Tuberculosis biomarkers discovery: developments, needs, and challenges

Robert S Wallis, Peter Kim, Stewart Cole, Debra Hanna, Bruno B Andrade, Markus Maeurer, Marco Schito, Alimuddin Zumla

Biomarkers are indispensable to the development of new tuberculosis therapeutics and vaccines. The most robust biomarkers measure factors that are essential to the underlying pathological process of the disease being treated, and thus can capture the full effects of many types of interventions on clinical outcomes in multiple prospective, randomised clinical trials. Many Mycobacterium tuberculosis and human biomarkers have been studied over the past decade. Present research focuses on three areas: biomarkers predicting treatment efficacy and cure of active tuberculosis, the reactivation of latent tuberculosis infection, and the induction of protective immune responses by vaccination. Many older, non-specific markers of inflammation, when considered in isolation, do not have sufficient predictive values for clinical use in tuberculosis. Although no new accurate, tuberculosis-specific biomarkers have yet been discovered, substantial progress has been made in some areas. However, the qualification of biomarkers as a surrogate for a clinical endpoint in tuberculosis is very challenging, and, for biomarkers that are non-culture-based, impossible to pursue without the availability of well characterised biobanks containing biospecimens from patients who have had adequate follow-up to establish long-term treatment outcome. We review progress in tuberculosis biomarker development and efforts being made to harness resources to meet future challenges.

Introduction

Over the past 5 years, increased donor, governmental, and corporate investment for the diagnosis, treatment, prevention, and control of tuberculosis have led to substantial advancement in the development of new diagnostics, drugs, and vaccines.1,2 Accelerated drug development is leading to a new portfolio of promising drugs against tuberculosis and regimens for drug-susceptible and drug-resistant disease, some of which are now under evaluation; however, discovery of tuberculosis biomarkers has lagged behind. Over the past decade both human and Mycobacterium tuberculosis biomarker studies have focused on three specific areas of research: biomarkers predicting treatment efficacy and cure of active tuberculosis, the reactivation of latent tuberculosis infection, and the induction of protective immune responses by vaccination. The non-specific markers of inflammation such as C-reactive protein, when considered in isolation, do not have sufficient predictive values for clinical use.

Biomarkers are objective characteristics that indicate a normal or pathogenic biological process, or a pharmacological response to a therapeutic intervention or vaccination. Thus they can provide information about disease status, risk of progression, likelihood of response to treatment or of drug toxicity, and protective immunity after vaccination (panel 1). Biomarkers can be the basis for surrogate endpoints in a clinical trial, replacing typical clinical endpoints that describe how a patient feels, functions, or survives. The biomarker-endpoint association can be shown by trials of antiretroviral therapy in which a biomarker (plasma HIV RNA) forms the basis of a surrogate endpoint (eg, the proportion of patients with undetectable plasma HIV RNA by week 48). The value of such an endpoint lies in its use for the prediction of clinically meaningful events (eg, opportunistic infection or mortality) in short trials with few patients, thus accelerating clinical research.

The most robust biomarkers measure factors that are essential to the underlying pathological process of the disease being treated, and thus can capture the full effects of many types of interventions on clinical outcomes in multiple prospective, randomised clinical trials. This...
proposed highest level of certainty is indicated for month 2 sputum culture status and interferon γ release (table). Prediction of outcomes in natural history (non-interventional) studies confers an intermediate level of certainty. The simple detection of a treatment effect not yet related to a clinical outcome has the lowest level of certainty, because it depends solely on biological plausibility for its interpretation. Biomarkers inevitably overlap with diagnostics, which, by contrast, inform present rather than future health status. Some biomarkers can have a dual role (eg, plasma HIV RNA, which can be used to both diagnose HIV-1 infection and monitor its treatment), whereas others cannot (eg, HIV antibody). In some situations, markers that have confirmed prognostic value when used to assess disease extent before treatment initiation can nonetheless fail as surrogate endpoints when used after treatment has started; they therefore cannot capture the effects of treatment. One such situation is quantitative detection of M tuberculosis DNA in sputum, which correlates with bacterial burden at the time of tuberculosis diagnosis, but, like the acid fast smear, cannot distinguish live from dead bacteria as treatment progresses.18

Several candidate biomarkers derived from M tuberculosis or human inflammatory immune responses have been studied over the past decade (table), describing the reactivation of latent tuberculosis infection, its durable eradication (relapse-free cure) in patients with active disease, and the induction of protective immune responses by vaccination.19-27 With the exception of month 2 culture status, the size of these studies falls far short of research needs. Many older, non-specific markers of inflammation, when used alone, can have insufficient predictive value for clinical use in tuberculosis, although a combination of the biomarkers highlighted in the table has a theoretical potential to help assessment of clinical cure, or risk of relapse or reactivation. For example, high levels of neopterin (a non-specific marker of macrophage activation) that persist despite appropriate tuberculosis treatment are associated with increased risk of relapse or reactivation, but only in people without concomitant HIV-1 infection or other complicating medical conditions.26 Although no new accurate, tuberculosis-specific biomarkers have yet been discovered, substantial progress has been made in some areas since this subject was last reviewed in this journal.27 In this Series paper we discuss this progress.

**Prediction of relapse-free tuberculosis cure**

**Sputum culture**

The introduction of rifampicin and pyrazinamide nearly 50 years ago transformed tuberculosis treatment, allowing the necessary duration of treatment to be shortened from 18 months to 6 months without an increase in the rate of recurrence due to relapse. Tuberculosis treatment is poised for a second such transformation,3 with development underway of several

---

Panel 1: Predictive roles for tuberculosis biomarkers

**Prediction of tuberculosis cure**
- Emergence of resistance
- Recurrence due to relapse
- Drug toxicity

**Prediction of tuberculosis reactivation**
- Progression from primary infection to disease
- Reactivation of latent infection
- Eradication of latent infection

**Prediction of protective immunity**
- Vaccine efficacy
- Adjunctive immunotherapy efficacy
- Recurrence due to reinfection

---

**Table:**

<table>
<thead>
<tr>
<th>Associated outcome</th>
<th>Proposed level of certainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month 2 culture status</td>
<td>III</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis DNA (GeneXpert MTB/RIF assay)</td>
<td>I</td>
</tr>
<tr>
<td>M tuberculosis RNA</td>
<td>I</td>
</tr>
<tr>
<td>Liquid culture time to positive in automated liquid culture</td>
<td>I</td>
</tr>
<tr>
<td>Early bactericidal activity, 7-14 days</td>
<td>I</td>
</tr>
<tr>
<td>Serum colony counts, 1–2 months</td>
<td>I</td>
</tr>
<tr>
<td>PET/CT imaging</td>
<td>I</td>
</tr>
<tr>
<td>Sporotub Ag85</td>
<td>II</td>
</tr>
<tr>
<td>Antiphospholipid antibody</td>
<td>I</td>
</tr>
<tr>
<td>Urine lipoarabinomannan</td>
<td>I</td>
</tr>
<tr>
<td>Urine tuberculosis DNA</td>
<td>I</td>
</tr>
<tr>
<td>Antilamine dehydrogenase antibody</td>
<td>II</td>
</tr>
<tr>
<td>Volatile organic compounds</td>
<td>I</td>
</tr>
<tr>
<td>Interferon γ release assay (ELISPOT or whole blood)</td>
<td>I</td>
</tr>
<tr>
<td>Neopterin</td>
<td>II</td>
</tr>
<tr>
<td>Soluble intercellular adhesion molecule-1</td>
<td>I</td>
</tr>
<tr>
<td>Soluble interleukin-2R</td>
<td>I</td>
</tr>
<tr>
<td>Soluble tumour necrosis factor receptor, granulocyte B</td>
<td>I</td>
</tr>
<tr>
<td>Sputum interferon γ</td>
<td>I</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>I</td>
</tr>
<tr>
<td>Soluble uronokase plasminogen activator receptor</td>
<td>I</td>
</tr>
</tbody>
</table>

(Continues on next page)
promising drugs with entirely novel mechanisms of action. A report qualifying month 2 sputum culture status as a biomarker for relapse has helped with early testing of new regimens containing these compounds.72 However, that analysis, which showed that changes in treatment that affected relapse risk were highly likely to also affect month 2 culture status (p<0·00001), did not directly inform the necessary duration of these new regimens—a potential stumbling block if they are to be efficiently made available to patients in greatest need.

No full synthesis has been done for the role of month 2 culture status as a biomarker predictor of required duration of treatment, although an analysis in which regimens were classified according to inclusion of rifampin and pyrazinamide suggested the problem could be addressed with modelling. Researchers investigated the connection between relapse risk, treatment duration, and month 2 culture status using meta-regression analysis (Wallis RS, unpublished). The model built from this study indicated an upper 80% prediction limit for relapse of less than 10% for a hypothetical phase 3 trial with 680 patients per group for a new regimen with a month 2 culture positive rate of 1%. The finding was proposed to support accelerated regulatory approval of new regimens for patients with drug-resistant tuberculosis.

By contrast with the month 2 endpoint, trials of early bactericidal activity (EBA) of 1–2 weeks in duration have repeatedly failed to capture important differences in clinical outcomes between treatment groups in prospective, randomised controlled trials, because they cannot distinguish between effective versus ineffective treatments (rifampicin plus isoniazid vs isoniazid alone) or regimens requiring 6 months versus 18 months for cure.30,31 The absence of sustained EBA of linezolid contrasts starkly with its ability to cure extensively drug-resistant tuberculosis.29,35 The discrepancy does not seem to be due to variability in measuring sputum colony counts, because independent EBA trials of a similar design have yielded similar results.36 Instead, EBA seems mainly to measure effects of treatment on extracellular mycobacteria in large lung cavities, which seem to play only a small part in relapse.

Automated liquid culture systems such as mycobacterial growth indicator tube are increasingly used worldwide for tuberculosis diagnosis. These standardised systems are attractive platforms for biomarker development, particularly because they routinely report the time interval needed for detection of growth—a variable that is strongly inversely correlated with inoculum size when tested with laboratory stock cultures (Pearson’s correlation coefficient -0·970; p=0·006).77 The correlation is reduced but remains significant when tested with sputum samples obtained during tuberculosis treatment.13-15 This correlation increases the feasibility of quantitative modelling of treatment effects in large clinical trials. Research suggests that the most informative time for this modelling will be during the second month of treatment.16 Questions remain about the optimum methods for specimen collection (pooled over 12–16 h vs spot) and processing (decontamination with sodium hydroxide variably decreases mycobacterial viability).

Other culture-based methods have been described with resuscitation-promoting factors to help with the detection of otherwise non-culturable mycobacteria that become more frequent in sputum as treatment progresses—eg, Mukamolova and colleagues17 used resuscitation-promoting factors in a preliminary study. The prognostic significance of such cultures is unknown. Further studies of the ability to resuscitate or recognise live but dormant non-replicating bacilli, and mechanisms behind relapse, could lead to improvements in existing culture-based detection systems, making them more powerful as possible intermediate endpoints in tuberculosis drug trials.

**Table: Candidate biomarkers in tuberculosis**

<table>
<thead>
<tr>
<th>Associated outcome</th>
<th>Proposed level of certainty†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural killer T cells</td>
<td>Extent of disease at start of treatment**</td>
</tr>
<tr>
<td>Mycobacterial growth inhibition assays</td>
<td>Vaccine effect**</td>
</tr>
<tr>
<td>Interleukin 18</td>
<td>Subsequent active tuberculosis***</td>
</tr>
<tr>
<td>Natural killer and CD4 T cells</td>
<td>Subsequent active tuberculosis***</td>
</tr>
<tr>
<td>Bcl2</td>
<td>Subsequent active tuberculosis***</td>
</tr>
<tr>
<td>Whole blood interferon release assay</td>
<td>Subsequent active tuberculosis***</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Diagnosis of active tuberculosis vs latent infection**</td>
</tr>
<tr>
<td>Proteomics</td>
<td>Diagnosis of active tuberculosis vs other chronic inflammatory diseases**</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Diagnosis of active tuberculosis vs latent disease**</td>
</tr>
<tr>
<td>microRNA</td>
<td>Diagnosis of active tuberculosis vs latent disease**</td>
</tr>
</tbody>
</table>

ELISPOT—enzyme-linked immunosorbent spot assay. *Data do not support a role as biomarker. **High-certainty, capturing differences in clinical outcomes across treatment groups in prospective randomised trials; †—intermediate certainty, predicting differences in outcomes in patients in non-interventional studies; and †—low certainty, biological plausibility without association with clinical outcome.
and pretreatment sputum samples. Studies are in progress to establish whether viable and non-viable *M tuberculosis* bacilli can be distinguished by pretreatment with propidium monoazide, which enters damaged mycobacteria, covalently binding DNA after light exposure, and thus preventing DNA amplification. Other, manual methods to measure viable mycobacteria in sputum based on amplification of RNA have been described that generally correlate well with colony-forming unit counts during early treatment.

**Serological alternatives**

Lipoarabinomannan is a major constituent of the mycobacterial cell wall that can be detected in a patient’s urine by commercial ELISA. Although the sensitivity of this assay is adequate for tuberculosis diagnosis only in patients with far-advanced HIV infection, its performance might be improved by combination with DNA detection or by modification of test variables. Results of a study by Wood and colleagues showed that concentrations of lipoarabinomannan decreased slowly, after 1–2 months of combined tuberculosis and HIV treatment, in a small set of patients with positive urine lipoarabinomannan results at start of treatment. Further studies of lipoarabinomannan as a candidate biomarker might be of interest, especially since new assays with lower detection thresholds could make the approach more widely applicable. Ethambutol acts by blocking lipoarabinomannan synthesis, and so more studies will be needed to establish whether its effects are overestimated by the monitoring of lipoarabinomannan.

**Imaging**

Imaging of lung lesions during treatment by combined PET and CT can influence early tuberculosis drug and regimen development and increase the accuracy of predictions of relapse in individual patients. Combined imaging allows the superposition of two types of data: structural data from CT (which differentiate lesions according to x-ray densities), and functional data from PET (which, when undertaken with 18fluorodeoxyglucose [¹⁸F-FDG], detect metabolic activity of mammalian inflammatory cells). Studies in the rabbit tuberculosis model show distinct types of lesions with different natural histories and different early responses to chemotherapy. ¹⁸F-FDG intensity reaches a maximum in the rabbit model 5 weeks after infection, but stabilises or decreases during the next month as the infection reaches a chronic stage. Individual lesions in the same animal have very different fates at this stage, ranging from complete resolution to striking progression. Chemotherapy with either isoniazid or rifampicin reduces ¹⁸F-FDG uptake and, more slowly, CT lesion density and volume. Findings from one study showed reduced ¹⁸F-FDG uptake after 1 month of tuberculosis treatment in 19 of 21 patients. Of the two PET non-responders, one had delayed sputum culture conversion (still positive at 3 months), and the other was later shown to have lymphoma. Clinical studies are underway to examine the link between changes in PET and CT scans and clinical outcomes during tuberculosis treatment. Despite the high costs associated with this approach, it could be an indicator of drug activity and an aid to dose and regimen selection in early drug trials, and a predictor of relapse risk at the end of treatment in appropriate settings.

**Gene expression profiles**

Changes in tuberculosis-specific gene and protein expression profiles (transcriptomics) could be of value in assessment of the early response to tuberculosis treatment. A study by Berry and colleagues showed that a multitranscript interferon-driven neutrophil signature differentiated patients with tuberculosis from controls, correlated with radiographic extent of disease, and diminished in seven patients after 2 months of ultimately successful tuberculosis treatment. A similar study assessed interferon-driven signatures earlier during treatment in 29 patients. Changes in gene expression levels were readily detectable after 2 weeks of treatment (figure).

A study of 27 patients with pulmonary tuberculosis assayed at diagnosis and during treatment showed significant changes in expression of more than 4000 genes in blood during treatment. Rapid, large-scale changes were detected, with downregulated expression of 1261 genes within the first week, including inflammatory markers such as complement components C1q and C2. Subsequent changes include B-cell signalling pathways. These results indicate the possibility that measurement of host factors such as gene expression profiles during the first few weeks of treatment could be suitable for biomarkers of treatment efficacy and would have benefits for early-phase and mid-phase clinical trials, providing more information about clinical outcome than would quantitative sputum microbiology. Further studies are warranted to assess the prognostic significance of these signatures for tuberculosis reactivation and cure.

**Prediction of reactivation of latent infection**

**Interferon γ release**

In most instances, infection with *M tuberculosis* is contained by the host immune response, preventing the progression to active disease. Tests for latent tuberculosis infection such as the tuberculin skin test and tuberculosis antigen-stimulated interferon γ release assay detect sensitisation to mycobacterial antigens, but do not differentiate between persistent and resolved latent infection. For example, two studies of latent infection treatment with isoniazid did not show a consistent effect on early secretory antigenic target-6 and culture filtrate protein-10-induced interferon γ responses with whole blood culture or enzyme-linked immunoabsorbent spot. As a result, interferon γ release assays cannot
specifically identify those with highest risk of reactivation. In one meta-analysis, the predictive value of a positive interferon γ release assay for subsequent progression to active tuberculosis increased to 8–15% if testing was not restricted to individuals with a positive tuberculin skin test. Findings from some studies have shown substantial increases in interferon γ release shortly before the diagnosis of active tuberculosis, suggesting that a higher threshold for positivity might improve its predictive value, at least in people who are not overtly immunocompromised.

Interleukin 18

The largest prospective study of individuals at high tuberculosis risk (defined as recent household contacts of active tuberculosis cases) who were not overtly immunocompromised identified only 26 incident tuberculosis cases in 2348 contacts over 4312 person-years of follow-up. Most cases occurred within the first year after study entry. Samples of plasma and viable cells were available for analysis to identify predictors of disease progression in more than half of these cases. Increased concentrations of interleukin 18, a marker of innate immune activation, differentiated those who developed tuberculosis from controls, as did significantly higher expression of chemokine (c-c motif) receptor (CCR7) and lower expression of Bcl2 in RNA extracted from blood. Interleukin 18 concentrations are increased in patients with active tuberculosis, in proportion to radiographic extent of disease. In tuberculosis and other chronic inflammatory diseases, interleukin 18 supports T-cell activation and interferon γ production. CC chemokines have an important role in the homing of lymphocytes to sites of inflammation, and are likely to be involved in the early responses to M. tuberculosis infection. These findings support the notion that latent infection reactivation might, at least in some cases, be a relatively slow, gradual process, in which measurable changes in the immune system precede the development of active disease by a long interval. The significance of reduced expression of Bcl2, an inhibitor of apoptosis, is uncertain, since antigen-driven T-cell apoptosis is increased in tuberculosis, both in blood and at the site of infection. Differential Bcl2 expression in monocytes and T cells might account for the contrary findings at gene and cellular levels.

These findings emphasise the importance of innate immunity in defences against tuberculosis and identify a set of biomarkers indicating potential tuberculosis risk. The findings also show the logistical challenges faced in the conduct of prospective, longitudinal studies of the natural history of M. tuberculosis infection. Most such studies have been hampered by identification of relatively more coprevalent cases than incident cases, even in high-risk individuals in tuberculosis households. Such coprevalent cases, sometimes termed subclinical tuberculosis, are uncovered by careful history, chest radiography, and sputum culture using liquid medium; patients generally have reduced symptoms and limited extent of disease compared with index cases. Subclinical cases occur in patients both with and without HIV. The extent to which these cases represent a stable disease phenotype is uncertain, particularly in people with HIV with low CD4 T-cell counts. Data from the pre-chemotherapy era in the USA indicate a distinct natural history in tuberculosis cases with minimal radiographic extent of disease, with two-thirds of individuals showing apparent spontaneous resolution of the illness (so-called

Figure: Changes in gene expression profiles during early tuberculosis treatment

(A) Profile plot of all detectable transcripts (15 837), obtained without any filtering, in the treated patients with active tuberculosis in the South Africa 2011 cohort. Gene expression changes after only 2 weeks of treatment. Normalised expression at 0 months. (B) Temporal molecular response showing the quantitative response to antituberculosis treatment in a 664-gene transcript using linear mixed models (dots represent mean and bars show 95% CI). Reproduced from Bloom and colleagues, by permission of PLoS One.

www.thelancet.com/infection Vol 13 April 2013
arrested tuberculosis) within 3 years. Nonetheless, using this population is an attractive alternative for researchers exploring the identification of early biomarkers of tuberculosis risk, because these patients represent an intermediate stage between the two traditional categories of latent and active infection that is amenable to study by host signatures. The study of subclinical cases to inform early tuberculosis reactivation is further supported by serological studies indicating differential recognition of stage-specific mycobacterial antigens in subclinical cases.99–102

**MicroRNA and metabolomic profiles**

Patient classification by gene expression or other profiles is inevitably imperfect. For example, the study by Berry and colleagues62 of transcriptional tuberculosis signatures misclassified about 10% each of cases and controls. The misclassified tuberculosis cases had minimally abnormal chest radiographs and minimally abnormal gene expression profiles. A reasonable question would be whether the misclassified controls represent early events in the process of reactivation of latent infection. A study of microRNA profiles in 29 patients with tuberculosis, 29 people with latent infection, and 18 controls identified 17 micro-RNAs that were differentially expressed among the groups.63 Two individuals with latent infection showed profiles similar to those with tuberculosis.

Similarly, Weiner III and colleagues66 explored the metabolomic profiles of more than 400 small molecules in serum of 46 people with latent infection and 44 patients with clinical signs of pulmonary tuberculosis. The investigators noted increased activity of indoleamine 2,3-dioxygenase 1, decreased phospholipase activity, increased abundance of adenosine metabolism products, and indicators of fibrotic lesions in patients with active disease compared with those with latent infection. They recorded that 20 metabolites were sufficient for robust but imperfect discrimination of patients with tuberculosis from healthy individuals. Furthermore, researchers have found that systemic concentrations of haemoxgenase-1 (HO-1) were increased in individuals with active pulmonary and extrapulmonary tuberculosis (BB Andrade, unpublished). HO-1 concentrations effectively discriminated active from latent tuberculosis and there was a marked reduction in HO-1 levels in active tuberculosis cases after antituberculous therapy, but not in those for whom treatment was unsuccessful.

The misclassified latent infection cases in these series could simply be due to test error, but they could represent early events in the reactivation process. Findings from further cross-sectional studies of these markers in people with latent infection, subclinical tuberculosis, and overt active disease will help to assess their potential value in prediction of both tuberculosis cure and reactivation; prospective longitudinal trials will ultimately be needed for their qualification.

**Prediction of protective immunity induction by vaccination**

Much data have been published on the role of CD8+ and CD4+ adaptive T-cell responses directed against M tuberculosis.103 Advances in response analyses showed that immune responses in infections are impaired and T cells express specific exhaustion markers, including PD-1 (programmed cell death protein 1) or TIM-3 (T-cell immunoglobulin and mucin domain-containing molecule 3).104 By contrast with the generally accepted model, TIM-3+ T cells show stronger immune effector functions, defined by Th1 and Th22 cytokine production, cytotoxic T-lymphocyte function, and reduced M tuberculosis replication in macrophages.105 Decreased cellular immune responses in patients with tuberculosis have also been associated with altered SOCS3 (suppressor of cytokine signalling 3) expression. SOCS3 regulates cytokine signalling and affects T-cell polarisation. SOCS3 is increased in M tuberculosis-specific T cells; it could therefore represent an exhaustion biomarker,106 or enhance expansion of interleukin-17-producing immune cells, or both.107 Perhaps the most surprising finding was identification of the antimicrobial activity of mucosal-associated invariant T cells. In individuals without active disease they express as the invariant TCRα (T cell receptor α) chain VA72-Jα33, are restricted by the MR1 (MHC class 1 related) antigen, can display M tuberculosis-specific effector functions,108 and are enriched in the lung (compared with the peripheral circulation in patients with active tuberculosis).109

16 new tuberculosis vaccine candidates have been clinically trialled in the past decade.110 In most instances, their development has proceeded by evaluation of protective efficacy in an animal model, and then assessment of immunogenicity in people by measurement of T-cell frequencies and cytokine profiles. However, studies showing the poor correlation of these measures (including polyfunctional T-cell responses) with protection against tuberculosis after BCG vaccination111 have focused attention on the need for other indicators of vaccine-induced protection.

Bactericidal or viral neutralisation assays have eased development of all other licensed vaccines. Several such assays have been described for M tuberculosis with mononuclear cell or whole blood culture.52,53,112–113 Immune control of growth in these assays is inferior in people who are tuberculin-skin-test-negative and in young children enhanced by BCG vaccination or vitamin D; impaired by chemokine receptor blockade, T-cell deletions, or HIV infection; and restored by antiretroviral therapy.53,114–119,111a–c. These findings indicate their plausibility as correlates of protection. Fletcher and colleagues119 examined the ability of whole blood and mononuclear cell growth inhibition assays to detect effects of BCG vaccination in 30 British adults with or without a previous history of BCG vaccination. They used time to detection in mycobacterial growth indicator
tube rather than colony-forming unit counting to assess mycobacterial viability in assays. Volunteers were followed up for 6 months to assess the reproducibility of growth inhibition and to examine the evolution of responses over time. A single BCG vaccination is protective in this population, particularly against severe forms of tuberculosis,\(^{128,129}\) whereas repeated vaccination is not.\(^{128,129}\) The study results showed that both assays were sufficiently reproducible, and investigators noted incremental vaccine-induced growth inhibition only in patients without a previous history of BCG vaccination. Furthermore, although T-cell frequencies increased after vaccination, they did not correlate with growth inhibitory activity. Further studies of these assays after vaccination in people in tuberculosis-endemic regions are warranted to assess the potential correlation with clinical outcomes.

**Future of tuberculosis biomarker research**

Whereas several possible biomarkers of treatment response, cure, and relapse have been proposed from small, geographically restricted patient cohorts, none has been validated, largely due to unavailability of well characterised biobanks for biomarker research. What is required are central biobanks (collections of biospecimens—eg, cells, tissue, blood, serum, sputa, DNA—with associated clinical and laboratory data and information from large cohorts of patients who have had adequate follow-up through to cure, relapse, or recurrent disease). Availability of these biobanks will allow a comprehensive set of analyses to be done using routine and advanced techniques. This will enable better understanding of tuberculosis pathogenesis (panel 2) and identification and evaluation of new biomarkers. Thus, the main barrier to the development of biomarkers into validated surrogates of treatment response has historically been insufficient funding rather than scientific knowledge. Further funder investments into research that overcome barriers to the development of novel tuberculosis biomarkers and to building biobanks for providing well characterised samples for the validation of these biomarkers are needed.\(^{121}\) Several funding agencies have increased investment into biomarker studies, including the US National Institutes of Allergy and Infectious Diseases (NIAID), US Food and Drug Administration (FDA), British Medical Research Council, European Developing Countries Clinical Trials Partnership, and Bill & Melinda Gates Foundation. Ten new projects funded by the Gates Foundation include several new areas of research, such as studies of *M tuberculosis* small RNAs,\(^{124}\) disease-specific cytokine profiles,\(^{125–127}\) and analysis of mycobacterial constituents released by exosomes of cells in infected tissues.\(^{126}\) The NIAID has also published an initiative to expand fundamental understanding of latent tuberculosis infection, especially in the setting of HIV co-infection.\(^{129,130}\)

An optimum setting in which putative surrogates of treatment response could be effectively assessed is within clinical drug trials or in non-interventional observational cohorts in which participants are well characterised and followed in a rigorous and standardised way. Cross-sectional studies of close tuberculosis contacts without HIV with minimally symptomatic subclinical disease could provide important information about candidate biomarkers, and inform the biological basis and stability of this phenotype. Ideally, such prospective studies, interventional or not, would have the following components: detailed clinical, radiographical, and microbiological participant characteristics; strict observation of treatment dosing; culture confirmation of tuberculosis at baseline and all treatment and follow-up timepoints; and, most importantly, systematic follow-up of study participants for at least 6–12 months after treatment completion. Additionally, clinical trials provide randomisation or assignment to investigational or standard-of-care antituberculosis regimens allowing comparisons of two therapies with different efficacies, and as such are ideally suited to establishment of surrogate capabilities of a biomarker.

As investment and activity in biomarker research increases, rapid communication of crucial findings and coordination of broad research activities are essential to avoid duplication of efforts, maximise resources, and accelerate the translation of basic discovery to clinical applications. Efforts are underway to establish collaborative and harmonised prospective cohort studies in several high-prevalence countries. Proper cooperation and collaboration will allow the creation of an international...
network of coordinated prospective cohorts that can not only facilitate biomarker discovery, but also allow the validation of candidate markers between different epidemiological settings and populations with samples and data collected in a standardised way. Any analytical technique used to measure a biomarker will probably need adaptation for routine clinical use, because the biomarker might prove to be useful for both clinical trials and clinical monitoring. Additionally, as the number of potential biomarkers grows, understanding the profile of desired characteristics (ie, target product profile) for each of the three biomarker research areas will be important.

In an effort to establish a tuberculosis biobank to be used in biomarker evaluation, the Global TB Alliance, the AIDS Clinical Trials Group, and the TB Trials Consortium have joined together to create the Consortium for TB Biomarkers (CTB2) whose goal is to collect specimens of sputum, serum, urine, and other tissue from people with tuberculosis, latent tuberculosis, and relapse; small sample numbers in cohorts studied; variations between study cohorts, and geographical location of study; poor quality-control of standard laboratory techniques and platforms, and their reproducibility; and heterogeneous datasets. We have an opportunity to coordinate the qualification of new tuberculosis biomarkers with the confirmatory phase 3 trials of new tuberculosis drugs; we should do our utmost to make this effort succeed.

Contributors
RSW and AZ wrote the initial, subsequent, and final drafts. All authors contributed to the finalisation of the article.

Conflicts of interest
RSW is a Pfizer employee and shareholder. All other authors declare that they have no conflicts of interest.

Acknowledgments
We acknowledge support from the European and Developing Countries Clinical Trials Partnership (EDCTP), Netherlands, EDCTP grants REMOX PANACEA (AZ), and TB-NEAT (MM, AZ); UK Medical Research Council (AZ); UBS Optimus Foundation, Switzerland (AZ, MM); University College London Hospitals (UCLH) Comprehensive Biomedical Research Centre (AZ). NIH Intramural Research Program (BBA); and the UCLH National Health Service Foundation Trust (AZ).

References


372


