Adjunctive therapy of *Mycobacterium vaccae* vaccine in the treatment of multidrug-resistant tuberculosis: A systematic review and meta-analysis

HONG WENG^{1*}, JING-YU HUANG^{1*}, XIANG-YU MENG¹, SHENG LI¹ and GU-QIN ZHANG²

¹Center for Evidence-Based and Translational Medicine; ²Department of Respiratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, P.R. China

Received December 15, 2015; Accepted February 16, 2016

DOI: 10.3892/br.2016.624

Abstract. A number of studies have suggested that the Mycobacterium vaccae (MV) vaccine as an adjunctive therapy has a positive effect in the treatment of multidrug-resistant tuberculosis (MDR-TB). However, the result is inconclusive. The aim of the present study was to systematically evaluate the effect and safety of MV as an adjunctive therapy in the treatment of MDR-TB. A computerized search of PubMed, Embase, Cochrane Central Register of Controlled Trials, CBM, CNKI and VIP until October 2014 was conducted to collect the relevant studies. The main outcome measures were the sputum smear positive-turned-negative rate, the absorption rate of TB foci and the closure situation of the TB cavity. Two investigators identified the eligible studies and extracted data independently. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated and pooled using the fixed effects model. A total of 25 studies involving 2,281 patients with MDR-TB were included. The pooled OR was 3.84 (95% CI, 3.84-4.73) for the sputum smear positive-turned-negative, 4.08 (95% CI, 3.08-5.45) for the absorption rate of TB foci, and 3.42 (95% CI, 2.68-4.37) for the closure situation of TB cavity. Therefore, MV has a significant effect as an adjunctive therapy in the treatment of MDR-TB. However, larger scale multicenter randomized controlled trials are required to confirm this evidence for limited latent bias at present.

Correspondence to: Dr Hong Weng, Center for Evidence-Based and Translational Medicine, Zhongnan Hospital of Wuhan University, 169 Donghu Road, Wuhan, Hubei 430071, P.R. China E-mail: wengh92@163.com

*Contributed equally

Key words: multidrug-resistant tuberculosis, Mycobacterium vaccae, randomized controlled trial, systematic review, meta-analysis

Introduction

Tuberculosis (TB) is a public health and social problem worldwide, and remains the main infectious disease with a serious effect on health in the current century. Since the 1960s, TB chemotherapy has replaced the previous negative health nutrition therapy and has become recognized worldwide as the main method for control of TB. However, TB deteriorated as a global trend from the middle of the 1980s, and currently, the majority of the TB epidemic is extremely low in developed countries. The TB epidemic situation is noticeably improving in numerous developing countries. In numerous countries and regions the occurrence of TB is associated with the human immunodeficiency virus infection, multiple drug-resistant Mycobacterium TB (MTB) infection, increased poverty, population growth and migration, and other objective factors. By contrast, the complexity of the TB epidemic has increased due to a lack of understanding of the vigilance and control of TB. The management of TB was also believed to be solved, and therefore, controlling TB and the input and control of other subjective factors was reduced. According to the World Health Organization (WHO), an estimate in 2008 indicates that the global annual incidence of drug-resistant TB is ~9.2 million, while the actual multidrug-resistant TB (MDR-TB) may approach >500,000. The number of patients in China with MDR-TB ranks second worldwide (1). MDR-TB treatment for TB prevention and control is an extremely difficult problem that is often clinically treated on the basis of anti-TB chemotherapy combined with immunotherapy.

Mycobacterium vaccae (MV) vaccine is an immunization of heat-killed MV that enhanced anti-TB mycobacterial infections in patients with cellular immune function, and combined with chemotherapy can enhance the efficacy of chemotherapy for the adjunctive treatment of TB. In 2011, Yang et al (2) performed a meta-analysis to evaluate the MV as an adjunctive therapy to anti-TB chemotherapy in never-treated TB patients. Certain studies have shown that MV can protect against MDR-TB (3-5). However, not all the studies reached the same or similar association, and no summary of the evidence of the effectiveness of MV as an adjunctive therapy in the treatment of MDR-TB exists. The

present study conducted a comprehensive systematic review and meta-analysis of the eligible studies on the adjunctive therapy of MV in the treatment of MDR-TB to indicate the critical effect of MV, further research and application.

Materials and methods

Literature search. The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement (6). A computerized search was conducted in the English databases of PubMed, Embase, Cochrane Central Register of Controlled Trials, CBM, CNKI and VIP until October 2014 for original studies using the following keywords: ('Mycobacterium vaccae' OR 'M. vaccae' OR MV) AND ('multidrug tuberculosis' OR MDR-TB OR 'refractory tuberculosis'). The performance was limited to human studies and manual retrieval was applied to the literature references or bibliographies of all the relevant studies.

Study selection. All the initial studies complied with the following criteria: i) Randomized controlled trials (RCTs) or clinical controlled trials with available data to investigating MV as an adjunctive therapy used as intervention versus general chemotherapy (without MV); ii) the general chemotherapy regimens in the MV group was the same as the control group (without MV); and iii) subjects without adverse disease, such as severe autoimmune disease, or pregnant women.

Data extraction. Two investigators (HW and SL) independently conducted the data extraction and quality assessment. Any disagreements were resolved by discussion. The first author, year of publication and the detailed information of PICOS (patient, intervention, comparison and study design) were recorded.

Quality assessment. The methodological quality of each study was evaluated using the Cochrane collaboration's tool for assessing the risk of bias. The following four items were mainly assessed: Adequate sequence generation, allocation concealment, blinding and incomplete outcome data addressed.

Statistical analysis. All the data were pooled using Stata 12.0 (Stata Corp., College Station, TX, USA). For the binary outcomes, the odds ratio (ORs) and 95% confidence intervals (CIs) were calculated; and for the continuous outcomes, the standard mean differences (SMDs) and their 95% CIs were calculated. The heterogeneity test was performed for the included studies and P<0.1 was considered to indicate a statistical significant difference, and subsequently a quantitative measure of heterogeneity across studies was conducted using the I² statistic. Studies with an I² of 25-50% were considered low heterogeneity, 50-70% were considered moderate heterogeneity and >75% were considered high heterogeneity (7). The fixed effects model was applied to pool the effects for studies or subgroups without heterogeneity. Sensitivity analysis was carried out by removing studies, and using fixed and random effect models. Subgroup analysis was conducted to investigate the potential source of between-study heterogeneity. A two-side P<0.05 in the Z-test was considered to indicate a statistically significant difference.

Results

Description of studies and quality assessment. The initial search yielded 178 relevant studies, of which 150 were excluded for various reasons depending on the abstracts, and finally 25 studies (8-32) were included. These 25 studies involved 2,281 Chinese patients. The characteristics of the eligible studies are shown in Table I. The course of treatment ranged from 6 to 24 months. The quality assessment of the eligible studies is shown in Table II.

Meta-analysis of outcome measures. In total, 23 studies (8-29,32) reported the sputum smear positive-turned-negative rate (Table III). There was no heterogeneity between these studies (I²=0.0%, P-value for heterogeneity=0.493). Subsequently, the fixed effects model was performed to pool the results. The aggregated result of these studies suggested that MV as an adjunctive therapy is associated with a significantly increased sputum smear positive-turned-negative (OR=3.84; 95% CI, 3.11-4.73). Following this, sensitivity analysis was carried out by changing the statistical model (OR=3.73; 95% CI, 3.01-4.61; I²=0.0%; P-value for heterogeneity=0.493).

Fifteen studies (8-13,17-22,24,26,27) reported the absorption rate of TB foci (Table III). There was no heterogeneity between these studies (I²=35.7%, P-value for heterogeneity=0.084). Following this, the fixed effects model was applied to pool the results. The pooled result suggested that MV as an adjunctive therapy is associated with a significantly increased absorption rate of the TB foci (OR=4.08; 95% CI, 3.06-5.45). Subsequently, sensitivity analysis was carried out by changing the statistical model (OR=4.08; 95% CI, 2.75-6.05; I²=35.7%; P-value for heterogeneity=0.084).

Eighteen studies (9-23,25,26,28) reported the closure situation of the TB cavity (Table III). There was no heterogeneity between these studies (I²=0.0%, P-value for heterogeneity=0.794). Subsequently, the fixed effects model was performed to pool the results. The summarized results of these studies suggest that MV as an adjunctive therapy is associated with a significant increase in the closure situation of the TB cavity (OR=3.42; 95% CI, 2.68-4.37). Following this, sensitivity analysis was carried out by changing the statistical model (OR=3.40; 95% CI, 2.65-4.36; I²=0.0%; P-value for heterogeneity=0.794).

Four studies (26,27,30,31) reported the curative effects of the adjunctive therapy of MV. The aggregated results of these studies suggest that MV as an adjunctive therapy is associated with a significant increase in the curative effects (OR=2.44; 95% CI, 1.29-4.60; I²=0.0%; P-value for heterogeneity=0.979) with the fixed effects model.

Four studies (10,13,17,21) reported the improvement of symptoms of the adjunctive therapy of MV. The aggregated results of these studies suggest that MV as an adjunctive therapy is associated with a significant increase in the improvement of symptoms (OR=6.15; 95% CI, 3.32-11.42; I²=15.0%; P-value for heterogeneity=0.317) with the fixed effects model.

Four studies (11,13,18,22) reported the T-lymphocyte subsets counts including cluster of differentiation 3^+ (CD3 $^+$), CD4 $^+$, CD8 $^+$ and CD4 $^+$ /CD8 $^+$. The pooled result of these

Table I. Characteristics of the studies included in the meta-analysis.

First author, Sample year size, n (m/f)		COT, months Interventions		Comparison	Outcomes	Refs.
Luo, 2000	28/28	6	Chemotherapy regimens + 0.1 mg MV	Km, Am, P, Cm, Ofx, Lfx and Pto	1-5	(8)
Song, 2002	36/35	9	Chemotherapy regimens + 22.5 µg MV	3Pa-Pto-Am-E-Lfx/6Pa-Pto-E-Lfx	1-3,5	(9)
Zheng, 2004	50/47	6	Chemotherapy regimens+MV	H, Z, K, Pto, P and RFT	1-3,5	(11)
Wu, 2004	43/41	18	Chemotherapy regimens + 22.5 μ g MV	Pa, RFT, Lfx, Pto and Am (or Z, P, E and Cm)	1-3	(10)
Yao, 2005	30/30	18	Chemotherapy regimens + 22.5 μ g MV	3Pa-RFT-Am-Z-Pto-Lfx/ 15Pa-RFT-Z-Pto-Lfx	1-3, 5	(13)
Chen, 2005	38/41	12	Chemotherapy regimens + 22.5 µg MV	2RFT-Lfx-Am-Pa-E/10RFT-Lfx-Pa	1-3, 5	(12)
Wang, 2006	36/35	12	Chemotherapy regimens + 22.5 µg/ml MV	3Cp-Ofx-Pa-Z-Pto/9Pa-Ofx-Pto	1-3	(15)
Liu, 2006	34/36	18	Chemotherapy regimens + 22.5 µg MV	3Pa-RFT-Pto-Lfx-Am/ 15Pa-RFT-Pto-Lfx	1, 3, 5	(14)
Lan, 2007	35/33	18	Chemotherapy regimens + 22.5 µg MV	3Pa-Pto-Lfx-Am-Z/15Pa-Pto-Lfx	1, 3, 5	(16)
Zhao, 2008	52/50	12	Chemotherapy regimens + 22.5 µg MV	3Pa-Cp-Pto-Lfx-E/9Pa-Pto-Lfx-E	1-3,5	(17)
Xi, 2009	43/41	18	Chemotherapy regimens + 22.5 μ g MV	3P-Lfx-Pto-Am-E/15P-Lfx-Pto-E	1-5	(22)
Chu, 2009	98/91	18	Chemotherapy regimens + 22.5 μ g MV	3Pa-RFT-Lfx-Pto-Am-Cm/ 15Pa-RFT-Lfx-Pto-Cm	1-6	(18)
Hong, 2009	47/45	12	Chemotherapy regimens + 22.5 μ g MV	3Pa-Am-Pto-Lfx-E/9Pa-Pto-Lfx-E	1-3, 5, 6	(20)
Gu, 2009	34/36	18	Chemotherapy regimens + 22.5 μ g MV	3Pa-RFT-Am-Z-Pto-Lfx/1 5Pa-RFT-Z-Pto-Lfx	1-3,5	(19)
Wang, 2009	120/120	18	Chemotherapy regimens + 22.5 µg MV	3Pa-RFT-Am-Z-Pto-Lfx/ 15Pa-RFT-Z-Pto-Lfx	1-3,5	(21)
Mei, 2010	40/41	18	Chemotherapy regimens + 22.5 µg MV	3Pa-Lfx-Am-Z/15Pa-Lfx	1, 2, 5	(24)
Zhang, 2010	56/52	12	Chemotherapy regimens + 22.5 µg MV	3Pa-Pto-Z-Lfx-Am/15Pa-Pto-Z-Lfx	1, 3, 6	(25)
Liu, 2010	32/34	9	Chemotherapy regimens + 22.5 µg MV	3Am-Pa-Z-Lfx-E/6Pa-E-Lfx	1,3	(23)
Zhou, 2011	72/71	21	Chemotherapy regimens + 22.5 μ g MV	3Pa-Lfx-Z-Pto-S/18Pa-Lfx-Z-Pto	1-3, 5, 6	(29)
Wang, 2011	60/60	12	Chemotherapy regimens + 22.5 μ g MV	RFT, Pa, and Lfx	1-3, 5, 7	(27)
Yang, 2011	30/30	12	Chemotherapy regimens + 22.5 μ g MV	Pa, Pto, Z, Lfx, and Am	1-3	(28)
Sheng, 2011	36/36	21	Chemotherapy regimens + 22.5 μ g MV	3H-P-Cm-Pto-Lfx/18Pa-Pto-Lfx	1-3, 5, 7	(26)
Yin, 2012	17/18	24	Chemotherapy regimens + 22.5 μ g MV	6Z-Am-Lfx-Pto-P/18Z-Lfx-Pto-P	7	(31)
Chen, 2012	43/40	12	Chemotherapy regimens + 22.5 μ g MV	Pro, RFT, Lfx, and Pa	7	(30)
Zhang, 2013	72/62	24	+ 22.5 μg MV Chemotherapy regimens + 22.5 μg MV	6Mfx-Z-Pto-Km-P/18Mfx-Z-Pto-P	1, 4, 5	(32)

Outcome 1, the sputum smear positive-turned-negative rate; 2, the absorption rate; 3, the cavity closure rate; 4, T cell subgroup counts; 5, adverse effects; 6, improvement of symptoms; 7, the effective rate. COT, course of treatment; COD, course of disease; MV, *Mycobacterium vaccae*; H, isoniazid; R, rifapentine; RFT, rifapentine; Am, amikacin; Z, pyrazinamide; E, ethambutol; S, streptomycin; Pa, pasinizid; P, p-aminosalicylic acid; Km, kanamycin; Cm, capreomycin; Ofx, ofloxacin; Lfx, levofloxacin; Mfx, moxifloxacin; Pto, protionamide; m, male; f, female.

Table II. Quality assessment of the included studies.

First author,	Adequate sequence	Allocation	Incomplete outcome			
year	generation	concealment	Blinding	data addressed	Refs.	
Luo, 2000	Unclear	Unclear	Unclear	Yes	(8)	
Song, 2002	Unclear	Unclear	Unclear	Yes	(9)	
Zheng, 2004	Unclear	Unclear	Unclear	Yes	(11)	
Wu, 2004	Unclear	Unclear	Unclear	Yes	(10)	
Yao, 2005	No	Unclear	Unclear	Yes	(13)	
Chen, 2005	Unclear	Unclear	Unclear	Yes	(12)	
Wang, 2006	Unclear	Unclear	Unclear	Yes	(15)	
Liu, 2006	Unclear	Unclear	Unclear	Yes	(14)	
Lan, 2007	Unclear	Unclear	Unclear	Yes	(16)	
Zhao, 2008	Unclear	Unclear	Unclear	Yes	(17)	
Xi, 2009	Unclear	Unclear	Unclear	Yes	(22)	
Chu, 2009	Yes	Unclear	Unclear	Yes	(18)	
Hong, 2009	Unclear	Unclear	Unclear	Yes	(20)	
Gu, 2009	Unclear	Unclear	Unclear	Yes	(19)	
Wang, 2009	No	Unclear	Unclear	Yes	(21)	
Mei, 2010	Unclear	Unclear	Unclear	Yes	(24)	
Zhang, 2010	Unclear	Unclear	Unclear	Yes	(25)	
Liu, 2010	Unclear	Unclear	Unclear	Yes	(23)	
Zhou, 2011	Unclear	Unclear	Unclear	Yes	(29)	
Wang, 2011	Unclear	Unclear	Unclear	Yes	(27)	
Yang, 2011	Unclear	Unclear	Unclear	Yes	(28)	
Sheng, 2011	Unclear	Unclear	Unclear	Yes	(26)	
Yin, 2012	Yes	Unclear	Unclear	Yes	(31)	
Chen, 2012	Unclear	Unclear	No	Yes	(30)	
Zhang, 2013	Unclear	Unclear	Unclear	Yes	(32)	

Table III. Sensitivity analysis of the pooled ORs of adjunctive therapy of MV vs. general chemotherapy (without MV).

	SSP		ATF		CSTBC	
Variable	Total, n	OR (95% CI)	Total, n	OR (95% CI)	Total, n	OR (95% CI)
Model						
FE	23	3.84 (3.11-4.73)	15	4.08 (3.06-5.45)	18	3.42 (2.68-4.37)
RE	23	3.73 (3.01-4.61)	15	4.08 (2.75-6.05)	18	3.40 (2.65-4.36)
COT, weeks						
<12	4	4.21 (2.49-7.12)	3	5.28 (2.89-9.66)	3	4.32 (2.42-7.71)
12	7	5.89 (3.79-9.16)	4	5.10 (2.27-11.45)	6	4.34 (2.72-6.92)
>12	12	3.15 (2.41-4.12)	8	3.31 (1.77-6.19)	9	2.79 (1.99-3.89)

MV, *Mycobacterium vaccae*; SSP, sputum smear positive-turned-negative; ATF, absorption of tuberculosis foci; CSTBC, closure situation of tuberculosis cavity; OR, odds ratio; CI, confidence interval; FE, fixed effect model; RE, random effect model; COT, course of treatment.

studies suggested that MV as an adjunctive therapy is associated with a significant increase in CD4⁺ counts (SMD=0.98; 95% CI, 0.04-1.93; I²=94.6%; P-value for heterogeneity <0.001) and specific value of CD4⁺/CD8⁺ (SMD=0.76; 95% CI, 0.06-1.45; I²=90.6; P-value for heterogeneity <0.001) with the random effects model, and MV as an adjunctive therapy had no statistical significance for increasing CD3⁺

counts (SMD=0.31; 95% CI, -0.05-0.68; I^2 =68.2%, P-value for heterogeneity=0.024) and CD8+ counts (SMD=-1.14; 95% CI, -2.81-0.53; I^2 =98.0%; P-value for heterogeneity <0.001).

The adverse effects of MV mainly include induration at the vaccine sites (8-9,13,16,17,19-22,24,26), fever (8,13,16,19,21,22) and local swelling (9,12,17,20,21,26). For the purified protein

derivative test, only 1 study (15) reported that no significance occurred for the changes of the scleroma diameter between the MV and control groups.

Discussion

The main aim of the present study, which included 25 eligible studies involving 2,281 subjects, was to critically evaluate the effects of MV as an adjunctive therapy in the treatment of MDR-TB patients. Based on the available studies, the meta-analysis showed that MV is an effective adjuvant when combined with general chemotherapy for treating MDR-TB. MV can improve the sputum smear positive-turned-negative, the absorption of TB foci and the closure situation of the TB cavity for some of the poor quality RCTs reported. In addition, MV as an adjunctive therapy has fewer adverse effects compared to the general anti-TB drugs. The clinical effects of MV have been confirmed presently using the previous studies.

Particularly in low-income countries and developing nations, MDR-TB is spreading with a series of negative consequences. MDR-TB had been reported in Eastern Europe, Western Europe and Central Asia (33,34). Control of TB by chemotherapy is mainly measured according to 'the long-term, combined, in moderation, appropriate and whole course', but resistance is frequently due to unreasonable chemotherapy regimens, poorer compliance and worse immune function of patients, resulting in TB bacterium that are resistant of MDR, and subsequently, searching for the correct treatment is the focus of clinical physicians and pharmacists. The T-cell [T-helper cells (Th cells)] immunity of patient immune function is closely associated with the onset and outcomes of TB disease; Th1 cells decrease in peripheral blood and increase in lesions, and furthermore, they are associated with the course of dynamic changes. Therefore, according to the immunological characteristics of TB, the study protocol of immune therapy application combined with chemotherapy has been more frequently applied in anti-TB treatment in clinical practice. In an early study by Stanford et al (35), MV as immune agents improved the pathological changes around the immune cell activity, reduced tissue necrosis, weight gain, shortened the chemotherapy treatment, and reduced the case fatality rate.

MV, a type of immune enhancer, mainly affects the immune response to adjust the human immune function and achieve therapeutic purposes. MV can remove and restrain MTB by activating the Th1 cytokine-mediated immune response, improving Th1/Th2, and activating the macrophage phagocytosis of MTB. From the perspective of pharmacoeconomics, although the treatment cost of MV combined with chemical therapy increased for MDR-TB, its curative effect significantly improved and the incremental cost was reduced (12). It is the only recommended immunization agents by the WHO in the Tuberculosis Strategic Development Plan of the 1990s (36).

The result of the present meta-analysis with little heterogeneity was in accordance with the study reporting that immunotherapy with MV offers hope for the treatment of MDR-TB pulmonary TB in Estonia, Iran, Kuwait, New Zealand, Romania, Vietnam and the United Kingdom (37). There are certain limitations that originated from the limited studies present in this meta-analysis, which should be taken into consideration. Firstly, these studies (8-32) were not

targeted globally (all studies were Chinese populations) and the course of treatment varied from 6 to 24 months, and the chemotherapy regimens in the control group were different forms in each trials. Secondly, the studies were all carried out in China and their qualities had a generally high risk of bias; all did not address the allocation concealment, which would magnify >40% effects of the results (38-40). Only 2 studies (18,31) reported the method of adequate sequence generation, including stratified random and using a random numbers table. Thirdly, the sample size of the included studies was relatively small. On the basis of the course of treatment in the subgroup analysis, a notable finding was that the effects of the sputum smear positive-turned-negative, the absorption of TB foci and the closure situation of TB cavity were reduced as the course of treatment increased. This was possibly due to the reducing effect of the general chemotherapy as the extension of treatment. Furthermore, the treatment of MDR-TB should always be prioritized above chemotherapy, and immunotherapy is a type of auxiliary treatment. Therefore, multi-central large RCTs are required to investigate the safety of MV as an adjunctive therapy in MDR-TB treatment in the future.

In conclusion, the present study demonstrates that MV has a significant effect and safety as an adjunctive therapy in the treatment of MDR-TB. Further studies are required to validate this conclusion.

References

- 1. Dye C, Floyd K and Uplekar M: Global tuberculosis control: Surveillance planning financing. WHO report 2008. World Health Organization, Geneva, p300, 2008.
- 2. Yang XY, Chen QF, Li YP and Wu SM: *Mycobacterium vaccae* as adjuvant therapy to anti-tuberculosis chemotherapy in never-treated tuberculosis patients: A meta-analysis. PLoS One 6: e23826, 2011.
- 3. Abou-Zeid C, Gares MP, Inwald J, Janssen R, Zhang Y, Young DB, Hetzel C, Lamb JR, Baldwin SL, Orme IM, et al: Induction of a type 1 immune response to a recombinant antigen from Mycobacterium tuberculosis expressed in Mycobacterium vaccae. Infect Immun 65: 1856-1862, 1997.
- Hernandez-Pando R, Pavön L, Arriaga K, Orozco H, Madrid-Marina V and Rook G: Pathogenesis of tuberculosis in mice exposed to low and high doses of an environmental mycobacterial saprophyte before infection. Infect Immun 65: 3317-3327, 1997.
- 5. Skinner MA, Yuan S, Prestidge R, Chuk D, Watson JD and Tan PL: Immunization with heat-killed *Mycobacterium vaccae* stimulates CD8+ cytotoxic T cells specific for macrophages infected with Mycobacterium tuberculosis. Infect Immun 65: 4525-4530, 1997.
- 6. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J and Moher D: The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ 339: b2700, 2009.
- Higgins JP, Thompson SG, Deeks JJ and Altman DG: Measuring inconsistency in meta-analyses. BMJ 327: 557-560, 2003.
- 8. Luo Y, Lu S and Guo S: Immunotherapeutic effect of *Mycobacterium vaccae* on multi-drug resistant pulmonary tuberculosis. Zhonghua Jie He He Hu Xi Za Zhi 23: 85-88, 2000 (In Chinese).
- Song XD, Ren YW, Zhu H, Ji Q and Xu Y: The effect of Mycobacterium vaccae on multi-drug resistant pulmonary tuberculosis of 36 cases. Zhongguo Zong He Lin Chuang 18: 48, 2002 (In Chinese).
- Wu C, Yu GC and Cheng CX: Effect of Mycobacterium vaccae on multi-drug resistant pulmonary tuberculosis of 43 cases. Zhongguo She Qu Yi Shi: Yi Xue Zhuan Ye 6: 15-16, 2004 (In Chinese).

- 11. Zheng XM, Li SH and Xing BC: Short-term effect of treatment protocol utilizing levofloxacin, pasiniazide and M. Vaccae on multi-drug resistant pulmonary tuberculosis. Di Yi Jun Yi Da Xue Xue Bao 24: 574-578, 2004 (In Chinese).
- 12. Chen SQ, Liu JY and Weng LZ: Pharmacoeconomics evaluation for the treatment of Mycobacterium vaccae on multidrug-tuber-
- culosis. Fu Jian Yi Yao Za Zhi 27: 129-131, 2005 (In Chinese). 13. Yao HB, Shang HZ, Guo L, Wang YL, Feng JM and Ma JM: The observation of the effect of the treatment of multi-drug resistant pulmonary tuberculosis with M Vaccae. Zhongguo Fang Lao Za Zhi 27: 389-392, 2005 (In Chinese).
- 14. Liu YH: Effect of Mycobacterium vaccae as an adjunctive therapy on multidrug resistant tuberculosis. Zhongguo Ji Ceng Yi Yao 13: 492-493, 2006 (In Chinese).
- 15. Wang JY and Lin DW: Effect of Mycobacterium vaccae on multidrug resistant tuberculosis. Hua Xia Yi Xue 19: 443-445, 2006 (In Chinese).
- 16. Lan K: Effect of Mycobacterium vaccae as an adjunctive therapy on multidrug resistant pulmonary tuberculosis. Nei Ke 2: 47-48, 2007 (In Chinese).
- 17. Zhao SX: Effect of Mycobacterium vaccae vaccine in treatment of multi-drug resistant tuberculosis. Lin Chuang Fei Ke Za Zhi 13: 927-928, 2008 (In Chinese).
- 18. Chu NH, Luo YA, Zhu LZ, Fu Y, Ye Z, Xiao H, Wang W, Yuan S, Zhang X, Lu S, et al: A controlled clinical study of long course chemotherapy regimens combined with immunopotentiator in the treatment of multi-drug resistant pulmonary tuberculosis. Zhongguo Fang Lao Za Zhi 31: 10-14, 2009 (In Chinese).
- 19. Gu XY: Effect of Mycobacterium vaccae as an adjunctive therapy on 70 multidrug resistant tuberculosis cases. Lin Chuang Fei Ke Za Zhi 14: 1677-1678, 2009 (In Chinese).
- 20. Hong Y and Lin XH: Effect of Mycobacterium vaccae vaccine plus anti-tuberculosis drugs on multi-resistant tuberculosis. Zhongguo Re Dai Yi Xue 9: 850-851, 2009 (In Chinese).
- 21. Wang YL: Study of microcalory united with chemotherapeutics on tuberculosis of more drugs fast. Xian Dai Zhong Xi Yi Jie He Za Zhi 18: 2362-2363, 2009 (In Chinese).
- 22. Xi XE, Zhang HQ and Zhang LG: Frozen-dried M. Vaccae Vavvine for therapy combined with general chemotherapy for multi-drug resistance pulmonary tuberculosis. Xin Xiang Yi Xue Yuan Xue Bao 26: 355-357, 2009 (In Chinese).
- 23. Liu XD: MV immune treatment of multi-drug resistant tuberculosis 32 cases of clinical curative effect observation. Gan Nan Yi Xue Yuan Xue Bao 30: 412, 2010 (In Chinese).
- 24. Mei YZ and Yang LM: Effects of Mycobacterium vaccae vaccine on multi-drug resistant pulmonary tuberculosis. Zhongguo Re Dai Yi Xue 10: 693-694, 2010 (In Chinese).
- 25. Zhang JD: MV with the chemotherapy drug therapy retreatment on case of multi-drug resistant tuberculosis curative effect observation. Lin Chuang Fei Ke Za Zhi 15: 1489-1490, 2010 (In Chinese).
- 26. Sheng GL: Clinical analysis of Vaccae combined anti-tuberculosis drugs in treatment of multidrug-resistant tuberculosis. Yi Yao Lun Tan Za Zhi 32: 37-38, 2011 (In Chinese).

- 27. Wang L and Jin ZD: Effect of Mycobacterium vaccae vaccine on multi-drug resistant pulmonary tuberculosis. Zhongguo She Qu Yi Shi: Yi Xue Zhuan Ye 13: 52, 2011 (In Chinese).
- 28. Yang SY: Clinical analysis of Vaccae combined anti-tuberculosis drugs in treatment of 60 cases of multidrug-resistant tuberculosis. Zhongguo Yi Yao Zhi Nan 9: 105-106, 2011 (In Chinese).
- 29. Zhou Y: Clinical observation of mycobacterium bovis combination with chemotherapy on multidrug-resistant mycobacterium. Chong Qin Yi Xue 40: 341-342, 2011 (In Chinese).
- 30. Chen YJ: Chemical joint MV clinical research for the treatment of senile multi-drug resistant tuberculosis. Zhong Wai Yi Xue Yan Jiu 10: 44, 2012 (In Chinese).
- 31. Yin LS, Zhu M and Zhou M: Clinical observation on buqin tablet combined with Western medicine for 17 cases of multidrug-resistant tuberculosis. Zhong Yi Za Zhi 53: 669-672, 2012 (In Chinese).
- 32. Zhang XL and Huang D: Effects of cellular immunity improvement on chemotherapy efficacy and prognosis of patients with multidrug-resistant tuberculosis. Shi Yong Yi Xue Za Zhi 29: 1964-1966, 2013 (In Chinese).
- 33. Skrahina A, Hurevich H, Zalutskaya A, Sahalchyk E, Astrauko A, van Gemert W, Hoffner S, Rusovich V and Zignol M: Alarming levels of drug-resistant tuberculosis in Belarus: Results of a survey in Minsk. Eur Respir J 39: 1425-1431, 2012.
- 34. Zignol M, van Gemert W, Falzon D, Sismanidis C, Glaziou P, Floyd K and Raviglione M: Surveillance of anti-tuberculosis drug resistance in the world: An updated analysis, 2007-2010. Bull World Health Organ 90: 111-119D, 2012.
- 35. Stanford JL, Bahr GM, Rook GA, Shaaban MA, Chugh TD, Gabriel M, al-Shimali B, Siddiqui Z, Ghardani F, Shahin A, et al: Immunotherapy with Mycobacterium vaccae as an adjunct to chemotherapy in the treatment of pulmonary tuberculosis. Tubercle 71: 87-93, 1990.
- 36. World Health Organization (WHO): Tuberculosis research and
- development: Report of a WHO meeting. WHO, Geneva, 1991. 37. Stanford JL, Stanford CA, Grange JM, Lan NN and Etemadi A: Does immunotherapy with heat-killed Mycobacterium vaccae offer hope for the treatment of multi-drug-resistant pulmonary tuberculosis? Respir Med 95: 444-447, 2001.
- 38. Schulz KF, Chalmers I, Hayes RJ and Altman DG: Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA 273: 408-412, 1995.
- 39. Moher D, Pham B, Jones A, Cook DJ, Jadad AR, Moher M, Tugwell P and Klassen TP: Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? Lancet 352: 609-613, 1998.
- 40. Jüni P, Altman DG and Egger M: Systematic reviews in health care: Assessing the quality of controlled clinical trials. BMJ 323: 42-46, 2001.