Title:	Assessment of whole blood bactericidal activity in the evaluation of new TB drugs
Short title:	Whole blood bactericidal activity
Author:	Robert S. Wallis, MD, FIDSA
Affiliation:	Pfizer
Correspondence:	50 Pequot Ave., B3140, New London, CT 06320 USA robert.wallis@pfizer.com or rswallis@gmail.com +1 860 732 2573
Word count:	3350
Figures:	3
Tables:	0
References:	29

Abstract

Intracellular mycobacterial subpopulations with altered metabolism and reduced replication arise *in vivo* as a result of pressure from host immune mechanisms. These subpopulations, which show altered susceptibility to the action of anti-TB drugs, play an important role in mycobacterial persistence and TB relapse. This chapter describes the use of whole blood cultures infected *ex vivo* with *Mycobacterium tuberculosis* to assess the effectiveness of administered anti-tuberculous treatment against non-replicating persisters. Studies in TB patients have shown that cumulative whole blood bactericidal activity is indirectly linked to outcome, and that drug activity in the *ex vivo* cultures is modulated by the intracellular immune milieu. Three studies in healthy volunteers have used the method for dose selection and regimen optimization. Further research in TB patients is warranted to explore the potential role of this method in accelerating the development of new TB drugs and regimens.

Introduction

Distinct subpopulations of *Mycobacterium tuberculosis* exist in patients with active tuberculosis. These organisms differ in metabolism, gene expression profiles, rates of replication, and other functional characteristics. Although these subpopulations can arise at random in small numbers in otherwise uniform bacterial populations, their numbers increase greatly in response to specific stresses created by the host immune response, often in distinct anatomic locations. These adaptations facilitate specific stages of the mycobacterial life cycle. For example, large numbers of metabolically active, replicating bacilli within caseous material in cavities are required for efficient aerosolization and transmission, whereas semi-dormant bacilli sequestered in hypoxic granulomas make possible persistence, reactivation and relapse. Current TB drugs act differentially on these subpopulations [1]. Isoniazid, for example, acts primarily on replicating, aerobic, extracellular bacilli, whereas rifampin appears equally active against both replicating and semi-dormant bacilli. Pyrazinamide requires low pH, found within granulomas and in macrophages after phagosome-lysosomal fusion, for its activity to be expressed [2]. Certain subpopulations can also emerge in response to specific stresses induced by drug treatments. Thus, the critical unit in tuberculosis is an unusually complex triad that includes host, pathogen, and therapy-related elements.

The existence of sequestered mycobacterial subpopulations poses a challenge for drug developers and clinicians who wish to meaningfully assess the therapeutic potential of a new drug or regimen. Monitoring tissue sterilization and predicting relapse risk presently can be accomplished only by measuring either rates of relapse after completion of therapy or by proportions with sputum culture conversion after 2 months of treatment [3]. These endpoints have relatively large sample size requirements, and in most circumstances cannot be used to study single new drugs. Measurement of serial sputum colony counts (SSCC) during the first several weeks of therapy has been proposed as an alternative endpoint, based on a retrospective analysis of 34 subjects enrolled in separate trials of HREZ

and STH [4]. These two regimens, which differ substantially in the duration of treatment required for cure (6 *vs*. 18 months), differed significantly in their effects on log colony counts from days 2 to 28 (-.163 *vs*. -.095 log/d). SSCC was measured prospectively in the OFLOTUB phase 2 study, which found moxifloxacin- and gatifloxacin-containing regimens to be superior to standard therapy (-.169 and -.166 log/d, respectively, *vs*. -.134) [5]. The effects of moxifloxacin and gatifloxacin on 2-month conversion and relapse rates remain to be determined, as studies to date have been contradictory [5-8]. These observations indicate an urgent need for other methods that can be used to efficiently evaluate the sterilizing activity of new compounds and regimens in early clinical trials.

Rationale

The *ex vivo* whole blood model is an alternative strategy to measure drug effects on otherwise elusive but critical microbial subpopulations that arise in the context of the host immune response. The approach benefits from the simplicity of whole blood culture to study cellular immune function. Whole blood cultures consist of blood collected in a heparinized tube that is diluted with an equal volume of tissue culture medium, to which a microbe or other immune stimulus is added. Mycobcteria added to the cultures rapidly undergo essentially complete phagocytosis by neutrophils and monocytes [9,10]. Mycobacterial antigens are processed, expressed, and recognized by CD4, CD8, NK, $\gamma\delta$, and other lymphocytes. Cytokines, adhesion ligands, activation markers, and effector molecules are expressed, resulting in restriction of mycobacterial replication. The extent of mycobacterial growth or killing in whole blood culture therefore reflects the balance between microbial pathogenicity and host immune mechanisms. In tuberculin skin test negative healthy donors, for example, the time required for doubling of *M. tuberculosis* H37Rv is increased to over 34 hrs from the from the usual 18-20 in simple broth culture. Superior restriction of growth is evident in blood of TST positive subjects. The balance between host and pathogen can be shifted by removal of CD4 or CD8 T cells, addition of prednisolone,

TNF antibodies, or dihydroxyvitamin D [9-12]. Growth inhibition in whole blood culture has improved in clinical trials in which patients were vaccinated with BCG or given oral vitamin D supplementation, or in AIDS patients following initiation of antiretroviral therapy [13-15].

Of greatest relevance to this review is the finding that whole blood cultures of patients with newly diagnosed active TB are capable of full restriction of growth of virulent clinical isolates, including the patient's own strain [16,17]. This presumably reflects the influence of antigen-driven *in vivo* expansion and activation of *M. tuberculosis*-reactive T cells. The resulting bacteriostasis can therefore serve to model the killing of non-replicating *M. tuberculosis* by a single drug or by drug combinations *in vitro* as it occurs in granulomas *in vivo*. This differs fundamentally from simple macrophage cultures, which are generally very permissive of intracellular mycobacterial replication.

Drug levels in whole blood culture reflect those in the blood at the time of phlebotomy. By collecting blood samples at various intervals before and after dosing, the full magnitude of therapeutic effects can be determined as drug concentrations rise and fall due to absorption, distribution, metabolism and elimination. The parameter of greatest interest therefore is the area under the curve over 24 hours of dosing.

Technical aspects

Fresh blood with viable cells is required. Blood can be collected in heparin-containing Vacutainers (BD) or similar pre-filled tubes. Sodium heparin is preferred, though lithium may be acceptable. Other anticoagulants such as ACD adversely affect cell function and should not be used. A total volume of approximately 1 ml blood per time point is sufficient. The specific times and number of time points depends on the kinetics of the drugs to be studied, but generally at least 4 and preferably 5 are preferred. Sampling at the same time points for PK sampling is desirable, as it facilitates additional analyses of the PK/PD relationship. Practical considerations favor reserving samples for transport as a

batch to the laboratory for processing by mid-afternoon. For drugs administered once daily in the morning, this can be facilitated by collecting specimens for later time points the prior evening. While awaiting transport, tubes are maintained at room temperature with slow constant rotation. Variability was increased in baseline (pretreatment) samples in one study in which samples were stored on a table top without mixing. Sixteen hours is the maximum recommended duration of such temporary storage.

Cultures are performed in sealed 2 ml tubes that are rotated slowly during incubation. The slow mixing enhances immune restriction of mycobacterial growth. At the conclusion of the period of incubation (usually 72 hrs), cells are pelleted. The supernatant is removed, and may be reserved for analysis of induced cytokines. Sterile distilled water is added to lyse red blood cells. The cells are pelleted again, and the liquid phase again removed. The remaining pellet resuspended in a small volume of 7H9 broth and added to a Mycobacterial Growth Indicator Tube (MGIT, Becton Dickinson). MGIT time to positivity (TTP) is recorded. In early studies, Bactec culture bottles were used instead. Mycobacterial growth in Bactec is measured daily as production of $^{14}CO_2$ from radiolabeled palmitic acid. A subsequent study has shown a high correlation between time to positivity in Bactec and MGIT. The disadvantages of the Bactec system are its requirements that $^{14}CO_2$ production by each culture must be measured daily, and that each completed culture must be disposed in a manner appropriate for low-level radioisotopes.

The MGIT 960 system has largely replaced Bactec in clinical mycobacteriology laboratories. MGIT tubes contain Middlebrook 7H9 liquid media plus an oxygen-quenched fluorochrome (tris 4, 7-diphenyl-1, 10-phenonthroline ruthenium chloride pentahydrate) embedded in silicone at the bottom of the tube. Bacterial growth within the tube results in depletion of free oxygen, which in turn results in fluorescence within the MGIT tube when visualized under UV light. The intensity of fluorescence is directly proportional to the extent of oxygen depletion. MGIT tubes placed in a MGIT 960 instrument are maintained at 37^{o}C and monitored every 60 minutes for increasing fluorescence. The instrument declares a culture positive if either it reaches a predetermined level of fluorescence or if it shows a rapid

rate of increase in fluorescence. Cultures of *M. tuberculosis* generally have 10^5 – 10^6 CFU per ml of medium at the time they are declared positive, although cultures with large inocula may be declared positive earlier. MGIT TTP is highly inversely related to the CFU count of the inoculum (figure 1). The main advantage of the MGIT system (aside from its automation) is that results are not affected by bacterial clumping and therefore show reduced variability. A secondary advantage is that the readout has a wide dynamic range (at least 10^5), such that measurement of serially diluted specimens is not necessary.

Hemoglobin can interfere with detection of growth in MGIT, both by increasing the starting oxygen content in the culture tube and by interfering with UV light transmission. However, the starting volume of blood in each whole blood culture is small (300 μl), and a sufficient proportion of hemoglobin is removed by the RBC lysis step to prevent its interference with the MGIT system. The lysis step also reduces the amount of drug carried over from the completed whole blood culture into the MGIT tube.

MGIT tubes are also used to prepare the inoculum and determine the appropriate volume for inoculation of whole blood cultures. This has the advantage that cultures are at a relatively early stage of growth when harvested, resulting in reduced bacterial clumping. In one study, this method reduced the variability among replicate cultures by half. The approach also facilitates the study of killing of unique patient isolates if desired. The inoculum size is specified as that calculated to be positive in 4.5 or 5.5 days in various experiments, based on the titration curve. In one experiment, changes as much as 50-fold in inoculum size had no effect on the extent of bactericidal activity [10].

Data analysis

Log change in viability in each whole blood culture is calculated as log(*final*) – log(*initial*), where *final* and *initial* are the volumes corresponding to TTP of the completed culture and its inoculum, respectively, based on a titration curve of the mycobacterial stock. Results are expressed on a log scale,

with zero indicating bacteriostasis, and negative values, killing. In recent studies, results have been calculated as log change per day of whole blood culture. In early studies, results were expressed simply as log change for the entire 3 day duration of culture. Those results here have been divided by 3 days for consistency in presentation.

Bactericidal activity *in vivo* varies as drug concentrations rise and fall during the dosing interval. These changes are not mirrored in individual whole blood cultures, each of which reflects levels in blood at the moment of phlebotomy. To better understand the total extent of killing throughout dosing, static values are integrated over time to determine the cumulative or total extent of killing throughout a 24 hour interval, equal to the area under the curve over 24 hrs ($AUC_{0\text{-}24}$). The units of this parameter are $\frac{\Delta log}{day} * day$ or simply log change. These data may be presented in tabular form, or illustrated as they evolve during the dosing interval, in which case they have the appearance of a dynamic time-kill curve. The data differ from a typical dynamic time-kill experiment, however, in that post-antibiotic effects (PAE) are not represented. PAE can delay the resumption of bacterial growth *in vivo* as drug concentrations fall. The lack of PAE in the whole blood model would be expected to result in underestimation of the cumulative WBA of drugs with long PAE such as the rifamycins, but would be anticipated to have little or no effect on measurement of activity of others such as linezolid with short PAE [18,19].

Studies in TB patients

One WBA trial has been conducted to date in patients with pulmonary tuberculosis [16]. In that study, WBA was evaluated in 36 patients with fully drug-sensitive disease treated with daily standard 4-drug therapy (HREZ). Each patient was studied with his or her own isolate, on week 8 (during the intensive 4-drug phase of treatment) and on week 12 (during the continuation phase). Blood was sampled for WBA determination at 0, 2, and 6 hrs post dose. Bactericidal activity was present

throughout the dosing interval during both the intensive and continuation phases. Peak activity was evident 2 hrs post dose (-1.6 to -2.0 log/d), when plasma drug concentrations were maximal. Minimal bactericidal activity was detected immediately pre-dose during the continuation phase; this time point was superior during the intensive phase (-0.25 log/d vs. -0.08 log/d), presumably reflecting the long intracellular half-life of pyrazinamide.

Cumulative WBA for the 24 hr dosing interval was calculated assuming that at steady state values at 0 and 24 hrs were equal. Cumulative WBA was greater during the intensive *vs*. continuation phases (-0.77 ± 0.27 vs. -0.56 ± 0.30, P <.001 by paired t test). Since ethambutol exerts only a bacteriostatic effect in the whole blood model, the difference between these two phases of therapy mainly reflects the activity of pyrazinamide. Cumulative activity throughout treatment, calculated as the weighted average of the 2 phases, was superior in patients whose sputum cultures converted by month 2 (-0.63 ± 0.26 *vs*. -0.46 ± 0.27, P=.04). Cumulative WBA also correlated with the rate of decline of log sputum CFU counts during the first month of treatment (R = .39, P=.018).

Patients in that study were also all tested against a single clinical isolate. In that case, variability among patients was reduced, but the extent of correlation with clinical microbiology was also reduced, indicating that strain characteristics – other than resistance, which was excluded by study entry criteria – contribute to outcome. This is consistent with other observations that differences among strains in phenotypic drug tolerance unrelated to MIC contribute to persistence and relapse in TB [20]. Thus differences in efficacy among treatments may be most efficiently measured when patients are tested using a single isolate, whereas clinical or microbiologic outcomes may be best predicted when patients are studied using their own isolates.

One study has directly compared bactericidal activity in patients and healthy volunteers [21]. In that report, whole blood cultures of *M. tuberculosis* H_{37}Rv were prepared with and without ofloxacin, added directly to the cultures at 1 μg/ml. Ofloxacin was highly active in blood of healthy volunteers, in

whom +0.21 log/d (growth) *vs*. -0.13 log/d (killing) was evident in cultures in the absence and presence of ofloxacin (net effect, -0.34 ± 0.08). In contrast, TB patients exerted full bacteriostasis in baseline cultures (-0.17 log/d) and showed a markedly reduced additional effect when drug was added (-0.09 ± 0.04, P<.001). This is consistent with a recent study by Sala *et al* indicating ofloxacin lacks activity against non-replicating *M. tuberculosis* [22]. These findings underscore the importance of the immunologic milieu as a modifier of drug activity during treatment of intracellular *M. tuberculosis* infection.

Studies in healthy volunteers

The first publication of the whole blood method described the activity of various drugs and drug combinations in 3 healthy volunteers [10]. Amoxicillin/sulbactam was inactive; ethambutol was bacteriostatic. The rank order of bactericidal activity was pyrazinamide < fluoroquinolones < isoniazid < rifampin. The cumulative WBA of standard therapy (HREZ) was approximately 3 times that of a fluoroquinolone-based regimen for MDR-TB, consistent with their respective required durations of curative treatment. Standard therapy was inactive against MDR strains.

One study conducted in 10 healthy volunteers compared the activity of orally administered clofazimine 200 mg QD vs. ofloxacin 600 mg QD, when given alone and when combined with standard doses of PZA and EMB [23]. The total dose of clofazimine administered during the study was 2 g. Whole blood cultures were inoculated with *M. tuberculosis* H37Rv. The objective was to determine the potential activity of a clofazimine-based regimen against MDR-TB. WBA was measured at 0, 2 and 6 hrs post dose on days 5 (monotherapy) and 10 (combination therapy). The study found clofazimine to be inactive at concentrations reached by oral dosing, and inferior to ofloxacin with regard to cumulative WBA (+0.15 ± 0.07 vs. -0.12 ± 0.10, P<.001). A trend toward inferiority of clofazimine persisted when combined with PZA and EMB (-0.01 ± 0.07 vs. -0.15 ± 0.13, P=.067). The basis of the lack of activity of clofazimine was examined further by directly adding it to whole blood culture. The MIC of clofazimine

against *M. tuberculosis* H37Rv has been reported as 0.1 μg/ml. Clofazimine accumulates in phagocytes during treatment. However, as indicated in figure 2, extracellular concentrations of 100 μg/ml were required to produce a bactericidal effect of -0.6 log/d against intracellular *M. tuberculosis* infection. These appear not to be achieved after short term oral administration of moderate clofazimine doses.

Two studies have been reported in which the whole blood model was used during early development of PNU-100480 [24,25], a linezolid analog that shows superior efficacy in the mouse TB model [26,27]. Linezolid 300 mg QD served as a comparator, based on one report that this dose may be adequately tolerated during treatment of MDR-TB [28]. The studies found that both drugs showed concentration-independent killing that reached 90% of maximal effect at approximately twice MIC, but that the maximal effect of PNU-100480 (approximately -0.42 log/d) was more than twice that of linezolid (-0.16 log/d, figure 3). The cumulative WBA of PNU-100480 600 mg BID was -0.316±0.04 vs. -0.072 ± 0.05 for linezolid 300 mg QD (P<.001). The activity of PNU-100480 was further enhanced to -0.420±.06 (P=.002) when administered together with pyrazinamide. These findings confirmed two key findings of PNU-100480 in the mouse model: superiority vs. linezolid, and synergy with PZA.

These 2 studies also illustrate the statistical power of the whole blood method. The multi-dose trial, for example, included 4 cohorts of 8 subjects dosed at 0, 100, 300, and 600 mg twice daily. Statistical analysis revealed highly significant differences among the cohorts by ANOVA (P<.001) and significant differences in all post hoc paired comparisons (all P<.05). In contrast, a study of the early bactericidal activity (EBA) of linezolid 600 mg given BID and QD in a total of 19 subjects was unable to distinguish changes in log sputum CFU counts of the two treatments from each other or from zero [29]. This indicates the strong potential of the whole blood method to rapidly evaluate new drugs and regimens in small, short trials.

Questions for future research

The body of evidence supporting a relationship of cumulative WBA to a clinical TB outcome is presently modest and comparable to that for serial CFU counts. For this reason, the method may be best described as an emerging candidate biomarker requiring validation. Further studies are warranted to determine the prognostic value of WBA for treatment arms in TB clinical trials and for individual TB patients.

Perhaps the most interesting and innovative use of the whole blood model may be in the sequential evaluation of new TB regimens tested in TB patients in a double cross-over manner. The use of within-subject (paired) comparisons would further reduce sample size requirements and permit rapid evaluation of multiple candidate regimens in as few as 5-6 subjects. Subsequent conventional trials would examine effects on 2-month sputum culture conversion, thus creating a highly efficient path to accelerated approval.

Modifications of the method may be of benefit in specific circumstances. For example, TMC207 shows a delay of up to several days in the time to onset of its bactericidal activity [30]. Whole blood cultures of longer duration may be required to accurately reflect its activity. It may be possible to modify culture conditions of whole blood cultures of healthy volunteers to make them more like those in TB granulomas, by altering pH, nutrient availability, and oxygen tension. Lastly, it may be possible to simplify aspects of the method to facilitate the study of patients with their own isolate, by reducing or eliminating the titration curve and simplifying the selection of the inoculum volume. This may ultimately result in a simple single assay with important prognostic value beyond that presently conferred by rifampin resistance testing, that may be incorporated into routine clinical care.

Figures

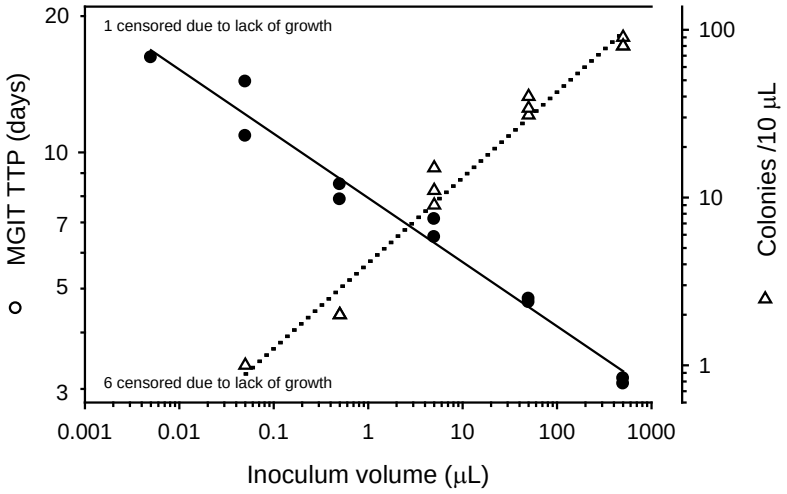

Figure 1. Relationship between time to positivity in Mycobacterial Growth Indicator Tubes (MGIT TTP) and CFU count.

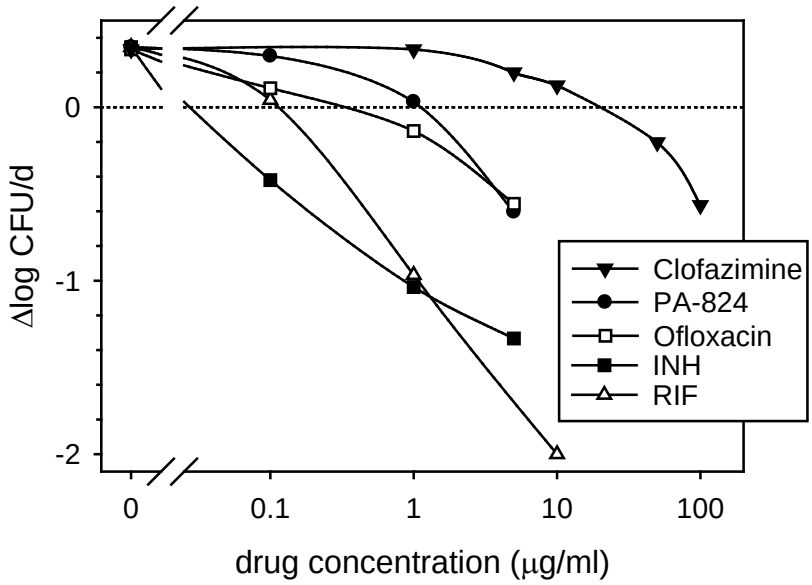

Figure 2. Relationship between drug concentration and bactericidal activity against *M. tuberculosis* H37Rv in blood of healthy volunteers. INH=isoniazid; RIF=rifampin.

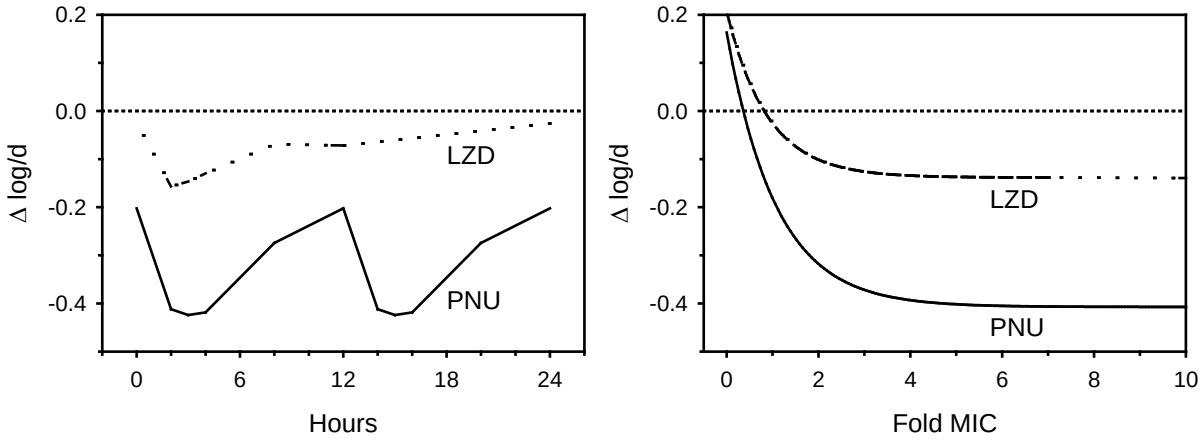

Figure 3. Whole blood bactericidal activity of linezolid (LZD) and PNU-100480 (PNU) against *M. tuberculosis* H37Rv after oral administration to healthy volunteers. Left: Mean WBA at steady state in subjects dosed with either LZD 300 mg QD (dashed line) or PNU-100480 600 mg BID (solid line). Right: Regression curves describing relationship between drug concentration and WBA for LZD and PNU. PNU concentrations represent summed MIC ratios for PNU-100480 and its metabolites.

Reference List

1. Mitchison DA: The action of antituberculosis drugs in short-course chemotherapy. Tubercle 1985;66(3):219-225.

2. Zhang Y, Mitchison D: The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis 2003;7(1):6-21.

3. Wallis RS, Wang C, Doherty TM, Onyebujoh P, Vahedi M, Laang H, Olesen O, Parida S, Zumla A: Biomarkers for tuberculosis disease activity, cure, and relapse. Lancet Infect Dis 2010;10(2):68-69.

4. Jindani A, Dore CJ, Mitchison DA: Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. Am J Respir Crit Care Med 2003;167(10):1348-1354.

5. Rustomjee R, Lienhardt C, Kanyok T, Davies GR, Levin J, Mthiyane T, Reddy C, Sturm AW, Sirgel FA, Allen J, Coleman DJ, Fourie B, Mitchison DA: A Phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis 2008;12(2):128-138.

6. Burman WJ, Goldberg S, Johnson JL, Muzanye G, Engle M, Mosher AW, Choudhri S, Daley CL, Munsiff SS, Zhao Z, Vernon A, Chaisson RE: Moxifloxacin versus Ethambutol in the First Two Months of Treatment for Pulmonary Tuberculosis. Am J Respir Crit Care Med 2006.

7. Conde MB, Efron A, Loredo C, De Souza GR, Graca NP, Cezar MC, Ram M, Chaudhary MA, Bishai WR, Kritski AL, Chaisson RE: Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomised, controlled phase II trial. Lancet 2009;373(9670):1183-1189.

8. Dorman SE, Johnson JL, Goldberg S, Muzanye G, Padayatchi N, Bozeman L, Heilig CM, Bernardo J, Choudhri S, Grosset JH, Guy E, Guyadeen P, Leus MC, Maltas G, Menzies D, Nuermberger EL, Villarino M, Vernon A, Chaisson RE: Substitution of Moxifloxacin for Isoniazid During Intensive Phase Treatment of Pulmonary Tuberculosis. Am J Respir Crit Care Med 2009;180(3):273-80.

9. Janulionis E, Sofer C, Schwander S, Simmons D, Kreiswirth B, Shashkina E, Wallis RS: Survival and replication of clinical *Mycobacterium tuberculosis* isolates in the context of human innate immunity. Infect Immun 2005;73(5):2595-2601.

10. Wallis RS, Palaci M, Vinhas S, Hise AG, Ribeiro FC, Landen K, Cheon SH, Song HY, Phillips M, Dietze R, Ellner JJ: A whole blood bactericidal assay for tuberculosis. J Infect Dis 2001;183(8):1300-1303.

11. Cheon SH, Kampmann B, Hise AG, Phillips M, Song HY, Landen K, Li Q, Larkin R, Ellner JJ, Silver RF, Hoft DF, Wallis RS: Bactericidal activity in whole blood as a potential surrogate marker of immunity after vaccination against tuberculosis. Clin Diagn Lab Immunol 2002;9(4):901-907.

12. Saliu O, Sofer C, Stein DS, Schwander SK, Wallis RS: Tumor Necrosis Factor Blockers: Differential effects on mycobacterial immunity. J Infect Dis 2006;194:486-492.

13. Hoft DF, Worku S, Kampmann B, Whalen CC, Ellner JJ, Hirsch CS, Brown RB, Larkin R, Li Q, Yun H, Silver RF: Investigation of the relationships between immune-mediated inhibition of mycobacterial

growth and other potential surrogate markers of protective mycobacterium tuberculosis immunity. J Infect Dis 2002;186(10):1448-1457.

14. Kampmann B, Tena-Coki GN, Nicol M, Levin M, Eley B: Reconstitution of antimycobacterial immune responses in HIV-infected children receiving HAART. AIDS 2006;20(7):1011-1018.

15. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, Rainbow SJ, Berry JL, Griffiths CJ: A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 2007;176(2):208-213.

16. Wallis RS, Vinhas SA, Johnson JL, Ribeiro FC, Palaci M, Peres RL, Sa RT, Dietze R, Chiunda A, Eisenach K, Ellner JJ: Whole blood bactericidal activity during treatment of pulmonary tuberculosis. J Infect Dis 2003;187:270-278.

17. Wallis RS, Vinhas S, Janulionis E: Strain specificity of antimycobacterial immunity in whole blood culture after cure of tuberculosis. Tuberculosis (Edinb) 2009;89(3):221-224.

18. Wallis RS, Weyer K, Fourie PB: Acquired rifamycin resistance: pharmacology and biology. Expert Rev Anti Infect Ther 2008;6(2):223-230.

19. Hui M, Au-Yeang C, Wong KT, Chan CY, Yew WW, Leung CC: Post-antibiotic effects of linezolid and other agents against Mycobacterium tuberculosis. Int J Antimicrob Agents 2008;31(4):395-396.

20. Wallis RS, Patil S, Cheon SH, Edmonds K, Phillips M, Perkins MD, Joloba M, Namale A, Johnson JL, Teixeira L, Dietze R, Siddiqi S, Mugerwa RD, Eisenach K, Ellner JJ: Drug tolerance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1999;43(11):2600-2606.

21. Wallis RS, Song HY, Whalen C, Okwera A: TB chemotherapy: Antagonism between immunity and sterilization. Amer J Respir Crit Care Med 2004;169(6):771-772.

22. Sala C, Dhar N, Hartkoorn RC, Zhang M, Ha YH, Schneider P, Cole ST: A Simple Model for Testing Drugs against Non-replicating Mycobacterium tuberculosis. Antimicrob Agents Chemother 2010.

23. Janulionis E, Sofer C, Song HY, Wallis RS: Lack of activity of oral clofazimine against intracellular M. tuberculosis in whole blood culture. Antimicrob Agents Chemother 2004;48(8):3133-3135.

24. Wallis RS, Kumar V, Jakubiec W, Silvia AM, Paige D, Bedarida G, Mitton-Fry M, Miller PF: Safety, tolerability, PK, and WBA of multiple ascending doses of PNU-100480. ICAAC 2010;50(Boston):A1-030a.

25. Wallis RS, Jakubiec W, Kumar V, Silvia AM, Paige D, Dimitrova D, Li X, Ladutko L, Campbell S, Friedland G, Mitton-Fry M, Miller PF: Pharmacokinetics and whole blood bactericidal activity against Mycobacterium tuberculosis of single ascending doses of PNU-100480 in healthy volunteers. J Infect Dis 2010;202(5):745-751.

26. Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, Nuermberger E: Promising anti-tuberculosis activity of the oxazolidinone PNU-100480 relative to linezolid in the murine model. Antimicrob Agents Chemother 2008;53(4):1314-1319.

27. Williams KN, Brickner SJ, Stover CK, Zhu T, Ogden A, Tasneen R, Tyagi S, Grosset JH, Nuermberger EL: Addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. Am J Respir Crit Care Med 2009;180(4):371-376.

28. Koh WJ, Kwon OJ, Gwak H, Chung JW, Cho SN, Kim WS, Shim TS: Daily 300 mg dose of linezolid for the treatment of intractable multidrug-resistant and extensively drug-resistant tuberculosis. J Antimicrob Chemother 2009;64(2):388-391.

29. Dietze R, Hadad DJ, McGee B, Molino LP, Maciel EL, Peloquin CA, Johnson DF, Debanne SM, Eisenach K, Boom WH, Palaci M, Johnson JL: Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. Am J Respir Crit Care Med 2008;178(11):1180-1185.

30. Rustomjee R, Diacon AH, Allen J, Venter A, Reddy C, Patientia RF, Mthiyane TC, De Marez T, van Heeswijk R, Kerstens R, Koul A, De Beule K, Donald PR, Mc Neeley DF: Early bactericidal activity and pharmacokinetics of the Diarylquinoline TMC 207 in pulmonary tuberculosis. Antimicrob Agents Chemother 2008;52(8):2831-2835.