OPEN ACCESS Freely available online

PLoS MEDICINE

Essay

Ensuring the Involvement of Children in the Evaluation of New Tuberculosis Treatment Regimens

William J. Burman*, Mark F. Cotton, Diana M. Gibb, A. Sarah Walker, Andrew A. Vernon, Peter R. Donald

Global tuberculosis control is threatened by dramatic increases in HIV-related tuberculosis and by the emergence of multidrug-resistant strains. Highly lethal outbreaks of extensively drug-resistant tuberculosis among HIV-infected persons in South Africa [1] demonstrate the public health emergency that results when these two forces converge in the same setting. Fortunately, at this time of great need, tuberculosis drug development has been roused from its decades-long slumber. New ways of using existing drugs and the development of new drug classes hold great promise for the treatment of both drug-susceptible [2–6] and drug-resistant tuberculosis [4–8].

Children are a critical part of the global tuberculosis pandemic, with an estimated 900,000 cases and 100,000 deaths per year [9]. In high-burden settings, children make up as much as 20% of incident cases of active tuberculosis [9,10]. Furthermore, young children have an increased risk of severe, rapidly progressive forms of tuberculosis, such as disseminated disease and meningitis (Figure 1) [11,12]. Therefore, it is imperative that children benefit from improvements made in tuberculosis treatment. However, children have only been included in one study of these new agents (a phase III trial of once-weekly rifapentine + isoniazid for latent tuberculosis) [13].

Barriers to Involving Children

Although not well articulated in the published medical literature, a number of barriers to the involvement of children have been raised in discussions of tuberculosis drug development (Box 1). Our concern is that these barriers may, once again, lead the field down the path of least resistance—the exclusion of children from tuberculosis drug development efforts.

Box 1. Barriers to Including Children in Tuberculosis Drug Development

- Infrequent transmission of tuberculosis from children to others
- Difficulty of confirming active tuberculosis among children
- Existence of effective therapy for drug-susceptible tuberculosis
- Concerns about pediatric-specific side effects
- Uncertainties about the appropriate time to involve children in drug development and the optimal trial designs for doing so
- Regulatory requirements engendered by the inclusion of children
- Concerns about further subdividing the limited resources available for drug development

What happens when children are not included in drug development. There is a rich history of clinical trials for tuberculosis treatment, beginning with the landmark streptomycin trial [14] and followed by a remarkable series of trials establishing that multidrug therapy could be curative, that it was possible to do so with ambulatory treatment, and that therapy could be shortened from two years to six months [15]. Children were almost completely left out of this series of clinical trials.

The result? Nearly 40 years after the development of short-course treatment in adults, there are still fundamental uncertainties about age-appropriate dosing of isoniazid, rifampicin, pyrazinamide, and ethambutol [16,17]. Children, particularly very young children, do not achieve adequate serum concentrations of these agents when given weight-based dosing based on pharmacokinetic data from adults. The uncertainties about pediatric dosing reflect the lamentable paucity of pharmacokinetic data for first-line drugs in children. Another consequence of the lack of involvement of children in the initial phase of tuberculosis drug development is that only in recent years has there been a substantial effort to manufacture child-friendly formulations of first-line tuberculosis drugs (crushable mini-pills, granules, oral suspensions). A number of controversies in the treatment of pediatric tuberculosis stem from the lack of clinical trials focused on child-specific questions

The Essay section contains opinion pieces on topics of broad interest to a general medical audience.

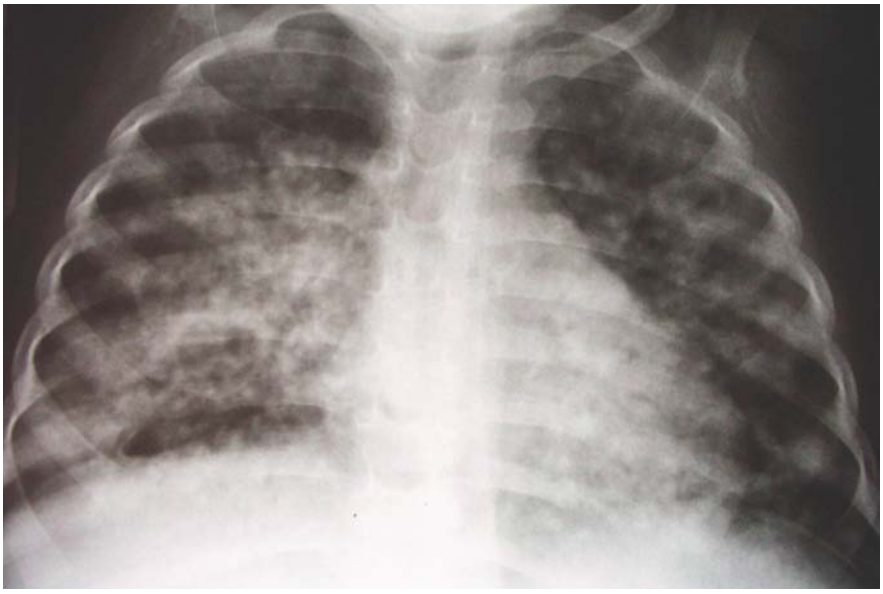
Funding: The authors received no specific funding for this article.

Competing Interests: The authors have declared that no competing interests exist.

Citation: Burman WJ, Cotton MF, Gibb DM, Walker AS, Vernon AA, et al. (2008) Ensuring the involvement of children in the evaluation of new tuberculosis treatment regimens. PLoS Med 5(8): e176. doi:10.1371/journal.pmed.0050176

William J. Burman is with Denver Public Health and the University of Colorado Health Sciences Center, Denver, Colorado, United States of America. Mark F. Cotton and Peter R. Donald are with the Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, Western Cape, South Africa. Diana M. Gibb and A. Sarah Walker are with the Medical Research Council Clinical Trials Unit, London, United Kingdom. Andrew A. Vernon is with the Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America.

* To whom correspondence should be addressed. E-mail: bburman@dhha.org



doi:10.1371/journal.pmed.0050176.g001

Figure 1. Chest Radiograph of an Infant with Pulmonary Tuberculosis, Complicated by an Immune Reconstitution Syndrome Event Following the Initiation of Antiretroviral Therapy

(e.g., the optimal duration and dosing frequency of tuberculosis treatment, how to ensure safe use of ethambutol in children). There are even greater uncertainties about less common therapeutic questions (e.g., the treatment or prevention of drug-resistant disease).

The history of tuberculosis drug development reflects the lack of involvement of children and the consequences of that omission. Concerted efforts are necessary to avoid repeating this unfortunate experience in this era of renewed interest in tuberculosis drug development.

The example of antiretroviral drug development. Antiretroviral drugs have been evaluated among children, age-specific pharmacokinetic data obtained, and child-friendly formulations developed and marketed. Throughout this process, the evaluation of antiretroviral drugs for children has not lagged far behind their development and licensure for adults. As a result, age-appropriate regimens are available in a range of formulations for children, and the rates of HIV-related morbidity and mortality decreased in concert for children [18–20] and adults [21]. The keys to this success were advocacy [22], earmarked funding for pediatric research, focused clinical trial designs on questions whose answers could not be extrapolated from research in adults, and incentives for the pharmaceutical industry to include children in drug development.

Overcoming the Barriers to the Involvement of Children

The first and perhaps the most important step toward involving children in tuberculosis drug development is to clearly articulate the necessity of doing so. To shine a light on the path of least resistance is to show how clearly unacceptable it is; children have the same right to benefit from research as do adults. Researchers, regulatory agencies, advocates, and government agencies and private foundations that fund drug development must insist that the development pathways for all new agents/regimens include specific plans for when and how children will be involved.

Once agreement has been reached on the necessity of including children in trials of new tuberculosis treatment regimens, the specific barriers to the involvement of children must be identified and then overcome (Table 1). The difficulty of culture confirmation of active tuberculosis among children is well known [17]. Because a positive culture is both an enrollment criterion and the primary endpoint of phase II and III clinical trials for tuberculosis treatment, some observers have concluded that tuberculosis treatment trials cannot be done among children. This limitation is quite real—because of it, pediatric trials are not the setting for the definitive evaluation of the efficacy of a new drug or treatment regimen. However, it does not mean that treatment trials cannot be done among children.

Improved specimen collection techniques (e.g., induced sputum and string test) can provide culture confirmation in a higher percentage of pediatric patients than previously thought possible [23–26]. Furthermore, case definitions for culture-negative pediatric tuberculosis can be used in clinical trials, as criteria both for enrollment and for evaluating efficacy [27,28]. While imperfect, these case definitions can be applied by an events committee blinded to treatment assignment to ensure unbiased assessment of diagnosis and response to treatment.

Although concerns about pediatric-specific side effects have led some to argue against inclusion of children in clinical trials, these concerns fail to recognize that new drugs will be used off-label among children, in the absence of data on pediatric pharmacokinetics and tolerability, as soon as they are approved for adults. Children are indeed vulnerable participants in research because of their inability to provide fully informed consent. However, an overzealous attempt to protect some children from the possible harms of research perversely causes harm, by either denying access to treatment or through exposing children to the risks of inappropriate dosages of new medications.

Effective therapy is available for drug-susceptible tuberculosis. However, the limitations of current first-line tuberculosis treatment should be recognized. Despite the appeal of our current nomenclature of "short-course therapy," a six-month treatment duration leads to worrisome numbers of patients who do not complete treatment in many programmatic situations [29–32]. The side effects of current regimens are appreciable as well: high rates of bothersome side effects, such as nausea and vomiting, and substantial rates of serious adverse events, such as hepatotoxicity [33]. In spite of the availability of effective therapy for drug-susceptible tuberculosis, new agents should still be evaluated among

Table 1. Summary of Barriers to the Involvement of Children in Tuberculosis Drug Development and Suggested Ways to Overcome Them

Barrier	Ways In Which Barriers Can Be Overcome
The difficulty of diagnosing active tuberculosis in children	Clinical trials of new drugs/regimens can proceed using validated case definitions for active disease, confirmed by blinded events review committees, even though many of the participants will not have culture-confirmed tuberculosis
Lack of pharmacokinetic data from children and the difficulty of performing pharmacokinetic sampling in young children	Validation of methods for doing pharmacokinetic studies using more easily obtained, very low-volume samples Identification of local sites that already have the capacity to perform such studies
Unwarranted complacency about the effectiveness of therapy for children with drug-susceptible tuberculosis	Initial evaluation of novel drug classes among children with proven or suspected drug-resistant tuberculosis Ensure that pharmacokinetics and tolerability of new drugs for drug-susceptible tuberculosis are evaluated among children while their efficacy is being investigated among adults
Trial design issues (endpoints, appropriate sample sizes)	Collaboration with pediatric trialists in the antiretroviral and cancer treatment fields
Concerns about pediatric-specific side effects	Appropriate monitoring during clinical trials involving children Recognition that drug toxicity may be less frequent among children
Concern that involving children in tuberculosis drug development will complicate the research oversight and regulatory aspects of clinical trials	Involvement of children in drug development is a requirement of some funding agencies (e.g., US National Institutes of Health) Provision of incentives by some regulatory authorities (e.g., US Food and Drug Administration and EU European Medicines Agency) to encourage the involvement of children in research in particular areas
Concerns about diffusing the very limited funds for tuberculosis drug development into too many areas (dividing an already small research pie into too many pieces)	Use of the involvement of children in tuberculosis drug development as a basis for more effective advocacy, and a way to increase overall funding for the field ("grow the pie")

doi:10.1371/journal.pmed.0050176.t001

children; there is much room for improvement in "short-course therapy."

At what points in tuberculosis drug development should studies be undertaken in children? This crucial question requires discussion among investigators, the pharmaceutical industry, advocates, and regulatory officials. As a starting point for such discussions, we offer initial suggestions in Table 2. If children are to be involved at specific points in drug development, appropriate timelines are needed for initial work on formulations and pharmacokinetic studies among children. It is inappropriate to wait until the drug development plan for a new drug or regimen has been completed in adults before beginning its evaluation in children.

What kinds of trials should be undertaken among children? Not all phase III trials in adults need repeating in children; it is highly likely that children will respond well to a new regimen if given a drug formulation and dose that achieves pharmacokinetic parameters comparable to those among adults. Questions that are specific to children, or where answers from adults are unlikely to extrapolate to children, require separate evaluation. Key examples of studies that must be done among children are those assessing the pharmacokinetics and tolerability of new drugs [17].

It can be difficult to obtain blood specimens by venipuncture from very young and very ill children. This challenge should not preclude pharmacokinetic studies of new antituberculosis drugs among children; more efficient study designs and techniques for use of ultra-small quantities of blood can overcome this limitation. Sparse sampling schemes analyzed with Bayesian statistical methods that incorporate pharmacokinetic data from adults, in addition to knowledge about maturation of metabolic pathways in children, facilitate the design and implementation of pharmacokinetic studies in children [34].

Table 2. Suggested Types of Research Activity among Children by the Stage of Clinical Trial Efforts among Adults for a New Antituberculosis Drug/Regimen

Clinical Trial Phase among Adults	Suggested Research Activities among Children
I (Single and multiple-dose PK and tolerability among healthy adults)	None
IIa (Early bactericidal activity and PK—patients with tuberculosis)	Initial work on possible formulations for young children
IIb (Sputum culture conversion over the first 2–4 months of therapy)	Initial PK studies among children with tuberculosis
III (Randomized trial with tuberculosis outcomes as the primary endpoint)	Randomized comparison of the new drug/regimen with PK and tolerability as primary endpoints; efficacy as a secondary endpoint Expanded access (compassionate use) protocol for children with known or suspected drug-resistant disease and poor treatment options
IV (Further evaluation of a regimen shown to be effective in an initial phase III trial)	Additional studies among key subgroups of children—those <3 years old, those with central nervous system involvement Validation of PK of formulations and doses chosen for clinical practice

PK, pharmacokinetics

doi:10.1371/journal.pmed.0050176.t002

An instructive example of optimizing methodologies for pharmacokinetic studies for children comes from malaria research. Severe malaria is predominantly a disease of young children, so it is critical that the pharmacokinetics of antimalarial drugs among infants and young children be well understood. In these studies, very small samples of blood (100 microliters) obtained by finger- or heel-stick are blotted onto filter paper, allowed to dry, stored at room temperature, and later used to determine concentrations of antimalarial drugs [34,35]. Thus, pharmacokinetic studies can be extended to infants and young children, and such samples can be obtained under field conditions where the disease is common. The extension of these techniques to the study of new antituberculosis drugs requires further research, but the challenge of pharmacokinetic sampling among young children calls for this kind of innovation.

There are many regulatory steps between the development of a study protocol and its implementation at study sites. Some have expressed concern that involving children in drug development will slow down the already lengthy timeline for study implementation by triggering more rigorous regulatory review. This concern may be well founded. However, key funding and regulatory agencies have policies and incentives to encourage evaluation of new drugs among children for conditions, like tuberculosis, that are common among that age group (the US National Institutes of Health requires specific justification if children are not included in a study). Some of those incentives, such as extended patent protection for the United States market, are unlikely to encourage tuberculosis drug development efforts for children. However, the US Food and Drug Administration has recently been authorized to take additional steps to promote the inclusion of children in drug development [36], and the European Union now requires that any new drug that could potentially be used in children have a Pediatric Implementation Plan for the drug to be licensed in adults [37]. Other groups are developing alternative incentive structures for diseases of poverty, such as tuberculosis [38].

Finally, there is the concern that including children in clinical trials will dilute the already inadequate funding for tuberculosis drug development [39], thus slowing down the pathway to licensure of new drugs. Proponents of this zero-sum argument may be willing to face difficult facts, but risk fostering the continued existence of an unacceptable situation. The expansion of antiretroviral therapy and the treatment of multidrug-resistant tuberculosis in high-burden settings are examples of two interventions which were said to be inadvisable, based on zero-sum arguments, but which have now been shown to be both feasible and critical for disease control [40–42]. Rather than being a detriment to funding for tuberculosis drug development, the inclusion of children may draw funding to the field.

Summary

We are on the threshold of revolutionary improvements in the treatment of tuberculosis. Within five to ten years, it is likely that highly effective three-month regimens will be available to treat both active and latent drug-susceptible tuberculosis. New drug classes that have the potential to dramatically improve the treatment of multidrug-resistant tuberculosis are entering clinical trials. Children have the same right as adults to benefit from research with these new treatments. By making a deliberate choice to avoid the path of least resistance, we can ensure that both adults and children benefit from these advances in tuberculosis treatment. ■

Acknowledgments

The authors thank Leonard Sacks, David McNeeley, and William Mac Kenzie for helpful discussions on this topic.

Author contributions. WJB wrote the initial draft of this essay and incorporated suggestions from other authors into the final version. MFC, DMG, ASW, and AAV revised the initial draft of the essay and provided references. PRD revised the initial draft of the essay and provided background on how children might best be included in studies of new drugs for tuberculosis.

References

1. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, et al. (2006) Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368: 1575-1580.
2. Rosenthal IM, Zhang M, Williams KN, Peloquin CA, Tyagi S, et al. (2007) Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. PLoS Med 4: e344. doi:10.1371/journal.pmed.0040344
3. Chapuis L, Ji B, Truffot-Pernot C, O'Brien RJ, Raviglione MC, et al. (1994) Preventive therapy of tuberculosis with rifapentine in immunocompetent and nude mice. Am J Respir Crit Care Med 150: 1355-1362.
4. Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, et al. (2006) OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med 3: e466. doi:10.1371/journal.pmed.0030466
5. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, et al. (2000) A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 405: 962-966.
6. Nikonenko BV, Protopopova M, Samala R, Einck L, Nacy CA (2007) Drug therapy of experimental tuberculosis (TB): Improved outcome by combining SQ109, a new diamine antibiotic, with existing TB drugs. Antimicrob Agents Chemother 51: 1563-1565.
7. Lounis N, Veziris N, Chauffour A, Truffot-Pernot C, Andries K, et al. (2006) Combinations of R207910 with drugs used to treat multidrug-resistant tuberculosis have the potential to shorten treatment duration. Antimicrob Agents Chemother 50: 3543-3547.
8. Ragno R, Marshall GR, Di Santo R, Costi R, Massa S, et al. (2000) Antimycobacterial pyrroles: synthesis, anti-*Mycobacterium tuberculosis* activity and QSAR studies. Bioorg Med Chem 8: 1423-1432.
9. Nelson LJ, Wells CD (2004) Global epidemiology of childhood tuberculosis. Int J Tuberc Lung Dis 8: 636-647.
10. Marais BJ, Graham SM, Cotton MF, Beyers N (2007) Diagnostic and management challenges for childhood tuberculosis in the era of HIV. J Infect Dis 196 (Suppl 1): S76-S85.
11. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Enarson DA, et al. (2006) The spectrum of disease in children treated for tuberculosis in a highly endemic area. Int J Tuberc Lung Dis 10: 732-738.
12. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, et al. (2004) The natural history of childhood intra-thoracic tuberculosis: A critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis 8: 392-402.
13. Tuberculosis Trials Consortium (2008) TBTC Study 26: Weekly RFP/INH for 3 mo. vs. daily INH for 9 mo. for the treatment of LTBI. Available: http://www.clinicaltrials.gov/ct2/show/NCT00023452?term=tuberculosis%2C+rifapentine&rank=5. Accessed 17 July 2008.
14. Medical Research Council (1948) Streptomycin treatment of pulmonary tuberculosis. BMJ 1: 769-782.
15. Fox W, Ellard GA, Mitchison DA (1999) Studies on the treatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946-1986, with relevant subsequent publications. Int J Tuberc Lung Dis 3: S231- S279.
16. Schaaf HS, Parkin DP, Seifart HI, Werely CJ, Hesseling PB, et al. (2005) Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. Arch Dis Child 90: 614-618.
17. Donald PR (2007) The assessment of new anti-tuberculosis drugs for a paediatric indication. Int J Tuberc Lung Dis 11: 1162-1165.
18. Gibb DM, Duong T, Tookey PA, Sharland M, Tudor-Williams G, et al. (2003) Decline in mortality, AIDS, and hospital admissions in perinatally HIV-1 infected children in the United Kingdom and Ireland. BMJ 327: 1019.
19. Bolton-Moore C, Mubiana-Mbewe M, Cantrell RA, Chintu N, Stringer EM, et al. (2007) Clinical outcomes and CD4 cell response in

children receiving antiretroviral therapy at primary health care facilities in Zambia. JAMA 298: 1888-1899.
20. Gortmaker SL, Hughes M, Cervia J, Brady M, Johnson GM, et al. (2001) Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 345: 1522-1528.
21. Palella F Jr, Delaney K, Moorman A, Loveless M, Fuhrer J, et al. (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 338: 853-860.
22. Havens PL, Gibb DM (2007) Increasing antiretroviral drug access for children with HIV infection. Pediatrics 119: 838-845.
23. Zar HJ, Tannenbaum E, Apolles P, Roux P, Hanslo D, et al. (2000) Sputum induction for the diagnosis of pulmonary tuberculosis in infants and young children in an urban setting in South Africa. Arch Dis Child 82: 305-308.
24. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, et al. (2006) The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis 42: e69-e71.
25. Chow F, Espiritu N, Gilman RH, Gutierrez R, Lopez S, et al. (2006) La cuerda dulce—A tolerability and acceptability study of a novel approach to specimen collection for diagnosis of paediatric pulmonary tuberculosis. BMC Infect Dis 6: 67.
26. Vargas D, Garcia L, Gilman RH, Evans C, Ticona E, et al. (2005) Diagnosis of sputum-scarce HIV-associated pulmonary tuberculosis in Lima, Peru. Lancet 365: 150-152.
27. Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Lombard C, et al. (2006) A refined symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics 118: e1350-e1359.
28. Zar HJ, Cotton MF, Strauss S, Karpakis J, Hussey G, et al. (2007) Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: Randomised controlled trial. BMJ 334: 136.
29. Lindtjorn B, Madebo T (2001) The outcome of tuberculosis treatment at a rural hospital in southern Ethiopia. Trop Doct 31: 132-135.
30. Tanguis HG, Cayla JA, Garcia de Olalla PG, Jansa JM, Brugal MT (2000) Factors predicting non-completion of tuberculosis treatment among HIV-infected patients in Barcelona (1987-1996). Int J Tuberc Lung Dis 4: 55-60.
31. Radilla-Chavez P, Laniado-Laborin R (2007) Results of directly observed treatment for tuberculosis in Ensenada, Mexico: Not all DOTS programs are created equally. Int J Tuberc Lung Dis 11: 289-292.
32. Hesseling AC, Schaaf SH, Westra AE, Werschkull H, Donald PR, et al. (2005) Outcome of HIV-infected children with culture-confirmed tuberculosis. Arch Dis Child.
33. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, et al. (2003) Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. Am J Respir Crit Care Med 167: 1472-1477.
34. Meibohm B, Laer S, Panetta JC, Barrett JS (2005) Population pharmacokinetic studies in pediatrics: Issues in design and analysis. AAPS J 7: E475-E487.
35. Barnes KI, Lindegardh N, Ogundahunsi O, Olliaro P, Plowe CV, et al. (2007) World Antimalarial Resistance Network (WARN) IV: Clinical pharmacology. Malar J 6: 122.
36. United States Congress (2007) Food and Drug Administration Amendments Act of 2007. HR 3580. 110th Cong. Available: http://www.fda.gov/oc/initiatives/HR3580.pdf. Accessed 17 July 2008.
37. European Medicines Agency (2007) The EU Paediatric Regulation. Available: http://www.emea.europa.eu/htms/human/paediatrics/regulation.htm. Accessed 17 July 2008.
38. World Health Organization (2006) Intergovernmental Working Group on Public health, Innovation and Intellectual Property. Available: http://www.who.int/mediacentre/events/2006/intellectual.property.meeting/en/index.html. Accessed 17 July 2008.
39. Feuer C (2007) Tuberculosis research and development: A critical analysis of funding trends, 2005-2006. Treatment Action Group. Available: http://www.aidsinfonyc.org/tag/tbhiv/tbrandd/2007tbranddreport.pdf. Accessed 17 July 2008.
40. Mitnick C, Bayona J, Palacios E, Shin S, Furin J, et al. (2003) Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. N Engl J Med 348: 119-128.
41. Badri M, Maartens G, Mandalia S, Bekker LG, Penrod JR, et al. (2006) Cost-effectiveness of highly active antiretroviral therapy in South Africa. PLoS Med 3: e4. doi:10.1371/journal.pmed.0030004
42. Fairall LR, Bachmann MO, Louwagie GM, van Vuuren C, Chikobvu P, et al. (2008) Effectiveness of antiretroviral treatment in a South African program: A cohort study. Arch Intern Med 168: 86-93.