AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

Moving Toward Tuberculosis Elimination: Critical Issues for Research in Diagnostics and Therapeutics for Tuberculosis Infection

Salmaan Keshavjee[1,2], Farhana Amanullah[3], Adithya Cattamanchi[4], Richard Chaisson[5], Karen M. Dobos[6], Gregory J. Fox[7], Howard E. Gendelman[8], Richard Gordon[9], Anneke Hesseling[10], Le Van Hoi[11], Beate Kampmann[12], Bavesh Kana[13], Gopal Khuller[14], David M. Lewinsohn[15], Deborah A. Lewinsohn[16], Philiana Ling Lin[17], Lenette Lin Lu, [18], Gary Maartens[19], Andrew Owen[20], Marina Protopopova[21], Jyothi Rengarajan[22], Eric Rubin[23], Padmini Salgame[24], Erwin Schurr[25], James A. Seddon[12], Susan Swindells[8], David M. Tobin[26], Zarir Udwadia[27], Gerhard Walzl[28], Sudha Srinivasan[21], Roxana Rustomjee[21], Payam Nahid[4].

Institutions:
[1] Harvard Medical School, Boston MA
[2] Harvard Medical School Center for Global Health Delivery-Dubai, Dubai, United Arab Emirates
[3] The Indus Hospital, Karachi, Pakistan
[4] University of California San Francisco, Zuckerberg San Francisco General Hospital
[5] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.
[6] Colorado State University
[7] Faculty of Medicine and Health, University of Sydney, NSW, Australia 2006
[8] University of Nebraska Medical Center, Omaha, NE 68198 USA
[9] South African Medical Research Council
[10] Desmond Tutu TB Centre, Stellenbosch University
[11] National Lung Hospital and the National TB Program in Vietnam
[12] MRC Unit The Gambia at the London School of Hygiene and Tropical Medicine
[13] DST/NRF Centre of Excellence for Biomedical TB Research, Faculty of Health Sciences University of the Witwatersrand, National Health Laboratory Service, Johannesburg, South Africa and
MRC-CAPRISA HIV-TB Pathogenesis and Treatment Research Unit, Centre for the AIDS Programme of Research in South Africa, CAPRISA, Durban, South Africa
[14] Postgraduate Institute of Medical Education & Research
[15] Oregon Health and Science University (OHSU) and Portland VA Medical Center (PVAMC)
[16] Oregon Health and Science University (OHSU)
[17] University of Pittsburgh, School of Medicine, Pittsburgh, PA
[18] Massachusetts General Hospital
[19] Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa
[20] University of Liverpool
[21] Division of AIDS/NIAID/NIH/DHH, National Institutes of Health
[22] Emory University School of Medicine
[23] Harvard T.H. Chan School of Public School
[24] Rutgers University
[25] McGill University

[26] Duke University School of Medicine
[27] Hinduja Hospital & Research Center, Mumbai
[28] DST-NRF Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for TB Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

Corresponding Author:
Salmaan Keshavjee MD, PhD, ScM
Department of Global Health and Social Medicine
Harvard Medical School
641 Huntington Avenue
Boston MA 02115
Salmaan_Keshavjee@hms.harvard.edu

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

ABSTRACT

Tuberculosis (TB) has surpassed HIV to become the leading infectious killer of adults globally, causing almost 2 million deaths annually.[1] Although this airborne disease has been treatable since 1948, global rates of TB have dropped less than two percent per year; an estimated 10 million incident cases continue to occur annually, including one million in children.[1,2] While transmission of active disease is an important driver of the epidemic, the seedbed that feeds the epidemic is the more than two billion people estimated to have TB infection, five to ten percent of whom will progress to active disease during their lifetime.[3] While any successful strategy aimed at TB elimination needs to address this reservoir of TB infection worldwide, much remains to be understood about host and pathogen factors that can be used to identify increased risk for progression to disease, and intervened upon to prevent progression from occurring.[4]

The Division of AIDS of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA, and the Harvard Medical School Center for Global Health Delivery–Dubai convened a group of scientists and stakeholders on September 28 and 29, 2017, to address knowledge gaps that affect our ability to rapidly find and treat individuals infected with *Mycobacterium tuberculosis* who are most likely to progress to active disease. The meeting identified a number of efforts underway to address this important gap in the collective ability to stop the global TB epidemic. Here, we review and outline the priority areas for research, diagnosis and treatment of TB infection that emerged from the meeting (Table 1), building on recent reviews in this area.[5,6,7]

Understanding the spectrum of TB infection and disease: a critical first step

Although epidemiological data from the late 1950s—and a number of randomized controlled trials since then—demonstrate the importance of treating TB infection before it becomes clinically apparent (preventive therapy for so-called "latent" TB),[8,9,10,11,12] very little is known about the spectrum of clinical states between asymptomatic infection and clinically apparent disease. It is becoming increasingly clear that conventional conceptions of TB as "latent" versus "active" are overly simplistic, and neither adequately reflect host immunology nor microbial pathogenesis.[13,14,15] Indeed, there is now increased appreciation that clinically apparent "TB disease" is assuredly preceded by a period of incipient TB—an asymptomatic phase that can been characterized by an evolving blood-based host RNA signature.[16] These observations confirm that the previous classification of "latent" infection is imperfect and limited by the characteristics of available diagnostic tests.[17] Moreover, it is hypothesized that some exposed individuals can mount effective immune responses that eradicate all viable bacilli, a phenotype distinct from persons in whom immune responses can only contain the infection, thereby harbouring populations of bacilli that intermittently replicate in macrophages, granulomata and other tissues.[4] Whether the newer terminology of "incipient" disease indicates early active TB that inevitably progresses to clinically apparent disease, or whether it is more of a dynamic state that reflects ongoing host-pathogen interactions, remain unknown. In addition, the definitions and pathophysiology of a pre-incipient state is also unknown. Being able to characterize and identify the full spectrum of "latency", including the dynamics of microbial and host interplay and the

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

role this relationship has in developing disease is a critical component to addressing the TB epidemic (Figure 1).

It has long been hypothesized that sub-clinical TB infection is characterized by a population of *M. tuberculosis* in a dormant or metabolically quiescent state.[18,19] Rather, recent data on changes in host immunity during TB infection and the efficacy of treatment for TB infection point towards a population of organisms that have substantive metabolic activity.[14,20] The true nature of bacterial metabolism, and associated vulnerability to anti-tubercular drugs, remains a critical knowledge gap that has been difficult to study in humans due to the inability to meaningfully sample bacteria prior to TB disease progression.[19] To address this, *in vitro, ex vivo* and *in vivo* models have been established to study various aspects of non-replicating persistence in mycobacteria. Passage of *M. tuberculosis* through these models reveals a dynamic and complex pathogen response to the immune stresses imposed during infection.[19] For example, *in vitro* models that simulate the non-replicative state demonstrate that carbon metabolism is extensively remodelled to accommodate for a reduction in simple glycolytic substrates and an increase in the availability of fatty acids and host derived cholesterol.[21,22,23,24] Similarly, nitrogen metabolism is rewired to respond to increasing availability of nitrate, a by-product of host-derived reactive nitrogen stress.[25,26] *In vitro* models and infection studies in animals show specific pathogen metabolic adaptations that address the reduced levels of oxygen present in the TB granuloma.[19,27,28,29] Collectively, data from these models of non-replicating mycobacterial persistence suggest that the bacteria are not metabolically quiescent. While this finding may provide an explanation for the effectiveness of preventative therapy with agents that target

active cellular processes given over an extended time-frame (e.g. isoniazid and rifampin),[4,8,9,10,11,12] it is not yet clear how much these *in vitro* models correspond to mycobacterial activity in humans.

There are many unanswered questions regarding the ability of tubercle bacteria to survive in an immune-active host. Gaps exist in understanding where bacteria are located during the early stages of TB infection and whether different locations and environments affect bacterial behaviour and treatment response.[19] How similar is the bacterial metabolism along the spectrum between infection and disease? If there are differences, how do we define vulnerable drug targets for TB infection? Would recognition of bacterial behaviour during early conditions allow us to define new growth/media conditions for diagnosis? These are all critical areas of inquiry.

The interplay between bacterial and host genetics and the subsequent combinatorial effect on host immune response during infection is another important area of research. First, we know that *M. tuberculosis* can interact with a large repertoire of innate host receptors that shape the immune response and outcome of infection and progression to disease.[30] For example, eicosanoid generation, considered as a host protective response to mycobacteria, is regulated by leukotriene A(4) hydrolase (LTA4H). In tuberculous meningitis, a genetic polymorphism in LTA4H is associated with both inflammation and responsiveness to adjunctive anti-inflammatory therapies.[31] Second, immune responses appear to also be influenced by *M. tuberculosis* strain diversity. *Mycobacterium tuberculosis* Complex (MTBC) comprises seven human adapted lineages and studies have reported that genotypic diversity is associated with differences in host immune responses.[32,33,34] That the interaction between host

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

polymorphisms and TB strain variation may dictate the outcome of infection is illustrated in a study that showed that the association of SNP TLR2 T597C with risk of TB meningitis is strongest among those infected with the Beijing lineage.[35]

Overall, it is plausible that the spectrum of infection is influenced by a combinatorial effect of both host and bacterial genotypes. A better understanding of the nuances of host-pathogen dynamics during different *M. tuberculosis* infection states and with *M. tuberculosis* genetic diversity would contribute to developing protective vaccines for TB infection and therapeutic vaccines for treatment. It would also help to identify biomarkers of pathogen clearance and of progression to TB, and advance our understanding of host innate and adaptive immunity to *M. tuberculosis.* This is critical for eradicating persistent infection, preventing reactivation of TB infection, shortening TB infection treatment and/or preventing re-infection.

Diagnosing TB Infection and Progression to Disease

Another major gap in addressing TB infection has been the inability to differentiate between asymptomatic individuals harboring viable persistent bacteria versus those that have cleared the infection. This is highly relevant for the accurate identification of infected individuals that are at greatest risk for progressing to TB disease. Whereas persons with positive interferon gamma release assays specific for TB antigens are at increased risk of progressing to clinically apparent disease, the number needed to treat to prevent a single active case can range as high as from 111 to 314 (depending on setting and the patient's risk for progression), an observation that

has dampened enthusiasm for universal treatment of TB infection.[36] While recent studies have identified host biomarkers and transcriptional profiles associated with active TB and signatures associated with TB disease risk,[37,38,39] signatures that distinguish persistent TB infection from cleared infection have not been identified. Moreover, the effect on these signatures by co-infection with HIV or helminths, or co-morbidities such as diabetes, is not known.

Overall, there are substantial problems with current definitions of infection since they are generally inferred from surrogate assays that measure the metabolic, transcriptomic or bimodal immunologic response of the host to an encounter with *M. tuberculosis*. The extent to which surrogate markers reflect characteristics of infection—versus "conversion"—remains unknown. It is recognized that individuals post-conversion exhibit both increased risk for progression and heterogeneity in timing of progression. Exposed individuals who do not convert may represent a "resistance to infection" phenotype. Genetic mapping efforts of *M. tuberculosis* infection offer particularly powerful approaches for identifying loci that confer resistance to infection or that control progression from conversion to clinical disease. These efforts may also be useful in fine-tuning surrogate markers to improve the accuracy of prediction of progression to clinical disease. For example, transcriptomic signatures have been identified that provide a correlate of risk for progression from conversion to disease with relatively good accuracy.[40] By including genetic markers that impact on transcript levels, so called eQTL loci, it may be possible to further improve the accuracy of these signatures. Much needs to be done in this area.

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

Despite the checkered history of serologic assays in TB, more refined understanding of the antigen binding (Fab) and constant (Fc) domains would support accurate antibody-based diagnostics. Most antibody work has focused on the Fab domain given the specificity of association with microbial antigens. Unfortunately, this approach has thus far given rise to commercial serological tests with poor sensitivity and specificity for TB infection and disease. However, it is well understood that immunogenic microbial antigens are not just proteins but also lipids and carbohydrates. Recent studies have shown that the "constant" Fc domain is actually diverse, capturing a plethora of host-associated processes.[41] More specifically, isotypes (n=5), subclasses (n=6), and post translational modifications such as glycosylation (n=at least 36) are dynamic throughout the course of disease, reflecting the host immune responses.[42] These Fc features, specifically glycosylation, have been successfully used in oncology to functionally enhance monoclonal antibody therapeutics, in rheumatology to provide more predictive and granular biomarkers, and in HIV diagnostics.[43,44,45] Overall, these important advances in our understanding of the role of antibodies suggest that further research is essential[46].

The measurement of other aspects of cellular immunity to mycobacterial infection may provide signals that discern TB infection from active disease, and hence might be used to define those with subclinical TB. As *M. tuberculosis* is an intracellular pathogen, CD8+ T-cells are uniquely poised to sample the intracellular environment. The prevalence of *M. tuberculosis*-specific CD8+ T-cells is associated with surrogates of bacterial burden such as sputum-smear positivity and is inversely correlated with treatment. Innate T-cells, such as invariant natural killer cells and mucosal associated

invariant T-cells, are diminished in the circulation of individuals with *M. tuberculosis*, possibly reflecting their movement to the site of active infection. Increased numbers of myeloid derived suppressor cells in blood of recently exposed individuals and in TB and increased numbers at the site of disease in pleural TB have been found, whereas these numbers normalize after prolonged asymptomatic infection and after successful TB treatment. These cells suppress innate and adaptive immune responses against TB.[47,48] Expression of immune activation markers such as CD38 and HLA-DR and intracellular proliferation markers such as Ki-67, on Mtb-apecific CD4 T cells have been shown to accurately discriminate between active TB disease and TB infection, suggesting that antigen-specific T cell phenotypes can be useful as biomarkers of TB disease.[49] Finally, the measurement of the functional status of *M. tuberculosis*-specific CD4+ T-cells—using either IL-2 production or cellular activation—has been demonstrated to discern TB from TB infection.[50] In summary, understanding better the host response to infection with *M. tuberculosis* has the potential to predict those at risk for progression, add specificity to RNA signatures, and guide therapy.

As high TB incidence areas are often characterized by high sensitization rates to *M. tuberculosis* antigens (i.e. TST or IGRA positivity), these tests are of limited value in selecting healthy individuals for targeted preventative treatment.[51] Recent publications have highlighted a new approach using gene signatures to predict future progression to clinically active disease in people who are apparently healthy at the time of testing.[40,52,53] The Adolescent Cohort Study, reported the generation of a risk signature from whole blood RNA sequencing followed by validation through multiplex quantitative real-time PCR in adolescents without a known exposure episode from a high TB

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

incident community. The expression of 16 mRNA transcripts constituted a correlate of risk (CoR) for active TB disease and was validated in a cohort of recent household contacts of active TB cases from South Africa and The Gambia (the GC6-74 study).[38] The CoR progression in the adolescent cohort was similar to CoR regression towards a control state in an independent TB treatment cohort that was recently reported from South Africa.[54] Subsequent further analysis of the GC6-74 cohort showed similar promising predictive performance across household contacts from multiple sites, suggesting that predictive signatures are indicative of incipient disease up to two years prior to the development of symptoms, and that signatures that perform reasonably well across different geographical areas should best be developed from data sets that include samples from multiple locations[55]. Such signatures are now being tested prospectively in clinical trials of biomarker-driven targeted preventative treatment.[40]**Error! Bookmark not defined.**

Newer discovery platforms, like next-generation biological mass spectrometry, coupled with methods for enhancing sensitivity such as exosome enrichment, are being evaluated for the detection of pathogen-specific markers across the spectrum of TB infection to disease.[56,57] Pathogen-specific diagnostics would provide a valuable alternative to surrogate marker approaches and be able to discern true “infection” in *M. tuberculosis* exposed patients from immunologic “conversion” after exposure.

Lastly, the fundamental challenge of how to optimize the evaluation of diagnostics for TB infection, for which there is no gold standard, needs to be addressed. Historically, active TB disease cohorts have been used, but the severe immunologic and pathogen features of this state are recognized as poorly reflective of the pre-disease,

infection state. Proxies for infection (e.g. based on TST or IGRA results) are also used in studies, including proposed new cut-offs for defining positive results,[58] and these can be further enhanced by determining whether a novel biomarker or diagnostic test separates the study population into two groups of size consistent with the known epidemiology of TB infection: the majority (80-95%) with stable TB infection and a minority (5-20%) identified as being at-risk (i.e., unstable TB infection or sub-clinical TB). This approach still has risk in terms of misclassification, but candidate biomarkers that achieve this goal could then be advanced to Phase 2 studies that assess changes in the candidate biomarker levels with treatment of TB infection. In the absence of a reference standard, assessing for treatment response is also a reasonable approach and is supported by prior studies demonstrating that gene expression is similar among patients who have completed treatment for TB disease and controls with TB infection.[59] Such Phase 1 and 2 studies require smaller sample sizes and shorter follow-up as compared to large epidemiologic cohorts, and could help identify potential candidate biomarkers or tests to advance to clinical impact studies that assess TB incidence.

Innovations in Treatment of Infection

Recently completed trials of duration shortening regimens for drug-susceptible TB infection have confirmed the importance of treating TB infection and provided viable alternatives to the conventional nine months of isoniazid (9H) used since the 1960's.[8,9,10,11,12] Several randomized controlled trials have shown that a new combination regimen of isoniazid (INH) and rifapentine (RPT) administered weekly for 12 weeks as directly observed therapy (DOT) or as self-administered therapy (SAT) is

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

as effective for preventing TB as other regimens and is more likely to be completed than the longer 9 months of INH daily.[60,61,62] A four month regimen of rifampicin (4R) has been evaluated in adults and children with TB infection (NCT00931736), and found not to be inferior to the standard 9-month regimen of isoniazid for the prevention of active tuberculosis and, notably, is associated with a higher rate of treatment completion and better safety.[63] Two ongoing studies are evaluating the effectiveness of preventive therapy among people living with HIV (PLHIV): A one month course of daily rifapentine and isoniazid (1HP) versus 9H (the A5279 study; NCT01404312) was recently completed and preliminary results indicate non-inferiority of the ultrashort regimen when applied under pragmatic strategy trial settings;[64] and a three arm comparison between 3HP, 9H and intermittent 3HP is being evaluated among people living with HIV (the WHIP3TB study; NCT02980016). The durability of protection achieved from a single six-month course of INH treatment for TB infection in PLHIV remains uncertain, with some trials showing up to seven years of protection,[65] while others found significant benefits from prolonged courses (36 months) of INH treatment in high-incidence settings as compared to the six-month regimen.[66] The CORTIS study is evaluating the effectiveness of 3HP for high-risk individuals, against surveillance, with the treatment decision based upon a blood-based RNA -expression profile (NCT02735590). Supervised weekly 3HP is being compared to isoniazid among pregnant and post-partum women (P2001; NCT02651259). Finally, six weeks of daily, self-administered rifapentine alone regimen is being evaluated in a new phase 3 clinical trial conducted by the CDC TB Trials Consortium.[67]

Currently, there are limited data on how best to treat contacts of individuals with multidrug-resistant (MDR)- and extensively drug-resistant (XDR)-TB. Several drugs or combinations of drugs including fluoroquinolones, ethambutol, pyrazinamide and ethionamide have been tested in modest sized trials that have not provided adequate evidence to impact practice guidelines. Currently, three large, randomized controlled trials are underway. Six months of daily levofloxacin is being compared to placebo for contacts of individuals with MDR-TB in the VQUIN MDR Trial (ACTRN12616000215426, in adults), and the TB CHAMP study (ISRCTN92634082, in children under five years). In the PHOENIX MDR-TB Trial, six months of daily delamanid will be compared with six months of daily isoniazid for contacts of individuals with MDR- or XDR-TB.

Current treatment studies focus on the use of orally-administered antibiotics for which susceptibility is presumed, however, there is interest in evaluating novel, non-antibiotic based approaches to treating infection and halting progression to disease, as well as alternative drug-delivery mechanisms. The idea of using host-directed therapies as adjunctive therapies for TB has gained traction.[68] Theoretically, host-directed therapies can address the challenges associated with antibiotic resistance, as bacterial mutations will be incapable of directly abrogating binding of the drug to a host target. In addition, these therapies open a new landscape of ways to harness or alter host immunity to attack the pathogen. A number of host-directed therapies have been proposed based on modulation of detrimental inflammation – more classically considered in the context of clinically apparent disease – including alterations of eicosanoid networks, lipid metabolism, and autophagy, among others.[69,70,71] Questions

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

remain about whether these newer host-directed approaches would be effective for addressing the spectrum of infection, where the inflammatory response may be different from active disease. Answering these questions is an important first step in determining the role of adjunctive host-directed medications or therapeutic vaccines in the prevention of progression from infection to disease.[5]

There remain a number of challenges in efficiently transforming pre-clinical results and clinical observations into specific adjunctive therapies, particularly given the heterogeneity in disease presentation and progression. Cell culture, animal models, human clinical data and long-term experiments in non-human primates (e.g. cynomolgus macaques or rhesus macaques, where infection with *M. tuberculosis* results in similar outcomes as the human infection/disease spectrum) are needed to capture the full spectrum of possibilities with adjunctive therapies. Ultimately, novel adjunctive therapies will require initial assessment of safety, consideration of potential interactions with front-line anti-TB drugs, and knowledge of efficacy in pre-clinical models.

Ensuring that treatment can be delivered is also a critical area of inquiry. Long-acting/extended release drug formulations have proved very successful in diverse areas of medicine including contraception, psychiatry, and most recently, HIV disease. While challenging, application of this technology to TB treatment could have substantial impact by improving treatment adherence. Although the properties of some TB drugs make them unsuitable for long-acting formulation, promising candidates have been identified through modelling and simulations.[72,73,74,75] An efficacious delivery mechanism for TB infection, particularly for those co-infected with HIV, would be an

important tool to accelerate progress towards TB elimination. Progress in this area is closely linked to the identification of markers associated with disease clearance and progression.

Understanding host-pathogen interactions in the setting of high transmission pressure is also an important facet of combatting the disease. While many of the early studies on preventive therapy were undertaken in high-burden settings,[8,9,10,11,12] and models of disease elimination have underscored the importance of using a comprehensive epidemic control approach strategy that includes active case finding, the treatment of active disease, and the treatment of infected contacts,[76,77,78] research is required to better understand how targeted identification of infected individuals in "hotspots" and other epidemiological contexts could advance the goal of TB elimination.[79]

Lastly it must be remembered that most of the studies on the relationship between *M. tuberculosis* and its human host have been conducted in adults. There are an estimated 10 million new pediatric TB infections each year.[80] Once infected with *M. tuberculosis* the risk of disease progression is much higher in children especially those <5 years of age.[81] Because of differences in host immunity between young children and older children and adults, this population faces a different series of gaps in diagnostics and therapeutics. Studying TB infection in children—who usually have a defined contact and hence defined time since exposure—is a unique "challenge model" to examine time-and possibly strain-related infection progression and protection.[82]

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

Conclusions

TB elimination can only be achieved if the reservoir of infected individuals at high risk of progression to disease are identified and treated. Recent advances in our understanding of host-pathogen interactions in TB, coupled with newer approaches for diagnosis, predictive biomarkers of risk for progression, and the promise of shorter duration treatments, adjunctive therapies and innovative modalities for drug-delivery are providing a new horizon for research. For the first time, the collective of these advances provide an exciting and viable platform on which to build the next generation of interventions for identifying and treating TB infection.

Funding

This publication and workshop have been funded in whole or in part with Federal Funds from the Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health (NIH), Department of Health and Human Services under contract number HHSN272201600001G Research Support Services for the Division of AIDS. Harvard Medical School Center for Global Health Delivery–Dubai received a grant from the Dubai Harvard Foundation for Medical Research. PN supported by the National Institutes of Health through National Institute of Allergy and Infectious Diseases funding (1R01AI104589: TB Surrogate Markers for Assessing Response to Treatment (TB SMART Study)), and by the Centers for Disease Control and Prevention, TB Trials Consortium Contract 200-2009-32597. Susan Swindells was supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under Award Number R24 AI 118397. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgements

The authors would like to thank the workshop participants for contributing to productive discussions relevant to this work. In addition, we would like to thank Ms. Suchitra Kulkarni from Department of Global Health and Social Medicine, Harvard Medical

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

School and Ms. Kathleen Muldoon from Division of AIDS, National Institute of Allergy and Infectious Diseases for their work towards developing this manuscript.

Table 1. Key research areas and questions in diagnostics and therapeutics.

1. Defining the spectrum between *M. tuberculosis* infection and clinically apparent disease, and identifying genetic and immunological markers associated with progression to disease?
• Where in the host is the *M. tuberculosis* population during TB infection, are there niche tissues, and do different locations and environments affect bacterial behavior, host response, and treatment response? • What is the physiologic and metabolic state of the population of *M. tuberculosis* along the spectrum between infection and clinically evident disease, and could this be used to target individuals for treatment? • Are there host and pathogen genetic markers ("gene signatures") that define these states, what is the interplay between the two? • Are there host immunological/cellular function responses that define these states? • Are their protein biomarkers that define these states? • Can any markers be identified that indicate the presence of live *M. tuberculosis*? • Can markers of bacterial clearance in response to chemotherapy or therapeutic vaccines be identified? • Are genetic, immunological and protein biomarkers the same between adults and children, and how can differenced between age groups be used to better understand the spectrum between TB infection and disease? • How is progression along the spectrum to disease affected by host and pathogen genetic diversity? • What are the correlates of protection from TB disease? • How do the above processes differ between young children and older children/adults, and what bearing will this have on identification of infection in this population?
2. Optimizing treatment for infection with *M. tuberculosis*?
• Which drugs and regimens are optimal for treatment of incipient forms of TB? • What are the drug mechanisms of action that could safely shorten duration of treatment for TB infection to 4 weeks or less?

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

- Is there a role for host-directed and adjunctive therapy to modulate inflammation, lipid metabolism, autophagy, and other cellular networks, to disrupt the transition between TB infection and disease?
- How can host-directed and adjunctive therapies be used to reduce the duration of treatment for TB infection?
- What anti-TB drugs are amenable to long-acting/extended release formulations that will help maintaining more constant antibiotic coverage and help with treatment adherence?
- What anti-TB drugs are amenable to alternative treatment delivery mechanisms (e.g. drug patches, depo injections)?
- What drugs should we use to treat individuals suspected of being infected with multidrug-resistant (MDR) strains of TB?
- What molecular markers, immunological responses and protein biomarkers can help define when infection and/or disease has been successfully treated?
- Do children and adults require the same treatments and durations for TB infection?

[1] World Health Organization. Tuberculosis fact sheet. 2016; http://www.who.int/mediacentre/factsheets/fs104/en/, November 2, 2016

[2] Jenkins HE, Pagano M, Yuen CM, Becerra MC, Cohen T. The burden of tuberculosis disease in children--Authors' reply. Lancet. 2014 Oct 11;384(9951):1343-4. doi:10.1016/S0140-6736(14)61811-0. PubMed PMID: 25307837.

[3] Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. *PLoS medicine.* 2016;13(10):e1002152.

[4] Rangaka MX, Cavalcante SC, Marais BJ, et al. Controlling the seedbeds of tuberculosis: diagnosis and treatment of tuberculosis infection. *The Lancet.*386(10010):2344-2353.

[5] Salgame P, Geadas C, Collins L, Jones-López E, Ellner JJ. Tuberculosis (Edinb). Latent tuberculosis infection--Revisiting and revising concepts. 2015 Jul; 95(4):373-84. doi: 0.1016/j.tube.2015.04.003. Epub 2015 May 15.

[6] Parekh MJ, Schluger NW. Treatment of latent tuberculosis infection. Ther Av Respir Dis. 2013 Dec; 7(6): 351-6.

[7] Getahun H, Matteelli A, Chaisson RE, Raviglione M. Latent Mycobacterium tuberculosis infection. N Engl J Med. 2015 May 28; 372(22): 2127-35.

[8] Smieja MJ, Marchetti CA, Cook DJ, Smaill FM. Isoniazid for preventing tuberculosis in non-HIV infected persons. Cochrane Database Syst Rev. 2000;(2):CD001363. Review. PubMed PMID: 10796642.

[9] Akolo C, Adetifa I, Shepperd S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. Cochrane Database Syst Rev. 2010 Jan 20;(1):CD000171. doi: 10.1002/14651858.CD000171.pub3. Review. PubMed PMID: 20091503.

[10] Kaplan GJ, Fraser RI, Comstock GW. (1972). Tuberculosis in Alaska, 1970: The continued decline of the tuberculosis epidemic. *American Review of Respiratory Disease*, Jun;105(6):920-6.

[11] Comstock GW, Baum C, Snider DE Jr. (1979). Isoniazid Prophylaxis among Alaskan Eskimos: A Final Report of the Bethel Isoniazid Studies. *American Review of Respiratory Disease*, May;119(5):827-30.

[12] Ferebee SH. (1970). Controlled chemoprophylaxis trials in tuberculosis. *Bibliotheca tuberculosea et medicinae thoracalis*, 26:28-106.

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

[13] Lillebaek T, Dirksen A, Baess I, Strunge B, Thomsen VØ, Andersen ÅB. Molecular evidence of endogenous reactivation of Mycobacterium tuberculosis after 33 years of latent infection. *The Journal of infectious diseases.* 2002;185(3):401-404.

[14] Barry, C. E., 3rd, Boshoff, H. I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., Schnappinger, D., Wilkinson, R. J. & Young, D. 2009. The Spectrum Of Latent Tuberculosis: Rethinking The Biology And Intervention Strategies. *Nat Rev Microbiol,* 7**,** 845-55.

[15] Ernst JD. The Immunological Life Cycle Of Tuberculosis. Nat Rev Immunol. 2012;12(8):581-91.

[16] Scriba, T. J., Penn-Nicholson, A., Shankar, S., Hraha, T., Thompson, E. G., Sterling, D., Nemes, E., Darboe, F., Suliman, S., Amon, L. M., Mahomed, H., Erasmus, M., Whatney, W., Johnson, J. L., Boom, W. H., Hatherill, M., Valvo, J., De Groote, M. A., Ochsner, U. A., Aderem, A., Hanekom, W. A., Zak, D. E. & Other Members Of The, A. C. S. C. S. T. 2017. Sequential Inflammatory Processes Define Human Progression From M. Tuberculosis Infection To Tuberculosis Disease. *Plos Pathog,* 13**,** E1006687.

[17] Pai, M. 2010. Spectrum Of Latent Tuberculosis - Existing Tests Cannot Resolve The Underlying Phenotypes. *Nat Rev Microbiol,* 8**,** 242; Author Reply 242.

[18] Nuermberger, E., Bishai, W. R. & Grosset, J. H. 2004. Latent Tuberculosis Infection. *Semin Respir Crit Care Med,* 25**,** 317-36.

[19] Dutta, N. K. & Karakousis, P. C. 2014. Latent Tuberculosis Infection: Myths, Models, And Molecular Mechanisms. *Microbiol Mol Biol Rev,* 78**,** 343-71.

[20] Schraufnagel DE. 2016. "Latent Tuberculosis Infection" Is A Term That Should Go Dormant, And The Significance Of Latent Tuberculosis Should Be Rethought. *Ann Am Thorac Soc,* 13**,** 593-4.

[21] Pandey AK, Sassetti CM. Mycobacterial Persistence Requires The Utilization Of Host Cholesterol. 2008. *Proc Natl Acad Sci U S A,* 105**,** 4376-80.

[22] Munoz-Elias EJ, McKinney JD. Carbon Metabolism Of Intracellular Bacteria. 2006. *Cell Microbiol,* 8**,** 10-22.

[23] Baek SH, Li AH, Sassetti CM. Metabolic Regulation Of Mycobacterial Growth And Antibiotic Sensitivity. 2011 *Plos Biol,* 9**,** E1001065.

[24] Boshoff HI, Barry CE 3rd. Tuberculosis - Metabolism And Respiration In The Absence Of Growth. 2005 *Nat Rev Microbiol,* 3**,** 70-80.

[25] Cunningham-Bussel A, Zhang T, Nathan CF. Nitrite Produced By Mycobacterium Tuberculosis In Human Macrophages In Physiologic Oxygen Impacts Bacterial ATP Consumption And Gene Expression. 2013; *Proc Natl Acad Sci U S A,* 110**,** E4256-65.

[26] Gouzy A, Poquet Y, Neyrolles O. Nitrogen Metabolism In Mycobacterium Tuberculosis Physiology And Virulence. 2014 *Nat Rev Microbiol,* 12**,** 729-37.

[27] Kesavan AK, Brooks M, Tufariello J, Chan J, Manabe YC. Tuberculosis Genes Expressed During Persistence And Reactivation In The Resistant Rabbit Model. 2009;*Tuberculosis (Edinb),* 89**,** 17-21.

[28] Muttucumaru DG, Roberts G, Hinds J, Stabler RA, Parish T. Gene Expression Profile Of Mycobacterium Tuberculosis In A Non-Replicating State. 2004; *Tuberculosis (Edinb),* 84**,** 239-46

[29] Via LE, Lin PL, Ray SM, Carrillo J, Allen SS, Eum SY, Taylor K, Klein E, Manjunatha U, Gonzales J, Lee EG, Park SK, Raleigh JA, Cho SN, McMurray DN, Flynn JL, Barry CE 3rd. Tuberculous Granulomas Are Hypoxic In Guinea Pigs, Rabbits, And Nonhuman Primates. 2008; *Infect Immun,* 76**,** 2333-40.

[30] Mortaz E, Adcock IM, Tabarsi P, Masjedi MR, Mansouri D, Velayati AA, Casanova JL, Barnes PJ. Interaction Of Pattern Recognition Receptors With Mycobacterium Tuberculosis. J Clin Immunol. 2015;35(1):1-10. Doi: 10.1007/S10875-014-0103-7. Pubmed PMID: 25312698; PMCID: PMC4306732.

[31] Tobin DM, Roca FJ, Oh SF, McFarland R, Vickery TW, Ray JP, Ko DC, Zou Y, Bang ND, Chau TT, Vary JC, Hawn TR, Dunstan SJ, Farrar JJ, Thwaites GE, King MC, Serhan CN, Ramakrishnan L. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. Cell. 2012 Feb 3;148(3):434-46. doi: 10.1016/j.cell.2011.12.023. PubMed PMID: 22304914; PubMed Central PMCID: PMC3433720.

[32] Portevin D, Gagneux S, Comas I, Young D. Human macrophage responses to clinical isolates from the Mycobacterium tuberculosis complex discriminate between ancient and modern lineages. PLoS Pathog. 2011;7(3):e1001307. doi: 10.1371/journal.ppat.1001307. PubMed PMID: 21408618; PMCID: PMC3048359.

[33] Krishnan N, Malaga W, Constant P, Caws M, Tran TH, Salmons J, Nguyen TN, Nguyen DB, Daffe M, Young DB, Robertson BD, Guilhot C, Thwaites GE.

Mycobacterium tuberculosis lineage influences innate immune response and virulence and is associated with distinct cell envelope lipid profiles. PLoS One. 2011;6(9):e23870. doi: 10.1371/journal.pone.0023870. PubMed PMID: 21931620; PMCID: PMC3169546.

[34] Carmona J, Cruz A, Moreira-Teixeira L, Sousa C, Sousa J, Osorio NS, Saraiva AL, Svenson S, Kallenius G, Pedrosa J, Rodrigues F, Castro AG, Saraiva M. Mycobacterium tuberculosis Strains Are Differentially Recognized by TLRs with an Impact on the Immune Response. PLoS One. 2013;8(6):e67277. doi: 10.1371/journal.pone.0067277. PubMed PMID: 23840651; PMCID: PMC3693941.

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

[35] Caws M, Thwaites G, Dunstan S, Hawn TR, Lan NT, Thuong NT, Stepniewska K, Huyen MN, Bang ND, Loc TH, Gagneux S, van Soolingen D, Kremer K, van der Sande M, Small P, Anh PT, Chinh NT, Quy HT, Duyen NT, Tho DQ, Hieu NT, Torok E, Hien TT, Dung NH, Nhu NT, Duy PM, van Vinh Chau N, Farrar J. The influence of host and bacterial genotype on the development of disseminated disease with Mycobacterium tuberculosis. PLoS Pathog. 2008 Mar 28;4(3):e1000034. doi: 10.1371/journal.ppat.1000034. PubMed PMID: 18369480; PubMed Central PMCID: PMC2268004.

[36] Kahwati LC, Feltner C, Halpern M, Woodell CL, Boland E, Amick HR, Weber RP, Jonas DE. Screening for Latent Tuberculosis Infection in Adults: An Evidence Review for the U.S. Preventive Services Task Force. Evidence Synthesis No. 142. AHRQ Publication No. 14-05212EF-1. Rockville, MD: Agency for Healthcare Research and Quality; 2016.

[37] Adekambi T, Ibegbu CC, Cagle S, Kalokhe AS, Wang YF, Hu Y, Day CL, Ray SM, Rengarajan J. Biomarkers on patient T cells diagnose active tuberculosis and monitor treatment response. J Clin Invest. 2015 Sep;125(9):3723. doi: 10.1172/JCI83279. Epub 2015 Sep 1. PubMed PMID: 26325038; PubMed Central PMCID: PMC4588276.

[38] Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, Mahomed H, Erasmus M, Whatney W, Hussey GD, Abrahams D, Kafaar F, Hawkridge T, Verver S, Hughes EJ, Ota M, Sutherland J, Howe R, Dockrell HM, Boom WH, Thiel B, Ottenhoff THM, Mayanja-Kizza H, Crampin AC, Downing K, Hatherill M, Valvo J, Shankar S, Parida SK, Kaufmann SHE, Walzl G, Aderem A, Hanekom WA; ACS and GC6-74 cohort study groups. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet. 2016 Jun 4;387(10035):2312-2322. doi: 10.1016/S0140-6736(15)01316-1. Epub 2016 Mar 24. PubMed PMID: 27017310; PubMed Central PMCID: PMC5392204.

[39] Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ, Quinn C, Blankenship D, Dhawan R, Cush JJ, Mejias A, Ramilo O, Kon OM, Pascual V, Banchereau J, Chaussabel D, O'Garra A. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. 2010 Aug 19;466(7309):973-7. doi: 10.1038/nature09247. PubMed PMID: 20725040; PubMed Central PMCID: PMC3492754.

[40] Suliman S, Thompson E, Sutherland J, Weiner Rd J, Ota MOC, Shankar S, Penn-Nicholson A, Thiel B, Erasmus M, Maertzdorf J, Duffy FJ, Hill PC, Hughes EJ, Stanley K, Downing K, Fisher ML, Valvo J, Parida SK, van der Spuy G, Tromp G, Adetifa IMO, Donkor S, Howe R, Mayanja-Kizza H, Boom WH, Dockrell H, Ottenhoff THM, Hatherill M, Aderem A, Hanekom WA, Scriba TJ, Kaufmann SH, Zak DE, Walzl G; and the GC6-74 and ACS cohort study groups. Four-gene Pan-African Blood Signature Predicts Progression to Tuberculosis. Am J Respir Crit Care Med. 2018 Apr 6. doi: 10.1164/rccm.201711-2340OC. [Epub ahead of print] PubMed PMID: 29624071.

[41] Dambrun M, Dechavanne C, Emmanuel A, Aussenac F, Leduc M, Giangrande C, Vinh J, Dugoujon JM, Lefranc MP, Guillonneau F, Migot-Nabias F. Human Immunoglobulin Heavy Gamma Chain Polymorphisms: Molecular Confirmation Of Proteomic Assessment. Mol Cell Proteomics. 2017 May;16(5):824-839. doi: 10.1074/mcp.M116.064733. Epub 2017 Mar 6. PubMed PMID: 28265047; PubMed Central PMCID: PMC5417824.

[42] Yu X, Chan HTC, Orr CM, Dadas O, Booth SG, Dahal LN, Penfold CA, O'Brien L, Mockridge CI, French RR, Duriez P, Douglas LR, Pearson AR, Cragg MS, Tews I, Glennie MJ, White AL. Complex Interplay between Epitope Specificity and Isotype Dictates the Biological Activity of Anti-human CD40 Antibodies. Cancer Cell. 2018 Apr 9;33(4):664-675.e4. doi: 10.1016/j.ccell.2018.02.009. Epub 2018 Mar 22. PubMed PMID: 29576376; PubMed Central PMCID: PMC5896247.

[43] Richardson SI, Chung AW, Natarajan H, Mabvakure B, Mkhize NN, Garrett N, Abdool Karim S, Moore PL, Ackerman ME, Alter G, Morris L. HIV-specific Fc effector function early in infection predicts the development of broadly neutralizing antibodies. PLoS Pathog. 2018 Apr 9;14(4):e1006987. doi: 10.1371/journal.ppat.1006987. eCollection 2018 Apr. PubMed PMID: 29630668; PubMed Central PMCID: PMC5908199.

[44] Alter G, Dowell KG, Brown EP, Suscovich TJ, Mikhailova A, Mahan AE, Walker BD, Nimmerjahn F, Bailey-Kellogg C, Ackerman ME. High-resolution definition of humoral immune response correlates of effective immunity against HIV. Mol Syst Biol. 2018 Mar 26;14(3):e7881. doi: 10.15252/msb.20177881. PubMed PMID: 29581149; PubMed Central PMCID: PMC5868198.

[45] Kaifu T, Nakamura A. Polymorphisms of immunoglobulin receptors and the effects on clinical outcome in cancer immunotherapy and other immune diseases: a general review. Int Immunol. 2017 Jul 1;29(7):319-325. doi: 10.1093/intimm/dxx041. Review. PubMed PMID: 28910969.

[46] Cell. 2016 Oct 6;167(2):433-443.e14. doi: 10.1016/j.cell.2016.08.072. Epub 2016 Sep 22. A Functional Role for Antibodies in Tuberculosis. Lu LL1, Chung AW2, Rosebrock TR3, Ghebremichael M4, Yu WH5, Grace PS4, Schoen MK4, Tafesse F4, Martin C3, Leung V3, Mahan AE4, Sips M6, Kumar MP7, Tedesco J4, Robinson H4, Tkachenko E4, Draghi M4, Freedberg KJ4, Streeck H8, Suscovich TJ4, Lauffenburger DA7, Restrepo BI9, Day C10, Fortune SM11, Alter G12.

[47] Du Plessis N, Jacobs R, Gutschmidt A, Fang Z, van Helden PD, Lutz MB, Hesseling AC, Walzl G. Phenotypically resembling myeloid derived suppressor cells are increased in children with HIV and exposed/infected with Mycobacterium tuberculosis. Eur J Immunol. 2017. 47(1):107-118. doi: 10.1002/eji.201646658. PMID: 27861788

[48] Du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, van Helden PD, Lutz MB, Walzl G. Increased Frequency of Myeloid Derived

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

Suppressor Cells during Active Tuberculosis and Following Recent Mycobacterium tuberculosis Infection Suppresses T Cell Function. *Am J Respir Crit Care Med*. 2013; 188(6):724-32. doi: 10.1164/rccm.201302-0249OC. PMID: 23885784

[49] Adekambi T, Ibegbu CC, Cagle S, Kalokhe AS, Wang YF, Hu Y, Day CL, Ray SM, Rengarajan J. Biomarkers on patient T cells diagnose active tuberculosis and monitor treatment response. J Clin Invest. 2015 Sep;125(9):3723. doi: 10.1172/JCI83279. Epub 2015 Sep 1. PubMed PMID: 26325038; PubMed Central PMCID: PMC4588276.

[50] Wergeland I, Assmus J, Dyrhol-Riise AM. Cytokine Patterns in Tuberculosis Infection; IL-1ra, IL-2 and IP-10 Differentiate Borderline QuantiFERON-TB Samples from Uninfected Controls. van Zyl-Smit R, ed. *PLoS ONE*. 2016;11(9):e0163848. doi:10.1371/journal.pone.0163848.

[51] Rangaka MZ, Wilkinson KA, Glynn JR, Ling D, Menzies D, Mwansa-Kambafwile J, Fielding K, Wilkinson RJ, Pai M. Predictive value of interferon-γ release assays for incident active tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012 Jan; 12(1):45-55.

[52] Pan L, Wei N, Jia H, Gao M, Chen X, Wei R, Sun Q, Gu S, Du B, Xing A, Zhang Z. Genome-wide transcriptional profiling identifies potential signatures in discriminating active tuberculosis from latent infection. Oncotarget. 2017 Dec 4;8(68):112907-112916. doi: 10.18632/oncotarget.22889. eCollection 2017 Dec 22. PubMed PMID: 29348876; PubMed Central PMCID: PMC5762561.

[53] Thompson EG, Shankar S, Gideon HP, Braun J, Valvo J, Skinner JA, Aderem A, Flynn JL, Lin PL, Zak DE. Prospective Discrimination of Controllers From Progressors Early After Low-Dose Mycobacterium tuberculosis Infection of Cynomolgus Macaques using Blood RNA Signatures. J Infect Dis. 2018 Mar 28; 217(8):1318-1322. doi: 10.1093/infdis/jiy006. PubMed PMID: 29325117.

[54] Thompson EG, Du Y, Malherbe ST, Shankar S, Braun J, Valvo J, Ronacher K, Tromp G, Tabb DL, Alland D, Shenai S, Via LE, Warwick J, Aderem A, Scriba TJ, Winter J, Walzl G, Zak DE; Catalysis TB–Biomarker Consortium. Host blood RNA signatures predict the outcome of tuberculosis treatment. Tuberculosis (Edinb). 2017 Dec;107:48-58. doi: 10.1016/j.tube.2017.08.004. Epub 2017 Aug 12. PubMed PMID: 29050771; PubMed Central PMCID: PMC5658513.

[55] Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ, Quinn C, Blankenship D, Dhawan R, Cush JJ, Mejias A, Ramilo O, Kon OM, Pascual V, Banchereau J, Chaussabel D, O'Garra A. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. 2010 Aug 19;466(7309):973-7. doi: 10.1038/nature09247. PubMed PMID: 20725040; PubMed Central PMCID: PMC3492754.

[56] Mehaffy C, Dobos KM, Nahid P, Kruh-Garcia NA. Clin Proteomics. 2017 Jun 5;14:21. doi: 10.1186/s12014-017-9156-y. eCollection 2017. PMID: 28592925

[57] Kruh-Garcia NA, Wolfe LM, Dobos KM. Deciphering the role of exosomes in tuberculosis. Tuberculosis (Edinb). 2015 Jan;95(1):26-30.

[58] Nemes E, Rozot V, Geldenhuys H, Bilek N, Mabwe S, Abrahams D, Makhethe L, Erasmus M, Keyser A, Toefy A, Cloete Y, Ratangee F, Blauenfeldt T, Ruhwald M, Walzl G, Smith B, Loxton AG, Hanekom WA, Andrews JR, Lempicki MD, Ellis R, Ginsberg AM, Hatherill M, Scriba TJ; C-040-404 Study Team and the Adolescent Cohort Study Team. Optimization and Interpretation of Serial QuantiFERON Testing to Measure Acquisition of Mycobacterium tuberculosis Infection. Am J Respir Crit Care Med. 2017 Sep 1;196(5):638-648. doi: 10.1164/rccm.201704-0817OC.

[59] Sutherland JS, Loxton AG, Haks MC, Kassa D, Ambrose L, Lee JS, Ran L, van Baarle D, Maertzdorf J, Howe R, Mayanja-Kizza H, Boom WH, Thiel BA, Crampin AC, Hanekom W, Ota MO, Dockrell H, Walzl G, Kaufmann SH, Ottenhoff TH; GCGH Biomarkers for TB consortium. Differential gene expression of activating Fcγ receptor classifies active tuberculosis regardless of human immunodeficiency virus status or ethnicity. Clin Microbiol Infect. 2014 Apr;20(4):O230-8. doi: 10.1111/1469-0691.12383. Epub 2013 Nov 11. PubMed PMID: 24205913.

[60] Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, Bliven-Sizemore E, Hackman J, Hamilton CD, Menzies D, Kerrigan A, Weis SE, Weiner M, Wing D, Conde MB, Bozeman L, Horsburgh CR Jr, Chaisson RE; TB Trials Consortium PREVENT TB Study Team. Three months of rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med. 2011 Dec 8;365(23):2155-66. doi: 10.1056/NEJMoa1104875. PubMed PMID: 22150035.

[61] Martinson NA, Barnes GL, Moulton LH, Msandiwa R, et al. New Regimens to Prevent Tuberculosis in Adults with HIV infection. N Engl J Med 2011; 365:11-20.

[62] Belknap R, Holland D, Feng PJ, Millet JP, et al. Self-administered Versus Directly Observed Once-Weekly Isoniazid and Rifapentine Treatment of Latent Tuberculosis Infection: A randomized Trial. Ann Intern Med. 2017 Nov 21; 167(10):689-697.

[63] Menzies D, Adjobimey M, Ruslami R, Trajman A, Sow O, Kim H, Obeng Baah J, Marks GB, Long R, Hoeppner V, Elwood K, Al-Jahdali H, Gninafon M, Apriani L, Koesoemadinata RC, Kritski A, Rolla V, Bah B, Camara A, Boakye I, Cook VJ, Goldberg H, Valiquette C, Hornby K, Dion MJ, Li PZ, Hill PC, Schwartzman K, Benedetti A. Four Months of Rifampin or Nine Months of Isoniazid for Latent Tuberculosis in Adults. N Engl J Med. 2018 Aug 2;379(5):440-453. doi: 10.1056/NEJMoa1714283.

[64] Swindells et al. CROI 2018, Abstract 37LB. One Month of Rifapentine/Isoniazid to Prevent TB in People with HIV: BRIEF-TB/A5279.

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

[65] Golub JE, Cohn S, Saraceni V, Cavalcante SC, Pacheco AG, Moulton LH, Durovni B, Chaisson RE. Long-term protection from isoniazid preventive therapy for tuberculosis in HIV-infected patients in a medium-burden tuberculosis setting: the TB/HIV in Rio (THRio) study. Clin Infect Dis. 2015 Feb 15;60(4):639-45. doi: 10.1093/cid/ciu849. Epub 2014 Nov 2.

[66] Samandari T, Agizew TB, Nyirenda S, Tedla Z, Sibanda T, Shang N, Mosimaneotsile B, Motsamai OI, Bozeman L, Davis MK, Talbot EA, Moeti TL, Moffat HJ, Kilmarx PH, Castro KG, Wells CD. 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. Lancet. 2011 May 7;377(9777):1588-98. doi: 10.1016/S0140-6736(11)60204-3. Epub 2011 Apr 12.

[67] Assessment of the Safety, Tolerability, and Effectiveness of Rifapentine Given Daily for LTBI (ASTERoiD)": NCT03474029

[68] Tiberi S, du Plessis N, Walzl G, Vjecha MJ, Rao M, Ntoumi F, Mfinanga S, Kapata N, Mwaba P, McHugh TD, Ippolito G, Migliori GB, Maeurer MJ, Zumla A. Tuberculosis: progress and advances in development of new drugs, treatment regimens, and host-directed therapies. Lancet Infect Dis. 2018 Jul;18(7):e183-e198. doi: 10.1016/S1473-3099(18)30110-5. Epub 2018 Mar 23. Review. Erratum in: Lancet Infect Dis. 2018 Apr 27;:

[69] Zumla A, Rao M, Parida SK, Keshavjee S, Cassell G, Wallis R, Axelsson-Robertsson R, Doherty M, Andersson J, Maeurer M. Inflammation and tuberculosis: host-directed therapies. J Intern Med. 2015 Apr;277(4):373-87. doi 10.1111/joim.12256. Epub 2014 May 19. Review. PubMed PMID: 24717092.

[70] Kaufmann SH, Lange C, Rao M, Balaji KN, Lotze M, Schito M, Zumla AI, Maeurer M. Progress in tuberculosis vaccine development and host-directed therapies—a state of the art review. Lancet Respir Med. 2014 Apr;2(4):301-20. doi:10.1016/S2213-2600(14)70033-5. Epub 2014 Mar 24. Review. PubMed PMID: 24717627.

[71] Wallis RS, Maeurer M, Mwaba P, Chakaya J, Rustomjee R, Migliori GB, Marais B, Schito M, Churchyard G, Swaminathan S, Hoelscher M, Zumla A. Tuberculosis--advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis. 2016 Apr;16(4):e34-46. doi: 10.1016/S1473-3099(16)00070-0. Review. PubMed PMID:27036358.

[72] Rajoli RKR, Podany AT, Moss DM, Swindells S, Flexner C, Owen A, Siccardi M. Modelling the long-acting administration of anti-tuberculosis agents using PBPK: a proof of concept study. Int J Tuberc Lung Dis. 2018 Aug 1;22(8):937-944. doi: 10.5588/ijtld.17.0515. PMID: 29991405

[73] Swindells S1, Siccardi M2, Barrett SE3, Olsen DB4, Grobler JA4, Podany AT1, Nuermberger E5, Kim P6, Barry CE7, Owen A2, Hazuda D4, Flexner C5.Long-acting formulations for the treatment of latent tuberculous infection: opportunities and challenges. Int J Tuberc Lung Dis. 2018 Feb 1;22(2):125-132. doi: 10.5588/ijtld.17.0486.

[74] Kailasam S, Wise DL, Gangadharam PR. Bioavailability and chemotherapeutic activity of clofazimine against Mycobacterium avium complex infections in beige mice following a single implant of a biodegradable polymer. J Antimicrob Chemother. 1994 Feb;33(2):273-9.

[75] Gangadharam PR, Kailasam S, Srinivasan S, Wise DL. Experimental chemotherapy of tuberculosis using single dose treatment with isoniazid in biodegradable polymers. J Antimicrob Chemother. 1994 Feb;33(2):265-71.

[76] Dye C, Glaziou P, Floyd K, Raviglione M. Prospects for tuberculosis elimination. Annu Rev Public Health. 2013;34:271-86. doi: 10.1146/annurev-publhealth-031912-114431. Epub 2012 Dec 14. Review. PMID: 23244049

[77] Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM Jr, Dye C, Halloran ME.Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc Natl Acad Sci U S A. 2009 Aug 18;106(33):13980-5. doi: 10.1073/pnas.0901720106. Epub 2009 Aug 3. PMID: 19666590

[78] Reid A, Grant AD, White RG, Dye C, Vynnycky E, Fielding K, Churchyard G, Pillay Y. Accelerating progress towards tuberculosis elimination: the need for combination treatment and prevention. Int J Tuberc Lung Dis. 2015 Jan;19(1):5-9. doi: 10.5588/ijtld.14.0078. PMID: 25519784

[79] Lönnroth K, Migliori GB, Abubakar I, D'Ambrosio L, de Vries G, Diel R, Douglas P, Falzon D, Gaudreau MA, Goletti D, González Ochoa ER, LoBue P, Matteelli A, Njoo H, Solovic I, Story A, Tayeb T, van der Werf MJ, Weil D, Zellweger JP, Abdel Aziz M, Al Lawati MR, Aliberti S, Arrazola de Oñate W, Barreira D, Bhatia V, Blasi F, Bloom A, Bruchfeld J, Castelli F, Centis R, Chemtob D, Cirillo DM, Colorado A, Dadu A, Dahle UR, De Paoli L, Dias HM, Duarte R, Fattorini L, Gaga M, Getahun H, Glaziou P, Goguadze L, Del Granado M, Haas W, Järvinen A, Kwon GY, Mosca D, Nahid P, Nishikiori N, Noguer I, O'Donnell J, Pace-Asciak A, Pompa MG, Popescu GG, Robalo Cordeiro C, Rønning K, Ruhwald M, Sculier JP, Simunović A, Smith-Palmer A, Sotgiu G, Sulis G, Torres-Duque CA, Umeki K, Uplekar M, van Weezenbeek C, Vasankari T, Vitillo RJ, Voniatis C, Wanlin M, Raviglione MC. Towards tuberculosis elimination: an action framework for low-incidence countries. Eur Respir J. 2015 Apr;45(4):928-52. doi: 10.1183/09031936.00214014. Review. PMID: 25792630

AJRCCM Articles in Press. Published on 18-October-2018 as 10.1164/rccm.201806-1053PP

[80] Dodd PJ, Gardiner E, Coghlan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. The Lancet Global health. 2014;2(8):e453-9

[81] Marais, B. J., et al. (2004). "The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era." Int J Tuberc Lung Dis 8(4): 392-402.

[82] Basu Roy, R., et al. (2012). "Current understanding of the immune response to tuberculosis in children." Curr Opin Infect Dis 25(3): 250-257.

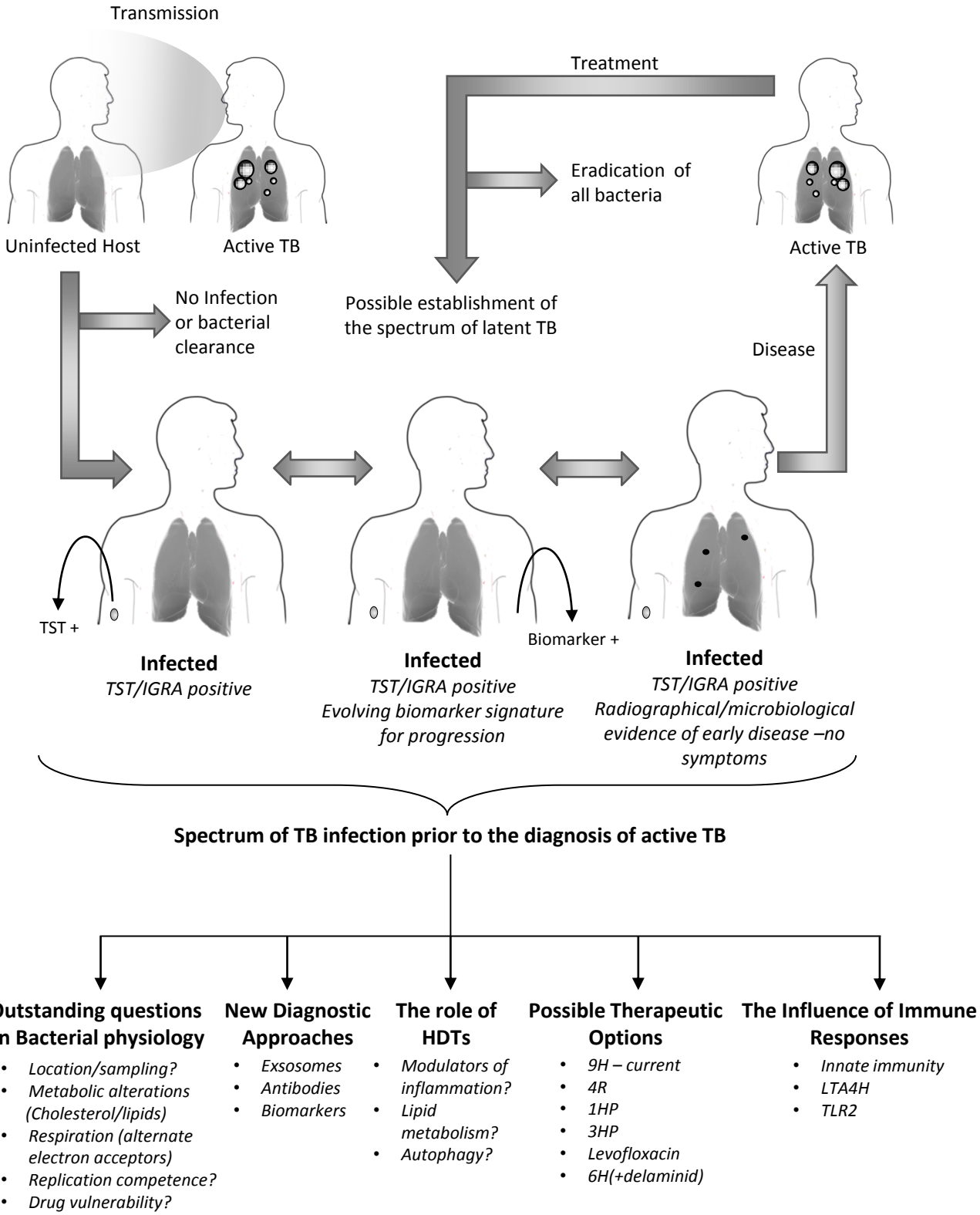

Figure 1. Spectrum of clinical states that comprise asymptomatic TB infection. Shown are the various manifestations of infection with *Mycobacterium tuberculosis*, prior to the diagnosis of active TB. The spectrum of TB infected states, which were previously all characterized as latent TB infection, represents a diversity of outcomes upon exposure to infectious particles. This appreciation of clinical complexity has been largely driven by the development of biomarkers to monitor risk of disease progression and refined imaging techniques. Text below the graphic details areas of investigation necessary to better characterize the latency spectrum including study of bacterial physiology, novel diagnostics, HDTs, new therapeutic options and host immunity. The ultimate goal of further understanding the spectrum of TB infection is to develop interventions that prevent progression to active TB disease. H-isoniazid, R-rifampin, P – rifapentine.