinding.

Incomplete outcome data: All included studies reported loss of participants. Only one study (17) applied the intention-to-treat (ITT) analysis.

All studies reported both negative and the positive results for the targeted indicator.

All studies got a "Y" for comparability of baseline.

Evidence quality (Table 3)

As an endpoint, incidence of TB was defined as "critical," while other indicators were defined as "important." The overall evidence quality of the included studies was only "moderate" for incidence of TB; other indicators' evidence quality was assessed as "low."

Incidence rate of tuberculosis

The incidence of tuberculosis was reported after a median 3.3-year follow-up period in one trial (17). Comparing MV with ("vs." is used hereafter) normal saline, the incidences of definite TB, disseminated TB, and probable TB were (33/1006 vs. 52/1007, P = 0.03), (7/1006 vs. 12/1007, P = 0.16), and (48/1006 vs. 40/1007, P = 0.46), respectively.

IFN-γ responses to Mycobacterium vaccae sonicate (MVS) (SI≥3)

Three trials (14–16) (n = 2096) reported a comparison of MV vs. baseline or normal saline (14, 16) or HBV vaccine (15) in IFN-γ responses to *Mycobacterium vaccae* sonicate (MVS). One trial (15) (n = 39) reported that after three doses of MV, IFN-γ response to MVS increased from 1,000 pg/ml (geometric mean) at baseline to 4,977 pg/ml (P value not provided). The other two trials (15, 16) (n = 2,052) reported IFN-γ response to MVS after five doses for both MV and CV groups. One trial (16) (n = 2,013) found P = 0.0038 for the comparison, while the other (15) did not report the P value. One trial (14) reported positive *M. vaccae* sonicate indices in 5 (23%) of 22 MV recipients and 1 (5%) of 22 normal saline patients, but this difference was not statistically significant (P = 0.19).

Impact of prior BCG immunization: Two trials (15, 16) (n = 2,052) included BCG-primed subjects. One (15) (n = 39) reported that IFN-γ response to MVS increased from 1000 pg/ml (geometric mean) at baseline to 4,977 pg/ml (MV) and 478 pg/ml (HBV) after three doses, a statistically significant difference (P = 0.001). The other trial (16) (n = 2,013) compared MV vs. normal saline and found a significant difference (P = 0.0038).

LPA responses to Mycobacterium vaccae sonicate (MVS)

Six trials (11–16) (n = 2,158) reported LPA responses to MVS. Two of these (11, 12) (n = 27) compared 3 doses of MV vs. normal saline or blank. The heterogeneity test for these trials yielded P = 0.73, I^2 = 0%, and a fixed effect model was applied. The RR was 6.66 with 95% CI (0.90, 49.21), P = 0.06 (Figure 2).

Two trials (13, 15) (n = 60) compared LPA response to MVS after inoculation with MV or HBV. The heterogeneity test yielded P = 0.02, I^2 = 83%, and a random effect model was applied. The results were RR = 1.13 (0.26, 4.91), P = 0.87 (Figure 3).

Table 1 Characteristics of the included studies

			Patients			Interventions			Comparisons			
Study	Country	Phase of trial*	Number of subjects	Age (years)	CD4+ counts	BCG-primed	Volume of single dose (mL)	Number of doses	MV administration regimen	Controls	Volume of doses (mL)	Outcome
Marsh 1997 (11)	USA	I	12	25-52	>300/mm3	NM	0.1	3	at 0 weeks, 2 months and 4 months	before-after comparison	-	③④⑤⑥⑦
Von Reyn 1998 (12)	USA	I	15	25-52	>300/mm3	NM	0.1	5	at 0 weeks, 2 months,4 months, 25 months, and 26 months	-	-	③⑤⑥⑦
Johnson 1999 (13)	USA	I	35	5-13	>300/mm3	NM	≥5 years: 0.1 <5 years: 0.05	5	at 0 weeks, 2 months, and 4 months	HBV	0.1	③④⑤⑥⑦
Waddell 2000 (14)	Zambia	II	44	24-51	>200/mm3	Y	0.1	5	at 0 weeks, 2 months, 4 months, 12 months, and 14 months	-	-	②③⑤⑥⑦
Vuola 2003 (15)	Finland	II	39	48	>200/mm3	Y	0.1	3	at 0 weeks, 2 months, and 4 months	HBV	0.1	②③⑤⑥⑦
Lahey 2010 (16)	Tanzania	III	2013	33	>300/mm3	Y	0.1	5	at 0 weeks, 2 months, 4 months, 6 months, and 12 months	NS	0.1	②③④⑥⑦
Von Reyn 2010 (17)	Tanzania	III	2013	33	>300/mm3	Y	0.1	5	at 0 weeks, 2 months, 4 months, 6 months, and 12 months	NS	0.1	①⑤

BCG: Bacille Calmette-Guerin vaccine; HBV: Hepatitis B vaccine; NM: not mentioned; Y: yes; *: see the first paragraph of Discussion for information on the definitions of the trial phases.
①Incidence of tuberculosis; ②IFN-γ responses to MVS; ③LPAs to MVS; ④Antibody converters titers to Ara-LAM; □ PPD conversion; □ CD4+ counts; □ HIV viral load.

Table 2 Overall quality assessment of included studies

Study	Adequate sequence generation	Allocation concealment	Blinding	Incomplete outcome data addressed	Selective report bias	Comparability of baseline
Marsh 1997 (11)	–	–	–	Y	N	–
Von Reyn 1998 (12)	–	–	U	Y	N	Y
Johnson 1999 (13)	U	U	Y	Y	N	Y
Waddell 2000 (14)	–	–	N	Y	N	Y
Vuola 2003 (15)	U	U	Y	Y	N	Y
Lahey 2010 (16)	U	U	Y	Y	N	Y

–: not applicable; Y: yes; N: no; U: unclear.

Table 3 Evidence quality of included studies

Indictor	Importance	Evidence quality	Limitations	Inconsistency	Indirectness	Imprecision
Incidence of TB	Critical	moderate	Serious[1]	not serious	not serious	not serious
IFN-γ response to MVS	Important	low	Serious[1]	not serious	Serious[2]	not serious
LPA response to MVS	Important	low	Serious[1]	not serious	Serious[2]	not serious
Antibodies to Ara-LAM	Important	low	Serious[1]	not serious	Serious[2]	not serious
PPD conversion	Important	low	Serious[1]	not serious	Serious[2]	not serious

[1]got U in term of "Adequate sequence generation" and "Allocation concealment".
[2]substituted surrogate for endpoint.

Two trials (12, 14) (n = 59) reported LPA responses to MVS after five doses of MV. Since the heterogeneity test showed P = 0.57, a fixed effect model was applied. The pooled RR and its 95% CI were 2.49 (1.40, 4.41), P = 0.002 (Figure 4). One of the trials (16) (n = 2,013) reported the median absolute responses to MVS of MV vs. baseline was statistically significant (P < 0.0001).

Impact of prior BCG immunization: Three trials (14–16) (n = 2,096) included BCG-primed subjects. Compared with CV, LPA response to MVS in these three trials was significantly different, with P values of 0.02 (14), 0.027 (15), and <0.0001 (16).

Antibody level changes

One trial (16) (n = 2013) reported that levels of the lgG antibody to Ara-lipoarabinomannin (LAM) in the MV group was higher than in the normal saline group (P < 0.0001). One trial (11) (n = 12) reported a detectable increase in titers of LAM, but the increase was less than twofold. One trial (13) (n = 35) reported that one MV recipient had a >2 fold increase in lgG Ara-LAM titer.

PPD skin test conversion rate

Two trials (n = 67) reported the PPD conversion rate of MV vs. HBV. The heterogeneity test yielded P = 0.92, I^2 = 0%, so a fixed effect model was applied (Figure 5). The pooled RR and its 95% CI were 0.20 (0.02, 1.77), P = 0.15.

Four trials (11, 12, 14, 17) (n = 235) reported the number of subjects with positive PPD skin test conversion in MV and normal saline groups. The heterogeneity test yielded P = 0.36, I^2 = 2%, so a fixed effect model was applied. The

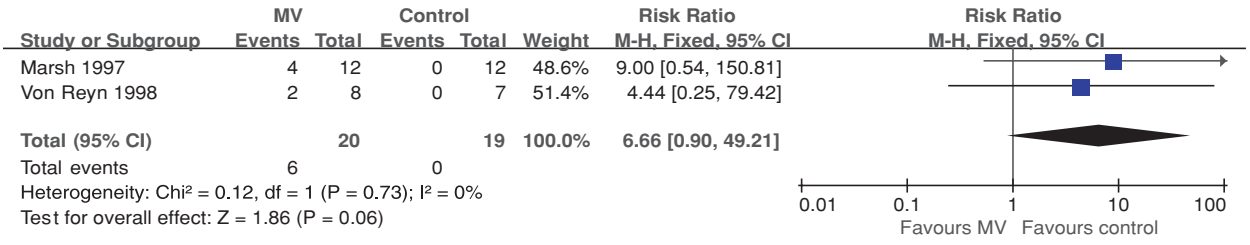

Figure 2 Pooled relative risk for LPA response to MVS of the MV groups versus normal saline groups (three doses).

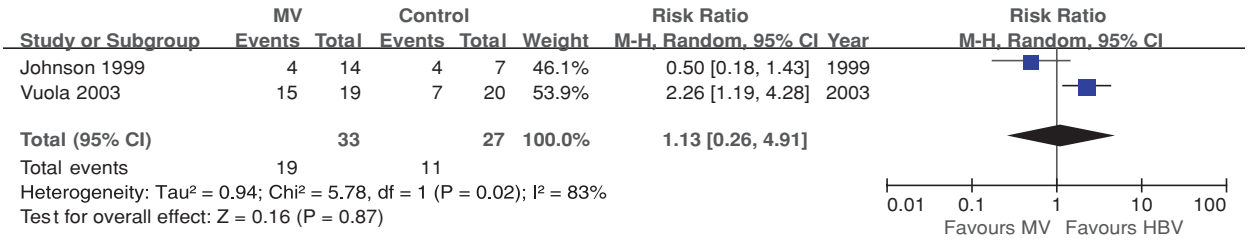

Figure 3 Pooled relative risk for LPA response to MVS of the MV groups versus HBV groups (three doses).

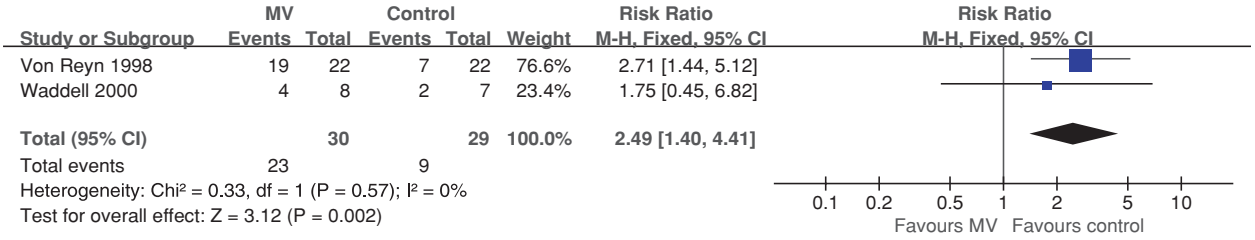

Figure 4 Pooled relative risk for LPA response to MVS of the MV groups versus the normal saline group (three doses).

pooled RR and its 95% CI were 0.56 (0.32, 0.99), P = 0.05 (Figure 6).

Safety evaluation

CD4+ lymphocyte counts: One trial (11) (n = 12) showed that the mean percent change in baseline and final absolute CD4+ was +8% (−18 to +32) (no P value provided). Four trials (12, 13, 15, 16) (n = 2102) found no statistically significant change in CD4+ count between the MV and CV groups P > 0.05.

One trial (11) (n = 12) showed that the mean change in baseline and final log10 for HIV viral load was +0.3 (−0.5 to +1.5) (P was reported). Five trials (12–16) (n = 2,146) showed that the change in HIV viral load before and after MV injection was not significantly different between the MV and CV groups (P > 0.05).

Reported adverse effects included induration at the vaccine site (11, 13, 15), skin irritation (13–15), erythema (14, 15), sore arm (13–15) and other local effects. No trials reported systemic side effecs after any dose.

Discussion

This review used endpoints and comprehensive surrogates to reevaluate the safety and efficacy of MV for HIV-associated TB. Our update includes a long-term follow-up study in addition to phase I and II studies. It is worth mentioning that phases I and II in the included studies were defined as different stages of single trials, differing from Good Clinical Practices (GCP) for the new drug approval process.

Although the incidence of TB is the critical patient-important indicator, due to the impaired immune system of HIV-infected persons, the preventive effect of MV is not manifested in the short term. So the included trials chose immune parameters, such as IFN-γ and LPA response to MVS and antibody responses to the TB glycolipid LAM, as surrogates. Several pieces of evidence (16, 18) support the hypothesis

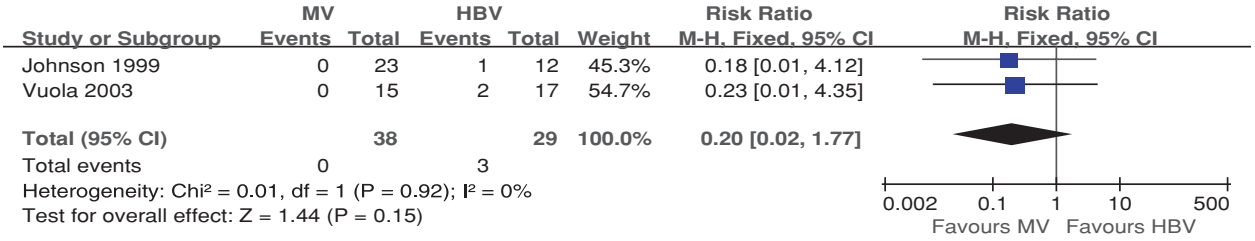

Figure 5 Pooled relative risk for PPD conversion of the MV group versus the HBV group (after five doses).

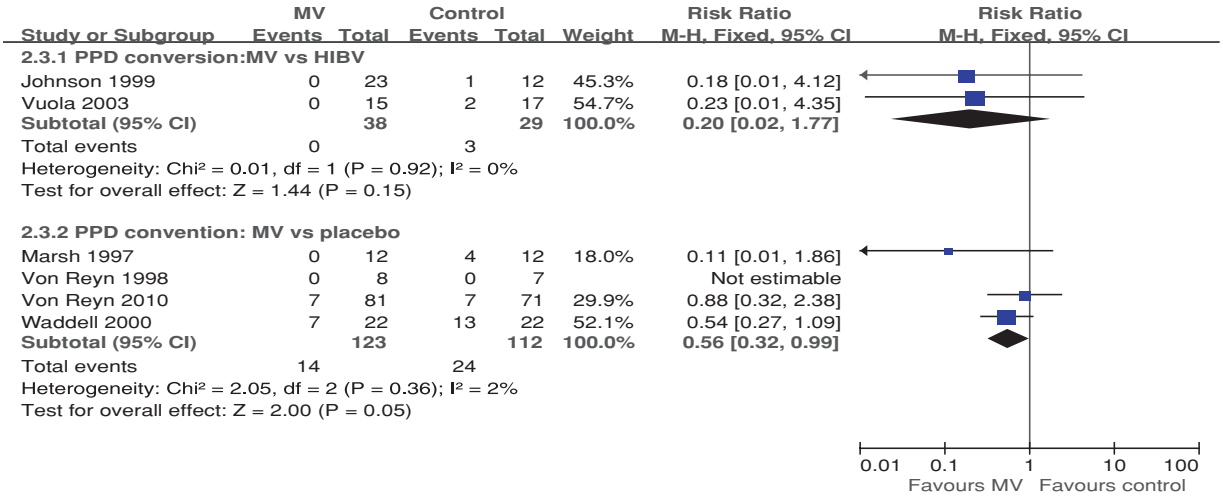

Figure 6 Pooled relative risk for PPD conversion of the MV group versus the normal saline group (after five doses).

that IFN-γ response to MVS contributes to TB prevention. First, there is a trend toward greater IFN-γ response to MV sonicate in MV recipients who did not develop TB compared to those who did develop TB. Second, placebo recipients who developed TB had significantly lower magnitude IFN-γ responses to MV. Third, among placebo recipients, detectable baseline IFN-γ response to mycobacterial antigens was also associated with lower risk of TB (16).

The incidence of definite TB was reported after a median 3.3-year follow-up period in one trial (16, 17) (n = 2013), which stated that the incidence of definite TB in the MV group was lower than that in normal saline controls, suggesting that MV can protect HIV-infected patients from TB. Although the immune system may be impaired in HIV-infected participants, the results of IFN-γ responses to MVS in the large sample trials (n = 2013) showed that the IFN-γ level of the MV group is significantly higher than that of the CV group after five doses. The pooled LPA response to MVS showed that the MV group had higher positive SI than did the CV group, which suggested that MV could induce immune response in HIV-infected patients. This result was confirmed in large sample trials (n = 2013). The antibody titer increased in MV recipients, and MV immunization did not result in PPD skin test conversions.

The HIV pandemic provided direct evidence that decline in $CD4^+$ T cell number and function results in progressive primary infection, reactivation of endogenous MTB, and enhanced susceptibility to re-infection (10), so we chose $CD4^+$ lymphocyte counts as one of the safety indicators for MV. The results suggested that there were no significant changes in CD4+ counts after inoculation. HIV viral load, another safety indicator, was essentially the same in the MV and CV groups, as well as before and after MV injection. Although MV had local adverse effects, such as sore arm and skin irritation, no systemic side effects were reported. It appears that MV is well-tolerated and safe for HIV-infected patients, as measured by its effect on $CD4^+$ T cell and viral load.

Still, this review has limitations:

- Only one included trial (n = 2013) analyzed patient-important outcomes (incidence of TB), while other trials (n = 145) applied surrogates to claim preventative effects for MV. The evidence quality of the endpoint and surrogates were moderate and low, respectively.
- Randomization, which could largely affect the results, was the key factor of clinical trials. However,few studies are RCTs after assessment, being limited in "Adequate sequence generation" and "Allocation concealment".
- Since the HIV burden of the included study sites varied, the extrapolation of our conclusion is limited. High-quality studies in different regions and among different populations are urgently needed.

Conclusion: Based on the available evidence, three or five doses of MV are safe and well-tolerated for HIV-infected patients with $CD4^+ \geq 200/mm^3$. The vaccine can protect HIV-infected patients from TB and induce biologically relevant immune responses against it. MV can also boost BCG-primed immunity for patients with a history of BCG inoculation.

High-quality trials should be conducted in countries with high HIV burdens to test the hypothesis that IFN-γ responses to shared mycobacterial antigens correlate with vaccine-mediated protection from TB and the anti-TB protective effect of MV.

References

1. WHO. Global tuberculosis control report 2010. Available from: URL: http://www.who.int/tb/publications/global_report/en/index.html 2010.
2. Ministry of Health of People's Republic of China. The estimated AIDS epidemic report in China (2009). Avalible from: URL: http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohjbyfkzj/s3586/201009/48858.htm
3. Clements CJ, Von Reyn CF, Mann JM. HIV infection and routine childhood immunization: a review. *Lancet* 1987; 65(6): 905–11.
4. Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, *et al*. The efficacy of bacillus Calmette Gue ´;r in vaccination of newborns and infants in the prevention of tuberculosis:meta-analyses of the published literature. *Pediatrics* 1995; 96(1 Pt 1): 29–35.
5. von Reyn CF, Vuola JM. New vaccines for the prevention of tuberculosis. *Clin Infect Dis*. 2002; 35(4): 465–74.
6. Stanford J, Stanford C, Grange J. Immunotherapy with Mycobacterium vaccae in the treatment of tuberculosis. *Front Biosci*. 2004; 9: 1701–19.
7. WHO. Tuberculosis Strategic Development Plan of the 1990s. Available from: URL: http://www.who.int/tb/strategy/en/index.html
8. Hernandez-Pando R, Pavön L, Arriaga K, Orozco H, Madrid-Marina V, Rook G. Pathogenesis of tuberculosis in mice exposed to low and high doses of an environmental mycobacterial saprophyte before infection. *Infect Immun*. 1997; 65(8): 3317–27.
9. Xu Y, Zhang BF, Zhang ZX, QP G. The effect of M.vaccae to prevent tuberculosis in aged mice. *Chinese Antituberculosis Association* 2009; 29(3): 230–32.
10. Yang XY, Chen QF, Cui XH, Yu Y, Li YP. Mycobacterium vaccae vaccine to prevent tuberculosis in high risk people: a meta-analysis. *J Infect*. 2010; 60(5): 320–30.
11. Marsh BJ, Fordham von Reyn C, Arbeit RD, Morin P. Immunization of HIV-infected adults with a three-dose series of inactivated Mycobacterium vaccae. *Am J Med Sci*. 1997; 313(6): 377–83.
12. von Reyn CF, Marsh BJ, Waddell R, Lein AD, Tvaroha S, Morin P, *et al*. Cellular immune responses to mycobacteria in healthy and human immunodeficiency virus-positive subjects in the United States after a five-dose schedule of Mycobacterium vaccae vaccine. *Clin Infect Dis*. 1998; 27(6): 1517–20.
13. Johnson D, Waddell RD, Pelton SI, Jaeger AS, Modlin JF, Yogev R, *et al*. Randomised trial of intradermal Mycobacterium vaccae or intradermal hepatitis B immunisation in children with HIV infection. *Vaccine*. 1999; 17(20–21): 2583–7.
14. Waddell RD, Chintu C, Lein AD, Zumla A, Karagas MR, Baboo KS, *et al*. Safety and immunogenicity of a five-dose series of inactivated Mycobacterium vaccae vaccination for the prevention of HIV-associated tuberculosis. *Clin Infect Dis*. 2000; 30(Suppl 3): S309–15.
15. Vuola JM, Ristola MA, Cole B, Järviluoma A, Tvaroha S, Rönkkö T, *et al*. Immunogenicity of an inactivated mycobacterial vaccine for the prevention of HIV-associated tuberculosis: a randomized, controlled trial. *AIDS*. 2003; 17(16): 2351–5.
16. Lahey T, Arbeit RD, Bakari M, Horsburgh CR, Matee M, Waddell R, *et al*. Immunogenicity of a protective whole cell mycobacterial vaccine in HIV-infected adults: a phase III study in Tanzania. *Vaccine*. 2010; 28(48): 7652–8.
17. von Reyn CF, Mtei L, Arbeit RD, Waddell R, Cole B, Mackenzie T, *et al*. Prevention of tuberculosis in Bacille Calmette-Guérin-primed, HIV-infected adults boosted with an inactivated whole-cell mycobacterial vaccine. *AIDS*. 2010; 24(5): 675–85.
18. Geldmacher C, Schuetz A, Ngwenyama N, Casazza JP, Sanga E, Saathoff E, *et al*. Early depletion of Mycobacterium tuberculosis-specific T helper 1 cell responses after HIV-1 infection. *J Infect Dis*. 2008; 198(11): 1590–8.