CONTRIBUTIONS to SCIENCE, **8** (2): 181–186 (2012)
Institut d'Estudis Catalans, Barcelona
DOI: 10.2436/20.7010.01.154 ISSN: 1575-6343
www.cat-science.cat

Global Implications of Personalised Medicine

Will personalised medicine be the key to eradicating TB?*

Pere-Joan Cardona
Experimental Tuberculosis Unit, Germans Trias i Pujol Health Sciences Research Institute, Barcelona
Archivel Farma, Barcelona

Resum. Cada any dos milions de persones moren de tuberculosi. Gairebé un terç de la població mundial està infectada, i un 10 % d'aquest grup desenvoluparà la malaltia. Els dos objectius principals del tractament de la tuberculosi són la prevenció de la resistència i el tractament de totes les poblacions de bacils. Però l'aparició de soques de tuberculosi multiresistent i tuberculosi extremament resistent s'està convertint en un problema cada vegada més preocupant, i, perquè els tractaments s'han de perllongar, en una enorme càrrega per als sistemes sanitaris. Des d'aquesta perspectiva, la medicina personalitzada pot millorar el tractament dels pacients amb tuberculosi. Mitjançant l'ús de proves genètiques que detecten polimorfismes específics podem identificar els pacients que responen més bé als fàrmacs disponibles, i considerar teràpies alternatives per als que no hi responen tan bé. La investigació continuada en biomarcadors donarà suport a la medicina personalitzada en el tractament de malalties infeccioses, especialment de la tuberculosi, una malaltia per la qual els tractaments disponibles actualment tenen moltes limitacions.

Paraules clau: medicina personalitzada · tuberculosi multiresistent (MDR-TB) · tuberculosi extremament resistent (XDR-TB) · teràpia d'observació directa de breu duració (DOTS) · polimorfisme gen SLCO1B1 · polimorfisme gen *LTA4H*

Summary. Every year, 2 million people die from tuberculosis. Nearly one third of the world's population is already infected, and 10 % of this group will go on to develop TB. The two basic goals of TB treatment are the avoidance of resistance and the treatment of all bacilli populations. But the appearance of multiple drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) strains is becoming an increasingly troublesome issue, and because treatments have to be prolonged, and enormous burden for health systems. From this perspective, personalised medicine can improve the treatment of patients with TB. By using genetic testing to detect specific polymorphisms and identify patients that best respond to the available drugs, and to consider therapeutic alternatives for those who are not. Further research for biomarkers will support personalised medicine in the treatment of infectious diseases, especially TB, where we are already confronted with the many limitations of the currently available treatments.

Keywords: personalised medicine · multiple drug resistant TB (MDR-TB) · extensively drug resistant TB (XDR-TB) · directly observed therapy- short course (DOTS) · *SLCO1B1* gene polymorphism · *LTA4H* gene polymorphisms

Introduction

At first glance, you may ask what do personalised medicine and tuberculosis (TB) have to do with each other? When I was first invited to give a talk at the 2012 EPTA annual meeting, I asked myself the same question. As a scientist, I have two jobs: carrying out research at the Experimental Tuberculosis Unit at the Germans Trias i Pujol Hospital, a public institution, but also working for the pharmaceutical industry in the development of a TB vaccine. Thus, in the common view of academia, I have 'crossed to the dark side,' meaning that I have come under the spell of the power of money and am under the control of private hands, a less 'pure' state than that of a true academic. But I think that the private sector is the way of the future. Indeed, I have gone so far as to try and convince my colleagues that it is only through the private route that one's scientific ideas and results can be transformed into direct benefits to society.

From this perspective, personalised medicine can strongly influence the prognosis of patients with TB, a disease of global incidence with a high mortality, mostly in Africa and Asia but also in first world countries. Every year, 2 million people die from TB; there are 10 million new cases of the disease and

* Based on the lecture given by the author at the Parliament of Catalonia, Barcelona, on 23 October 2012 for the annual conference of the EPTA network, 'From genes to jeans: challenges on the road to personalised medicine.'

Correspondence: P-J Cardona, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Ctra. De Can Ruti, Camí de les Escoles s/n, E-08916, Badalona, Catalonia, EU. Tel. +34-934978653. Fax +34-934978654. E-mail: pjcardona@igtp.com

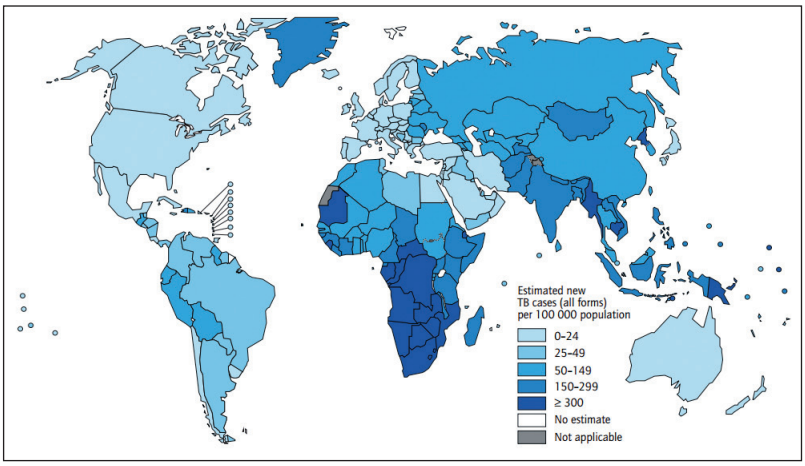

Fig 1. Estimated TB incidence rates, 2011. Source: World Health Organization [10].

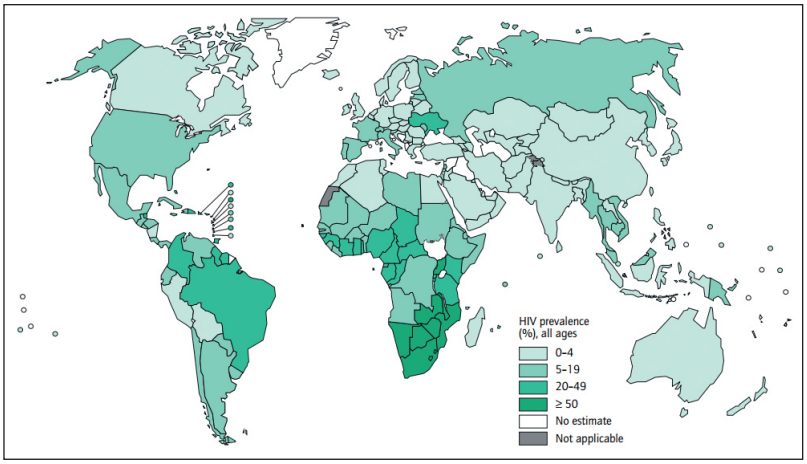

Fig 2. Estimated HIV prevalence in new TB cases, 2011. Source: World Health Organization [10].

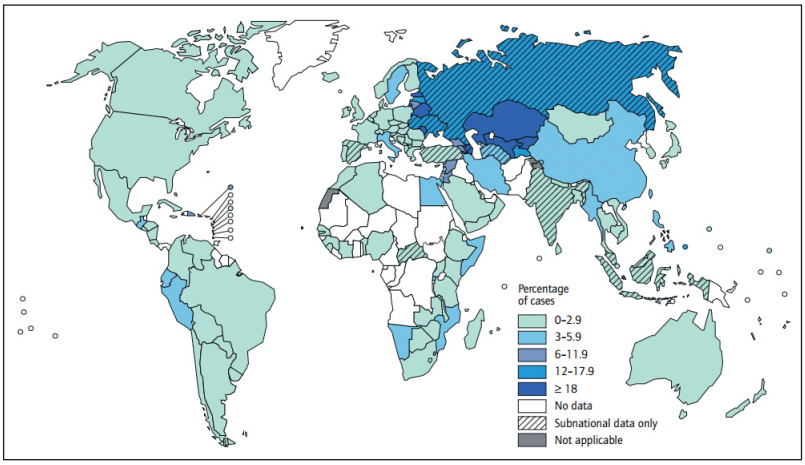

Fig. 3. Percentage of new TB cases with MDR-TB. Figures are based on the most recent year for which data have been reported, which varies among countries. Source: World Health Organization [10].

100 million cases of latent TB infection. In the documented history of TB, it accounts for 1000 million deaths. The problem with TB is that most infections are asymptomatic and latent. Thus, nearly one third of the world's population is already infected, and 10 % of this group will go on to develop TB. More worrying still is that none of these infected individuals are aware that they have the infection.

Figure 1 shows the data for the estimated TB incidence rates for 2011. The highest rates are in Asia and Africa and are very much related to HIV prevalence [10]. Even more alarming is the rate in South Africa, where HIV prevalence is as high as 30% in a population with an incidence of TB infection of more than 300 per 100,000 inhabitants (Fig. 2). That translates to approximately 1 % of the population of South Africa becoming infected with TB every year: half a million people in a population of 50 million is a massive incidence.

The situation is further complicated by the efforts of the World Health Organisation's (WHO) to treat as many cases of active TB as possible. But the treatment of active TB favours the selection of *Mycobacterium tuberculosis* strains with multi-drug resistance. Thus, while every year approximately 80 % of the 10 million cases of TB are treated, at the same time half a million people will develop multi-drug resistant TB (MDR-TB) (Fig. 3, Table 1). This is a frightening situation because TB

Table 1. Definitions of TB resistance (WHO/CDC 2006)

Multidrug-resistant TB (MDR-TB)	Resistance to isoniazid and rifampicin, the two most powerful first-line treatment drugs
Extensively drug-resistant TB (XDR-TB)	MDR with resistance to isoniazid and rifampin and at least three of the six main classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicyclic acid)

Table 2. Anti-TB drug classes

should have been eradicated by the year 2000. This was the prediction made in the 1970s and 1980s, when short term chemotherapy of TB consisted of a six-month course of treatment. But TB has become more complicated because of the presence of MDR-TB, which cannot be treated easily.

The areas of the world where MDR-TB is most problematic are the Baltic countries and Russia [9]. Despite adequate healthcare systems, treatment of these patients often fails because of logistical problems. Moreover, in cases in which the drug combination has not been efficacious, there has been a huge increase in MDR-TB, which now threatens to spread to other countries. Worse still are the extremely drug resistant strains, which are resistant to the main TB drugs available, isoniazid and rifampicin, and to other first-line drugs such as aminoglycosides. In India, for example, totally drug resistant strains or extensively drug resistant TB (XDR-TB) strains have been detected. For these patients, there is no available treatment other than surgery, in which diseased portions of the lungs are removed; but these patients will suffer from chronic TB for the rest of their lives.

Around the world, the number of countries that have reported at least one case of XDR-TB is growing. Resistance too, and thus mortality, is an increasingly troublesome issue, one that is not far from Europe (Fig. 4). Of particular concern are the countries of the former Soviet Union, where mortality is as high as 10 per 100,000 inhabitants [3]. But there are places in Western Europe where there is also cause for concern. For example in different quarters of London, there are areas with an alarming incidence of TB.

In Spain, immigration from African countries such as Algeria and Morocco plays a role in the overall incidence. In Barcelona, in the Raval quarter, located in the centre of the city, the incidence of TB is of 120 per 100,000 inhabitants. In other words, the risk of infection is 50 times higher than in any other quarter of the city. Fortunately, there are health programs that are especially vigilant in this and other vulnerable urban areas. The Catalan Government and the City Council have invested enormous sums of money to create an effective program to combat TB in Barcelona. Thanks to these efforts, we can monitor the evolution of TB in this populous city, where during the 1980s the high incidence mostly involved HIV-positive injection-drug users (HIV-IDU) and especially prison inmates. Since 1990, there has been an approximate annual decline of 10 %, reflecting lower rates of HIV-IDU among prison inmates, and the treatment of HIV with highly active retroviral therapy (HAART); thus, today, the incidence of TB is more closely related to immigration [1].

Not surprisingly, TB is also very much related to poverty. Figure 5 shows the relationship between the incidence of TB and individual income. According to predictions for the year 2030, this trend will continue to increase [4]. However, as noted above, the paradox is that the only strategy against TB, which is to treat the disease, leads to an even worse problem, the promotion of MDR-TB. To address this problem, the WHO has set up a TB control strategy, referred to as directly observed therapy – short course (DOTS), that combines five components:

1. Government commitment, including political will at all levels, and the establishment of a centralised and prioritised system of TB monitoring, recording, and training.
2. Case detection by sputum smear microscopy.
3. A standardised treatment regimen, with adherence directly observed by a healthcare worker or community health worker for at least the first two months.
4. A stable drug supply.
5. A standardised recording and reporting system that allows the assessment of treatment results. [11]

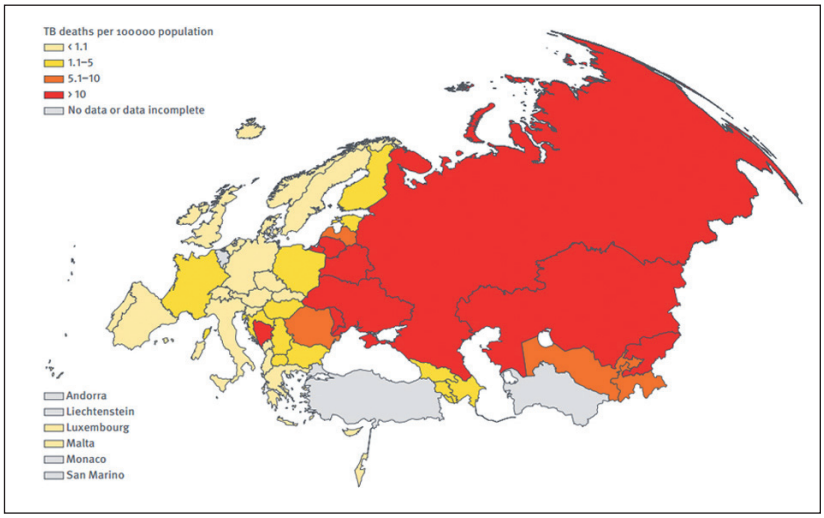

Fig. 4. TB mortality rates, Europe, 2001–2005. Source: European Centre for Disease Prevention and Control/ WHO Regional Office for Europe [3]

Table 2. Anti-TB drug classes

First-line anti-TB drug	Second-line anti-TB drugs	Third-line anti-TB drugs
Group 1 *Oral* isoniazid (H/Inh) rifampicin/rifampin (R/Rif) pyrazinamide (Z/Pza) ethambutol (E/Emb) rifapentine (P/Rpt) rifabutin (Rfb)	Group 2 *Injectable aminoglycosides* streptomycin (S/Stm) kanamycin (Km) amikacin (Amk) *Injectable polypeptides* capreomycin (Cm) viomycin (Vim)	Group 5 clofazimine (Cfz) linezolid (Lzd) amoxicillin plus clavulanate (Amx/Clv) imipenem plus cilastatin (Ipm/Cln) clarithromycin (Clr)
	Group 3 *Oral and injectable fluoroquinolones* ciprofloxacin (Cfx) levofloxacin (Lfx) moxifloxacin (Mfx) ofloxacin (Ofx) gatifloxacin (Gfx)	
	Group 4 *Oral* para-aminosalicylic acid (Pas) cycloserine (Dcs) terizidone (Trd) ethionamide (Eto) prothionamide (Pto) thioacetazone (Thz) linezolid (Lzd)	

However, this implies that, for the six months of chemotherapy, a healthcare worker must go to the residence of each patient in order to administer the medication. Obviously, this has huge logistical costs, but it is the only way to avoid MDR-TB. The two basic goals of TB treatment are the avoidance of resistance and the treatment of all bacilli populations. Table 2 shows the basis of TB treatment with the available first-line, second-line and third-line drugs [12]. Each TB lesion contains up to 10^9 bacilli, so a combination of these drugs is needed to avoid the development of spontaneous bacterial mutation. The administered drugs must have bactericidal activity, with the most effective agents being rifampicin and isoniazid. But, for instance, imagine an infection with 10^9 bacilli in which 10^2 bacilli are already resistant to isoniazid (INH). If the patient is treated with INH, the main bulk of the bacilli load will be eradicated but at the same time the resistant strain is spared and goes on to reproduce and to perpetuate the disease. The main problem is not the continuous growth of the TB bacilli, which are in fact susceptible to INH, but their low rate of INH metabolism. This means that long term treatment is required to kill these bacilli. Thus, in order to treat TB, four drugs, INH, rifampicin, pyrazinamide, and ethambutol, are initially administered for 6 months to massively reduce the amount of replicating bacilli, followed by another four months of INH and rifampicin to complete treatment [5].

What happens if the patient is infected with a MDR-TB strain? If INH is no longer effective, then neither is rifampicin and treatment with the other drugs must be prolonged, up to 21 months. This is an enormous burden for health systems. Worse still, is that the main antibiotic available in such cases is an aminoglycoside, which is mostly delivered intramuscularly. So patients must be treated intramuscularly for three months every day, in order to control the bacillary load, followed by approximately 18 more months of non-injectable anti-TB drugs, to make treatment more affordable [2]. In other words, there is a problem with the six-month treatment that can only be solved by 21 months of treatment. Furthermore, the number of highly drug-resistant (HDR) strains of TB is expected to increase.

Personalised medicine and TB

Regarding personalised medicine and TB, there is good and bad news. The bad news is that it is no longer clear whether treatment adherence is the key to the effective eradication of active TB. Data from experiments in which different treatments

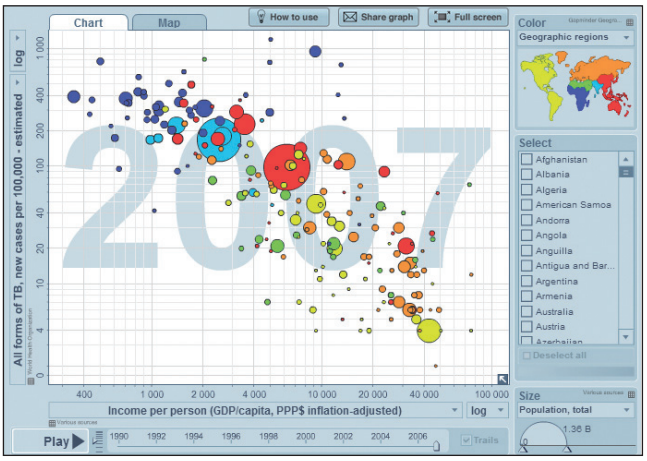

Fig. 5. Relationship between the number of new cases of TB and individual income. Source: Gapminder [www.gapminder.com].

in different types of patients were simulated showed that even if adherence was moderate, there was still an adequate reduction of the bacillary load [7]. Thus, DOTS, may not be the only option. The clue actually lies in the pharmacokinetics of the various drugs, i.e., the fate of these drugs in the body. Even with the best DOTS, there are differences between patients in the degree of destruction of the bacillary load, which can only be explained by pharmacokinetics. Again, this is a frightening consideration because it includes the two aforementioned critical drugs, INH and rifampicin.

People can be grouped as slow or rapid INH acetylators, depending on the rate at which the drug is inactivated. This is crucial to achieve peaks of INH concentration. Low acetylators have larger peaks of the drugs than rapid acetylators [6]. This difference translates into a difference in drug response. In a patient who is a rapid acetylator, a higher dose would probably be needed. Slow vs. rapid INH acetylators can be determined genetically. Yet, although the existence of this gene has long been known and the benefits of genetic testing in the treatment of TB are clear, it is rarely performed. Similarly, certain common variants (polymorphisms) in the *SLCO1B1* gene mean that some patients have higher peaks of rifampicin and that the drug will be more effective in some patients than in others. Both of these examples demonstrate how personalised medicine can improve the treatment of patients with TB, by using genetic testing to identify drug responders and to consider the therapeutic alternatives for those who are not.

The good news is related to the natural history of TB. Approximately 90 % of the individuals who are infected with *M. tuberculosis* have latent, asymptomatic infections and only 10 % have active TB. Among the latter, 5 % of the cases occur because the patient is a child or in the setting of AIDS or other risk factors such as diabetes, tobacco smoking, alcohol consumption or air pollution, all of which increase the likelihood of developing TB. And then there is the remaining 5 % of cases, in which the reasons for the development of active TB are unclear. These patients typically develop the disease 10–20 years after being infected, but why the delay occurs is not understood. Immunosuppressed individuals have higher chances of contracting TB, pneumonia and other diseases but this does not completely explain these cases, nor does malnutrition of poverty; instead, a genetic factor is suspected.

A latent infection requires a constant reinfection process, in which the mycobacteria invade and replicate within endosomes of the pulmonary alveolar macrophages, with the subsequent formation of encapsulated granulomas that prevent mycobacterial dissemination. Many of the granulomas persist in a balanced state. Thus, granulomas typically drain into the alveolar fluid and then into the gastrointestinal tract, but in progressive disease bacilli are released into the airways after cavitation of the granulomas and are able to re-infect the lungs. However, there are also local mechanisms that prevent constant re-infection. Thus, in addition to encapsulation the development of new lesions is controlled over time such that their formation occurs at a low rate. Latent infections are usually not treated at all, because this would imply treating some 2000 million people, which is simply not practical.

The risk of having active TB is higher soon after infection. In the active stage, new lesions form constantly, which increases the likelihood that they involve the upper lobes, where cavities are generated. In fact, in adults active disease tends to focus on the upper lobes, probably because of the high oxygen pressure, which favours bacterial growth, and the less dense capillary network, which delays the immune response. This is less frequent in the chronic phase. How the tiny lesions in a latent infection become the huge lesions that lead to cavitation in active TB, with lesions ranging in size from 0.5 mm to 20 mm in diameter, is unknown. Studies in mice models have shown that the lesions coalesce, a process that may be promoted by the less dense capillary network in the upper lobes rather than the reduced oxygen pressure.

There is also a genetic background that favours less capillary density in the upper lobe, thereby reducing drainage and increasing the coalescence of TB lesions. Furthermore, studies in mice have shown that in some cases there is an abundance of neutrophils in the lesions. The excessive inflammation results in the greater induction of active TB. Thus, host hyperactivity may be the clue to the 5 % of previously unexplained cases of TB. Genetically, these individuals have a polymorphism in the gene *LTA4H*, encoding leukotriene-A4 hydrolase. This bifunctional enzyme converts leukotriene A4 to leukotriene B4, causing higher levels of tumour necrosis factor and subsequently excessive inflammation and tissue damage. In people who are homozygous for this polymorphism, TB meningitis is more likely to be fatal [12].

If we were able to identify patients who are high acetylators or who are homozygous for *LTA4H* gene polymorphisms, we could then offer them more specific treatments. Additionally, we now have a biomarker that indicates the risk of conversion from a latent infection to active TB. Further research for biomarkers will support personalised medicine in the treatment of infectious diseases, especially TB, where we are already confronted with the many limitations of the currently available treatments. In a personalised medicine approach, patients could be stratified before they start chemotherapy, in order to avoid ineffective treatments and prevent adverse drug reactions, resulting in more efficient therapy and the reduction of resistance.

References

1. Cayla JA, Orcau A (2011) Control of tuberculosis in large cities in developed countries: an organizational problem. BMC Medicine 9:127
2. Espinal M (2004) What is the “fall and rise” phenomenon and the “sequential regimen” mechanism? In: Friedan TR (ed) Toman’s tuberculosis: case detection, treatment, and monitoring: questions and answers. 2nd ed. World Health Organization, Geneva, Switzerland, pp 200-202
3. European Centre for Disease Prevention and Control/ WHO Regional Office for Europe (2009) Tuberculosis surveillance in Europe 2007. European Centre for Disease Prevention, Stockholm
4. Gapminder [www.gapminder.org]
5. National Institute of Allergy and Infectious Diseases (2012) Drug-Resistant TB Visual Tour [http://www.niaid.nih.gov/topics/tuberculosis/Understanding/WhatIsTB/VisualTour/Pages/firstLine.aspx]
6. Ramachandran G, Swaminathan S (2012) Role of pharmacogenomics in the treatment of tuberculosis: a review. Pharmacogenomics and Personalized Medicine 5:89-98
7. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T (2011) Multidrug-resistant tuberculosis not due to compliance but to between-patient pharmacokinetic variability. J Infect Dis 204:1951-1959
8. Tobin DM, Vary JC Jr, Ray JP, Walsh GS, Dunstan SJ, Bang ND, Hagge DA, Khadge S, King MC, Hawn TR, Moens CB, Ramakrishnan L (2010) The lta4h locus modulates susceptibility to mycobacterial infection in zebrafish and humans. Cell 140:717-730
9. World Health Organization (2011) WHO Report
10. World Health Organization (2012) Global tuberculosis report 2012 [http://www.who.int/tb/publications/global_report/en/]
11. World Health Organization – The five elements of DOTS [http://www.who.int/tb/dots/whatisdots/en/index.html]
12. Zumla A, Nahid P, Cole ST (2013) Advances in the development of new tuberculosis drugs and treatment regimens. Nature Reviews Drug Discovery 12:388-404