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REVIEW



Accelerating tuberculosis vaccine trials with diagnostic and prognostic biomarkers

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ABSTRACT

Introduction: The most recent estimates on tuberculosis (TB) morbidity and mortality reveal that the global disease burden is even higher than previously assumed. Better drugs, diagnostics and vaccines are major requirements to control the ongoing TB pandemic. The high complexity of the infectious process and the underlying pathology, however, challenge elucidation of protective immune mechanisms at the various stages towards active TB disease, which need to be understood for rational design of novel intervention measures.

Areas covered: Next to the more classical approaches, host biomarkers increasingly receive attention as promising tools on our way to control the disease. In the area of diagnosis, host biomarkers are recognized as promising new means because the identification of small biosignatures with high discriminatory and even prognostic potential has stimulated the hope that rapid and easy-to-perform diagnosis and prognosis will become possible in the near future. For rational design of new vaccine candidates, correlates of protection are highly desirable. High-throughput systems-vaccinology will boost the identification of such biomarker profiles.

Expert commentary: Considering their potential to accelerate development of better diagnostics and vaccines, host biomarkers should be firmly integrated into future TB research.

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1. Introduction

Every year, the World Health Organization (WHO) publishes the Global Tuberculosis Report including an overview on the epidemiology of the disease in the previous year. The Report of 2016 noted 10.4 million new cases of active tuberculosis (TB) and 1.8 million deaths in the year 2015 [1]. Despite gradually falling numbers of TB cases and deaths over the past decade worldwide, the global TB burden remains disturbing. The WHO had proposed to reduce TB morbidity by 90% and TB mortality by 95% by 2035 [2]. Most likely, this goal cannot be achieved by currently available measures. Rather, we need to develop better interventions including all three medical pillars of infectious disease control: diagnostics, therapeutics, and vaccines. To these, host biomarkers need to be added since they emerge as highly promising tools for better diagnosis and prognosis of TB disease. Diagnostic and prognostic biomarkers can also provide important guidelines for the rational design of novel vaccines and accelerate clinical vaccine trials.

Defined as measurable indicators of a biological process or condition, biomarkers can be used to identify a particular disease state or immune response. Often, specific identification requires a combination of such biomarkers, termed a (bio) signature. Biomarkers that are in use or under development for diagnostic purposes usually indicate a state of disease. In the field of vaccinology, biosignatures that reflect protective immune responses are often referred to as correlates of protection.

TB is typically a pulmonary disease caused by mycobacterial pathogens characterized by their acid-fast staining [3]. *Mycobacterium tuberculosis* (Mtb) is by far the most frequent cause of TB in humans, although zoonotic mycobacteria like *M. bovis* need to be included, as well. *M. africanum* is another mycobacterial species affecting humans, which is primarily prevalent in western Africa [4]. Whereas most infected individuals remain healthy, active TB disease can gradually develop over time in around 10% of these people [5]. Thus, the stage of latent TB infection (LTBI) is variable and frequently much longer lasting than for most other infectious diseases. Importantly, the estimated 2 billion people with LTBI on this globe are healthy and noncontagious because they contain the pathogen in secluded niches, so-called 'granulomas' [6]. During LTBI, the coordinated interactions of macrophages, dendritic cells, neutrophils as well as T and B lymphocytes induce the formation of solid granulomas which keep Mtb growth and spread in check. In patients with clinical TB, these granulomas become necrotic and eventually form caseous lesions as a result of a failing immune response. Once this containment of the infection breaks down, TB patients become contagious, spreading the pathogen [7,8]. Between these two stages of LTBI and active TB, subclinical TB disease is increasingly recognized as a stage where individuals still do not show clinical signs of active TB, but may already spread Mtb. Notably, transition is not a one-way road from LTBI to subclinical to active TB, but reversions likely take place as well. At the organ level, this continuum is reflected by the

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coexistence of different forms of lesions including solid, necrotic, and caseous granulomas [9,10]. Therefore, it is the proportion and the location of these different lesions that define the stage of the infection and determine the disease outcome [11]. It is generally accepted that T lymphocytes are the major coordinators and macrophages are the major effectors of immune defense in TB. This has led to the dogma that T cells need to be targeted by vaccines. Yet, T cells are also considered to play critical roles in pathogenesis [12]. These complex interactions between different immune cells cause a profound challenge for the development of new vaccines. Similarly, interferon (IFN) signaling is crucial for an effective antimicrobial immune response, but more recent biomarker studies suggest that IFNs also play a role in the development of

active TB [13]. Although TB typically is a localized disease of the lung, biomarker studies have shown that cells and molecules in the blood reflect the activities at the site of infection (Figure 1). Blood-derived transcriptomic and metabolomic biomarkers can thus likely serve as reliable indicators for LTBI, subclinical TB, or active TB.

The current review summarizes recent progress in research on biomarkers for diagnostic or prognostic settings. In particular, we focus on biomarkers in the context of preclinical design and clinical development of vaccines against TB. Some aspects addressed in this review have partially also been covered in some recent reviews focusing more particular on TB diagnosis [21], risk [22], and vaccine development [23].

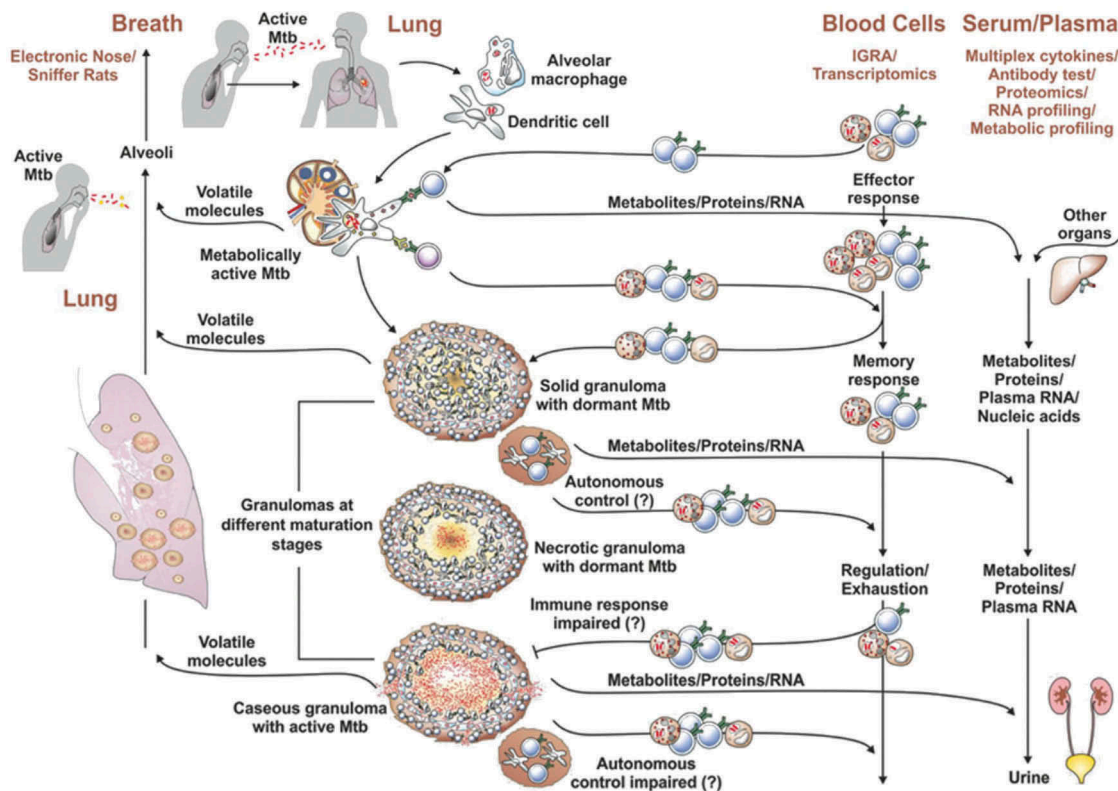


Figure 1. Major hallmarks of TB pathology and immunity and their relation to biomarkers.

Center of figure depicts major steps from initial infection, to latency and active disease. Aerosols containing live Mtb bacilli, that are expectorated by a person with pulmonary TB, are inhaled by a healthy individual. Alveolar macrophages engulf the pathogen and leukocytes like dendritic cells transport bacilli from the alveoli to the draining lymph node, where T lymphocytes are primed [14]. Some bacilli enter the lung parenchyma, eliciting inflammatory signals that attract mononuclear phagocytes and T lymphocytes to the site of Mtb deposition [8]. Antigen-specific interactions between mononuclear phagocytes, dendritic cells and T lymphocytes orchestrate the formation of a solid granuloma to which further immune cells are attracted [7]. The solid granuloma contains Mtb in a dormant stage and likely is autonomous [15]. Yet, leukocytes can enter and leave granulomas thereby linking it with the general immune system via the blood stream. The solid granuloma is a characteristic feature of individuals with LTBI. In some of these individuals granulomas become necrotic and later caseous, which is characteristic of active TB. Simultaneously, Mtb progresses from a dormant to a metabolically active stage. Rupture of caseous granulomas into the alveolar space results in spread of the bacillus into the environment. Some individuals in which LTBI progresses into subclinical TB may occasionally be contagious without showing any clinical signs of disease. In contagious individuals volatile molecules are exhaled that likely originate from Mtb, and which can be used for TB diagnosis (left part of figure) using electronic noses or sniffer rats trained for bouquets of such molecules. In blood, effector cells with different phenotypes, including effector and memory T cells, can be identified (right part of figure). Active TB disease is preceded by an increase of regulatory and exhausted T cells that likely are critical for disease progression. The most commonly used source for biomarkers is the blood (far right part of figure). The IGRA determines IFN-gamma secretion by T cells from the blood in response to selected Mtb-specific antigens and is widely used to diagnose infection. However it cannot reliably distinguish between LTBI, subclinical TB or active TB disease. Transcriptomic biomarkers have been widely studied in TB by determining gene expression profiles in blood cells. Biosignatures comprising few transcriptomic markers can identify individuals with active TB disease with high sensitivity and specificity [16]. Evidence has also been presented that a transcriptomic biosignature can be harnessed for prognosis of active TB, probably by detecting subclinical TB [17]. In serum or plasma, metabolites and proteins can be harnessed for biomarker analysis [18]. A biosignature composed of metabolites has been shown to accurately identify active TB patients and evidence has been obtained that metabolites can also be harnessed for a prognostic biosignature of active TB [19]. Although current metabolite studies harness serum, urine may be considered as a noninvasive alternative. Principally the whole serum proteome can be employed for biomarker studies. Yet, immune mediators such as cytokines and chemokines that are released in response to infection and inflammation are more widely used [20]. These include cytokines secreted by antigen-specific and activated T cells as well as cytokines released by mononuclear phagocytes in response to inflammatory stimuli. Abbreviations: TB, tuberculosis; LTBI, latent tuberculosis infection; Mtb, *Mycobacterium tuberculosis*; IGRA, interferon-gamma release assay.

2. The clinical TB vaccine trial pipeline

Several vaccine candidates have entered the clinical trial pipeline, pursuing various strategies and using different vaccine formulations (Table 1). Although the first candidate to be tested for efficacy, MVA85A (a modified vaccinia Ankara virus vector expressing the Mtb antigen 85A), failed to show protection [24,25], other candidates remain in the clinical pipeline, and several will enter efficacy trials in the near future [26,27]. Principally, current TB vaccine candidates can be categorized into two groups: subunit vaccines encompassing one or several antigens and whole-cell vaccines. Subunit vaccines are either attenuated viral vectors such as MVA, adenovirus, or influenza virus expressing Mtb-specific antigens or adjuvanted fusion proteins comprising several Mtb antigens. The latter ones include vaccines of the hybrid (H) series as well as the M72 and ID93 vaccines. Whole-cell vaccines comprise viable organisms such as the recombinant bacillus Calmette–Guérin (BCG) vaccine VPM1002 and the genetically attenuated Mtb vaccine MTBVAC. Inactivated vaccines also belong to this group. They are mostly considered in combination with standard drug therapy, but DAR-901 is currently in a Phase II prevention of infection trial (NCT02712424). Although most vaccine studies are currently aimed at preventing progression from LTBI to active TB, more recent approaches also consider preventing establishment of stable infection upon *Mtb* exposure [26,28]. Successful pre-exposure vaccines should prevent infection, in contrast to postinfection vaccines, which should prevent progression from LTBI to active TB.

3. Biomarkers

3.1. Biomarkers as guides for vaccine design

Traditional vaccines have so far mostly been developed empirically [29]. This approach was successful for those infectious diseases that are controlled primarily by antibodies. These include toxoid vaccines against diphtheria and tetanus; the inactivated vaccine against polio; attenuated viable vaccines against measles, mumps,

and rubella; the acellular vaccine against pertussis; and the conjugate vaccine against pneumonia, to name a few. For immunologically more complex diseases where T cells are often critically needed for protection such as malaria, acquired immune deficiency syndrome (AIDS), and TB, such empirical designs have more or less failed. For these diseases, a better understanding of how the immune system is stimulated is imperative. A study aimed at profiling the responses elicited by several preventative vaccines is currently underway [30]. Within this project BIOVACSAFE (<http://www.biovacsafe.eu/>), both human and animal models are used to identify biomarkers of inflammatory responses to different adjuvanted immunizations. The results from this study could help select the best combination of vaccine and adjuvant and identify biomarkers related to protective efficacy and safety of different vaccine types. For influenza for example, similar studies have already provided valuable and detailed data on vaccine-induced immune responses related to protection [31], and variations in human dendritic cell responses to *in vitro* stimulation with different microbial vaccines have been described [32]. Moreover, the adjuvant component of a vaccine can have a profound effect on the molecular signatures induced by the vaccine [33].

Since defense against different infectious diseases requires a balanced combination of unique immune mechanisms, new vaccines will need to elicit an equally fine-tuned immune reaction. Gaps in our knowledge of the qualitative aspects of the human immune response which prevents infection or progression to clinical disease have so far severely hampered the design of efficacious vaccines against several pandemics such as AIDS [34], hepatitis C infection [35,36], and malaria [37,38]. Similarly for TB, the fine-tuned mechanisms that are responsible for sterile eradication or at least lifelong containment of Mtb remain elusive, making it hard to design a vaccine that achieves elimination of Mtb or sustains LTBI lifelong, thereby preventing progression to clinical TB [29,39,40]. An alternative approach would be the prevention of infection. The identification of key biomarkers that correlate with LTBI, subclinical TB, or clinical TB or sterile pathogen eradication will

Table 1. Major vaccine candidates currently in clinical trials.

Vaccine	Comment	Clinical phase
MVA85A	Modified vaccinia Ankara virus (MVA) vector expressing Ag85A ^a of <i>M. tuberculosis</i> (Mtb) administered by aerosol	I
Ad5Ag85A	Adenovirus (Ad) type 5 vector expressing Ag85A ^a	I
Ad35 + MVA85A	Prime with Ad type 35 vector followed by boost with MVA vector both expressing Ag85A ^a	I
ChAdOx1.85A ± MVA85A	Prime with simian Ad vector alone or followed by boost with MVA both expressing Ag85A ^a	I
TB-FLU-04L	Replication-deficient influenza H1N1 vector expressing Ag85A ^a + ESAT-6 ^b administered intranasally	I
Hybrid 1 + IC31	Fusion protein comprising Ag85B ^a + ESAT-6 ^b adjuvanted in IC31 ^c	II
Hybrid 4 + IC31	Fusion protein comprising Ag85B ^a + TB10.4 ^d adjuvanted in IC31 ^c	II
Hybrid 56 + IC31	Fusion protein comprising Ag85B ^a , ESAT-6 ^b + Rv2660c adjuvanted in IC31 ^c	II
ID93 + GLA-SE	Fusion protein comprising Rv2608, Rv3619 + Rv3620 adjuvanted in GLA-SE ^e	II
M72 + AS01E	Fusion protein comprising Rv1196 + Rv0125 adjuvanted in AS01E ^f	II
MTBVAC	Genetically attenuated <i>M. tuberculosis</i> with deletions in <i>phoP</i> ^g and <i>fadD26</i> ^h	I
VPM1002	Recombinant bacillus Calmette–Guérin (BCG) expressing <i>hly</i> ⁱ in a <i>UreC</i> -deficient background	II
Dar-901	Whole-cell vaccine based on <i>M. obuense</i> given as booster of BCG prime	II

^aAg85A, Ag85B: mycolyl transferases which are immunodominant antigens of Mtb and BCG.

^bESAT-6: immunodominant antigen of Mtb.

^cIC31: formulation of cationic peptides + synthetic toll-like receptor-9 agonist.

^dTB 10.4: immunodominant antigen of Mtb and BCG.

^eGLA-SE: synthetic toll-like receptor-4 agonist in-oil-in-water emulsion.

^fAS01E: toll-like receptor-4 agonist incorporated in liposomes.

^g*phoP*: transcription factor for Mtb virulence factors.

^h*fadD26*: part of synthesis machinery of phthiocerol dimycocerosates.

ⁱ*hly*: listeriolysin from *Listeria monocytogenes*

^j*UreC*: urease C.

have profound impact on the development and design of new vaccines. In this respect, biomarkers can be harnessed to characterize their biological function *in vitro* and in experimental animals to more rapidly identify clinical end points, assess safety, and predict protective efficacy of new vaccines [18]. An additional value of biomarkers is the prediction of disease risk and the monitoring of progression from LTBI to active TB to accelerate clinical trials [22,41,42].

It is difficult to define vaccine-induced protective mechanisms in TB in the absence of an efficacious vaccine. Yet, the currently used vaccine BCG protects against extrapulmonary TB in infants [43], and hence, studies in this group could reveal information relevant to novel vaccine development and testing. Although the markers associated with the protective properties of BCG are poorly understood, several correlations with immunological biomarkers in BCG-vaccinated infants have been described. The dual role of T cells in protection and pathogenesis is illustrated by the association of higher frequencies of activated HLA-DR⁺CD4⁺ T cells with increased risk of TB disease in BCG-vaccinated infants [44]. On the other hand, higher numbers of IFN- γ -secreting T cells is correlated with a reduced risk of TB [44]. In a large case-control study, Fletcher et al. identified two major clusters of gene expression in BCG-vaccinated infants upon *ex vivo* peripheral blood mononuclear cell (PBMC) restimulation, reflecting different myeloid-cell and inflammatory responses [45]. This could suggest that TB vaccine candidates can elicit distinct responses in subpopulations of infants. Responses to vaccination can also be influenced by geographic location and other factors [46]. Moreover, the response to vaccination in young children can be influenced by mycobacterial exposure and immune status of their mother. For example, BCG-vaccinated infants from mothers with LTBI show increased IFN and inflammation-related biosignatures [47]. In summary, many factors can impact the immune response to vaccination in infants and should be taken into account in future TB vaccine development.

Alternatively, signatures from natural infection and clinical disease can be used to predict whether an induced signature reflects protection or pathology. Some vaccines induce a biosignature that mirrors responses in natural infection [32], thereby providing valuable information on desired and undesired mechanisms induced by the vaccine.

3.2. Diagnostic value of biomarkers of infection and disease

The detection of acid-fast bacilli in sputum still is the most widely used diagnostic test for clinical TB [20]. The tuberculin skin test (TST) measures the immune response against Mtb and remains the test of choice for screening and epidemiologic purposes of Mtb infection. Both stem back to discoveries in the late nineteenth century [48]. The IFN gamma release assay (IGRA) is a more reliable alternative to the TST for diagnosis of Mtb infection in BCG-vaccinated populations [49]. However, neither TST nor IGRA distinguishes between LTBI and clinical TB, let alone subclinical TB. More recently, conventional markers including inflammatory mediators and antigen-induced cellular responses, as well as high-throughput transcriptomics and metabolomics [50] have developed into well-established

research platforms. Although less well established yet than the former two, epigenetics and proteomics also hold promise as biomarker discovery tools [51,52]. Diagnosis of active TB disease, while reliable and cost-effective, is still a slow process. Combined with the fact that it usually takes several weeks before patients consult a health-care practice, the average delay between the onset of symptoms and the actual diagnosis ranges around 8 weeks [53]. Most earlier high-throughput biomarker studies mainly focused on adult TB patients, yielding a number of insights into the molecular pathology of TB [54–57]. Biomarker signatures have also been investigated in childhood cohorts [58] and in relation to HIV coinfection [59,60]. And of high relevance for diagnostics, several biomarker signatures have been identified that can differentiate TB from other pulmonary diseases [59,61,62]. Partially based on these studies, several small transcriptomic signatures have been identified for potential use in TB diagnosis [16,63,64].

These advances will pave the way for a pathognomonic TB transcriptome biosignature, which does not only distinguish between TB patients and healthy controls (even in HIV-coinfected individuals) but also between TB and other diseases.

More recently, it was shown that host biomarkers of TB also possess prognostic potential. Biomarkers characteristic for active TB disease can already be detected in blood transcriptomes of infected individuals prior to onset of clinical symptoms [17]. This phenomenon is time dependent, as the abundance of disease-related biomarkers increases closer to onset of clinical TB. Essentially, this indicates that TB biomarkers can detect subclinical TB which generally progresses to active TB disease [50].

Several other candidate biomarkers of TB risk have been described as well, including the before-mentioned enhanced T-cell activation [44]. Elevated monocyte/lymphocyte ratios are related to mycobacterial growth *in vitro* [65] and have been associated with risk of TB in both infants and HIV-infected adults [66,67]. These and several other correlates of TB risk have recently been reviewed by Petruccioli et al. [22].

Eventually, such biomarkers will facilitate the identification of individuals at risk of developing clinical TB, thereby allowing preventive drug therapy. In this way, high-risk individuals could be treated prophylactically before they develop severe symptoms and spread the disease.

In general, numerous biomarkers with diagnostic or even prognostic potential have been suggested, but it is crucial that the most promising ones are validated, requiring increased efforts and funding [21].

3.3. Biomarkers of protection and vaccine efficacy

For some infectious diseases, biomarkers are already in use measuring the immunologic outcome or protective efficacy of vaccines [68]. Historically, these primary markers in vaccine assessment are antibody titers and their neutralizing activity. Later on, T-cell functions and inflammatory markers were also integrated.

One such classic prototypic biomarker is the hemagglutination inhibition assay, used for evaluating influenza virus vaccine efficacy. Currently, it is the only universally accepted immune correlate of protection against influenza. However,

its relevance may vary between different vaccines and target populations [69].

In recent years, systems biology has been increasingly applied to decipher cellular and humoral host immune responses to infection and vaccination [70]. Such approaches have demonstrated that different classes of human vaccines induce distinct transcriptional signatures [71]. In the case of influenza, immunogenicity of a seasonal vaccine can be predicted based on early induced molecular signatures correlating with humoral responses [72,73]. Hemagglutination efficacy 1 month after vaccination can even be read from innate response signatures a single day postvaccination [31]. In contrast, in infants, these responses appear to be attenuated, in which case the responses can be enhanced by administering an MF59-adjuvanted vaccine [31]. One of the best studied vaccines by systems biology is the yellow fever vaccine YF-17D, which was shown to induce early gene signatures predicting immunogenicity in terms of neutralizing antibodies in humans [74]. However, antibody responses induced by YF-17D are variable [75], and vaccine efficacy may be affected by an activated immune microenvironment [76]. As with influenza, induction of CD8 T cells by YF-17D vaccination complements the antiviral protection conferred by antibodies [77]. In the case of malaria, efficacy of the vaccine candidate RTS,S has been correlated with several immunological processes [78,79], suggesting that multiple mechanisms play a role in protection.

3.4. Correlates of protection

Human challenge studies currently provide the most powerful framework for identification and characterization of correlates of protection. Obviously, for certain infectious diseases like HIV and TB, this poses serious safety concerns, but studies using other pathogens have proven the potential of the human challenge approach [78–87]. In several cases, a correlation with increased IFN signaling has been identified. In malaria, such a signature appears to be correlated with partial protection [88], whereas for influenza, it is associated with more severe symptoms at the early stage after challenge [89]. For dengue, a drop in IFN gamma levels is associated with disease development [81]. Also in several studies, increased IFN signatures have been associated with TB disease, rather than latency [17,54,90].

In TB, most systems biology studies have focused on decrypting signatures which discriminate between patients and healthy individuals with or without LTBI [91]. However, little information has been gained thus far about functional roles of markers related to protection to natural infection, let alone to vaccination. More recently, a study based on transcriptomic profiles from Mtb-exposed individuals revealed that progression toward active TB can be detected up to 12 months prior to onset of active TB disease [17]. The biomarker signature identified could distinguish individuals who developed TB later on from those who remained healthy and likely reflect underlying subclinical infection or retarded progression to disease. These findings suggest that transcriptomic signatures are capable of predicting Mtb-infected individuals with a high risk of developing clinical TB versus individuals who sustain LTBI. Such risk signatures

could be exploited to stratify high-risk individuals for recruitment into vaccine trials. This also raises the ethical question whether individuals with strong positive signatures should be offered treatment before the onset of clinical signs, thereby excluding them from enrollment into such studies. Clinical TB vaccine trials are prohibitively costly due to the relatively low proportion and the prolonged incubation period of study participants who develop active TB disease during the trial period. By focusing on high-risk individuals, the cohort size can be reduced and the duration of the trial shortened [92,93]. Further reduction of trial duration can be achieved by biomarker monitoring of study participants. Altogether, this will undoubtedly reduce total costs.

3.5. Biomarkers to overcome the wall between preclinical models and clinical trials

To accelerate the development of new vaccines and increase our understanding about their modes of action, biomarkers are becoming ever more crucial. Animal and other preclinical models remain indispensable for the development of a safe and efficacious generation of TB vaccines. Many species differences can affect the relevance of animal models for predicting the outcome in humans. Furthermore, there are fundamental differences in the design of animal experiments and human efficacy trials, including environmental factors, the mode of vaccination, the type of exposure, and the Mtb strain involved [94]. Mouse models are an important first step, but they do not reflect the full spectrum of pathology and protection of human TB. Thus, vaccine candidates that appear promising in a distinct mouse strain may be less successful in the genetically much more variable human population. On the other hand, BCG vaccination induces variable responses in genetically different mouse strains [95], stressing the importance of selecting the most appropriate strain in animal vaccine testing. It also illustrates the value of the ‘Collaborative cross project’ as a resource for genetic variability in mouse strains. Although human efficacy trials ultimately remain essential for final evaluation of new candidate vaccines, additional preclinical models can add information which will allow gating for the most promising vaccine for further clinical development [96]. One such approach to evaluate protective immunity and efficacy of vaccine candidates is mycobacterial growth inhibition assays (MGIs). These *ex vivo* assays assess the capacity of whole blood or isolated blood cells from vaccinated individuals to inhibit Mtb growth *in vitro*. The development of such MGIs dates back several decades, and different models have been employed in TB vaccine studies to identify protective immune markers [97]. Combined with transcriptomic analyses, MGIs can aid in identifying potentially relevant biosignatures induced by immunization [98]. However, additional selection criteria will be needed to allow a calculated decision on which vaccine candidates have the best chance of success in clinical trials.

Another helpful test would be a human challenge model [99]. Thus far, intradermal BCG is used as a surrogate for Mtb infection in the only TB challenge model tested so far [100]. However, BCG is attenuated and therefore much less of a challenge for the human immune system than Mtb.

Moreover, it is obvious that skin and lung provide very different environments for *Mtb*. With this caveat, such a model could contribute to better understanding of antimycobacterial immunity [101]. Currently, efforts are being undertaken to develop a human *Mtb* challenge model exploiting a mutant *Mtb* strain which is fully virulent but will die once internal energy resources have been used up [102]. While this approach sounds attractive, numerous hurdles will have to be overcome before it can be applied in a safe and ethical way [103].

4. Conclusion

Host biomarkers have emerged as promising new tools in TB research aimed at developing better intervention methods. We believe that such signatures become indispensable for unraveling the complexity of infection, pathology, and protection at various stages from exposure with *Mtb* via LTBI toward active TB disease. Integration of biomarkers in vaccine development will help to assess the potential of new vaccine candidates in preclinical models and clinical trials. Moreover, host biomarkers have become intriguing ingredients for novel rapid and easy diagnostic tests. Assays based on only few biomarkers can be harnessed to identify *Mtb*-exposed individuals with elevated risk of developing clinical TB for prophylactic treatment. It is encouraging to see the increased efforts over the years to develop new candidate TB vaccines and to test their safety and efficacy in clinical trials. A crucial gap still affecting the rational design and development of new TB vaccines, however, is the insufficient understanding of the complex immunologic processes in TB and, therefore, the lack of correlates of protection. We are confident that by robust implementation of host biomarkers in future vaccine research and development, these gaps can be overcome. For the development of better diagnostics, effective biomarkers are in closer reach. Promising biosignatures based on just a few markers have now been identified with the potential to rapidly triage suspected TB patients. Equally important from a clinical point of view, these biomarkers have a substantial potential of distinguishing TB from other pulmonary diseases. Hopefully, such diagnostic biomarkers will soon be validated on a larger scale to accelerate their introduction into relevant clinical settings.

5. Expert commentary

The WHO has set the ambitious goal to drastically reduce TB morbidity and mortality within the next two decades [2]. Yet, it is quite clear that with the currently available intervention measures, such a sharp decrease in disease burden will not be accomplished. The most effective and cost-efficient way to halt the TB pandemic would be the implementation of a highly efficient vaccine against TB.

- The currently available TB vaccine BCG is administered to newborns in virtually all countries with high TB prevalence. Although this vaccine protects children from extrapulmonary TB, it fails to prevent lung TB in all age groups, notably adolescents and adults. As a live vaccine, BCG is not recommended for HIV-exposed newborns,

due to the increased risk of mycobacterial dissemination. Considering the high HIV prevalence in many TB-endemic countries, the urgent need for a safer alternative is obvious. Ideal would be a vaccine that minimizes establishment of infection and prevents disease progression in the infected population. Currently, most new TB vaccines under development target prevention of active TB disease rather than infection. Furthermore, BCG's efficacy varies between regions [104], so different vaccine formulations may be needed for different geographic regions and populations of vaccinees.

- The current tools for TB diagnosis remain suboptimal. Differential diagnosis of TB versus other pulmonary diseases can be challenging, and a considerable number of TB cases remain undetected. An average delay of 4 weeks between first visit to a health practice and final TB diagnosis [53] is unacceptable. Encouraging in this respect are the various TB biomarker signatures that are rapidly emerging. Such signatures were first thought to reflect general signs of inflammation and pathology. But evidence is now surfacing that small biosignatures can be designed to be disease specific, with remarkable power to distinguish TB from health and from other pulmonary diseases at the same time. In the authors' view, such small biomarker signatures provide the opportunity to implement a simple, rapid, and cost-effective tool for screening of TB suspect cases. Slightly less advanced are predictive biosignatures. However, there is reason to foresee prognosis of clinical TB with the recent identification of a transcriptome-based biosignature that can describe subclinical TB progressing to clinical disease.
- A greater challenge will be the application of biomarkers for vaccine development. Most valuable in this respect would be the identification of markers related to protective immunity. Recent studies in other infectious diseases indicate that this ambitious goal is achievable by using advanced systems biology approaches [105,106]. Ideally, correlates of protection would be derived directly from clinical vaccine trials. Due to the long duration of such trials, it will probably take many more years before sensitive biomarkers of vaccine-induced protection will become available. Therefore, new biomarkers should also be sought in clinical studies on natural infection and in preclinical studies. This ambitious goal will only become possible if high-quality biorepositories for these valuable samples can be established and sustained. Two Phase II efficacy trials (ClinicalTrials.gov identifiers: NCT02075203 and NCT01755598) on TB candidates [28,107] are currently ongoing which could provide promising first biospecimens for such a repository. If successful, such studies will open new avenues toward the design of novel vaccines and their clinical evaluation.

6. Five-year view

Biomarker research and discovery have made tremendous progress over the past few years.

Now it is the time to more strongly implement biomarkers in research and development of vaccines and diagnostics. Small biosignatures have been identified which can be introduced as triage tool for screening suspected TB cases. Slightly less advanced are prognostic markers. However, identification of a signature which identifies individuals with subclinical TB who will progress to clinical TB within subsequent months provides proof of principle for feasibility of a tailor-made prognostic signature in TB in the years to come. These accomplishments can be achieved in the next 5 years and will form the basis for the design of a biosignature of vaccine efficacy. A combination of observational studies on outcome of natural *Mtb* infection and extensive monitoring of clinical TB vaccine trials by means of biomarkers and supported by sophisticated computational biology will be needed for this ambitious goal to be fulfilled before the end of the decade to come.

Key issues

- Current intervention measures are inadequate to end the TB pandemic.
- Multiple new TB vaccine candidates are currently being tested for safety and efficacy in clinical trials.
- The biological complexity underlying *Mtb* infection and progression to TB disease challenges the development of new vaccines and diagnostics.
- Systems-biology analyses can reveal biological processes underlying different stages of TB.
- Signatures for risk of clinical TB will accelerate preclinical and clinical research and development of TB vaccines.
- Broad-scale implementation of biomarkers in research and development on TB intervention measures is strongly encouraged.

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Declaration of interest

S.H.E Kaufman is a co-inventor of the TB vaccine candidate VPM1002. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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