OPEN LETTER

REVISED Intervening along the spectrum of tuberculosis: meeting report from the World TB Day nanosymposium in the Institute of Infectious Disease and Molecular Medicine at the University of Cape Town [version 4; peer review: 2 approved]

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Abstract

Tuberculosis (TB), caused by the highly infectious *Mycobacterium tuberculosis*, remains a leading cause of death worldwide, with an estimated 1.6 million associated deaths reported in 2017. In South Africa, an estimated 322,000 (range 230,000-428,000) people were infected with TB in 2017, and a quarter of them lost their lives due to the disease. Bacille Calmette-Guérin (BCG) remains the only effective vaccine against disseminated TB, but its inability to confer complete

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protection against pulmonary TB in adolescents and adults calls for an urgent need to develop new and better vaccines. There is also a need to identify markers of disease protection and develop novel drugs. It is within this backdrop that we convened a nanosymposium at the Institute of Infectious Disease and Molecular Medicine at the University of Cape Town to commemorate World TB Day and showcase recent findings generated by early career scientists in the institute. The speakers spoke on four broad topics: identification of novel drug targets, development of host-directed drug therapies, transmission of TB and immunology of TB/HIV co-infections.

Keywords
Tuberculosis, TB/HIV co-infections, Host directed Therapies, transmission, new tools

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 3**

In the revised manuscript we have made minor changes that were suggested by the reviewer. These include typos, grammar, references 2 and 3 and word substitution.

Any further responses from the reviewers can be found at the end of the article

Disclaimer

The views expressed in this article are those of the author(s). Publication in Gates Open Research does not imply endorsement by the Gates Foundation.

Tuberculosis is a debilitating communicable disease affecting millions of people worldwide, with the highest incidence and mortality rates in Southern Africa. In 2017 an estimated 1.6 million people died of TB worldwide, making it the leading cause of death due to a single infectious agent[1]. TB is transmitted through airborne inhalation of *Mycobacterium tuberculosis* (Mtb) that seeds in alveolar spaces in the lungs. Realizing the limitations of the TST and IGRA, it has been estimated that almost one-third of the world population that is exposed to Mtb infection is able to control the infection and remain asymptomatic, otherwise known as latently infected[2]. However, 5–10% of these latently infected individuals progress to active TB and manifest symptoms of the disease[3]. More recently, the situation has been made more complex by the emergence of drug-resistant bacteria and co-infections with the human immunodeficiency virus (HIV). In 2017, the WHO estimated that 558 000 (range 483,000–639,000) people developed resistance to rifampicin (RR-TB), and 82% of these people had multi-drug resistant TB (MDR-TB)[4]. This underpins an urgent need to develop better and efficacious vaccines than the currently available BCG and to develop novel drugs for TB.

Identifying new drug targets

Mtb has a sophisticated metabolic repertoire: it is able generate its own nutrients, but it can also scavenge for some nutrients from the host[5]. Studies have shown that Mtb is able to synthesise essential amino acids such as L-arginine and tryptophan, where deletion of these key metabolites restricts Mtb growth in culture and renders it more susceptible to host immune pressure reducing its survival[6,7]. Dr Melissa Chengalroyen, a research officer in the Molecular Mycobacteriology Research Unit, under the directorship/supervision of Prof Valerie Mizrahi, opened the morning session and spoke about the key elements required for Mtb survival and how Mtb sequesters these elements to evade host-recognition by non-conventional T cells. She then described a complex *de novo* riboflavin metabolic pathway and different tools to create Mtb mutants lacking key enzymes involved in this pathway to facilitate understanding of each step in the pathway. Her study showed that not all the enzymes involved in the riboflavin pathway are essential—some play redundant roles, while others are absolutely necessary for Mtb survival, and these may hold promise as new candidate drug targets for TB or play an essential role in alerting non-restricted T cell immune arm for faster clearance of the bacteria.

The second speaker in the morning session was Dr Kehilwe Nakedi, a postdoctoral research fellow in the laboratory of Prof Jonathan Blackburn. Her research project was aimed at identifying novel substrates for mycobacterial protein kinase G (PknG) using a mass spectrometry-based phosphoproteomics approach to elucidate mechanisms by which mycobacteria interfere with the host signalling during LTBI. She identified 3164 phosphopeptides with high confidence using label-free data analysis[8]. Moreover, she identified 63 host phosphopeptides that were phosphorylated in macrophages infected with *M. bovis* BCG only and not those infected with the mutant lacking PknG. Further analysis of the data revealed that these substrates phosphorylated in the presence of PknG play a key role in regulating actin polymerisation and cytoskeleton integrity[8]. This work suggest that pathogenic mycobacteria survive inside the host macrophages during early TB infection through interfering with the host's cytoskeletal dynamics mediated by PknG.

Development of host-directed drug therapies

Although the currently available TB treatment regimens are effective at killing the bacteria, the emergence of drug resistance and the long duration of treatment threaten their long-term efficacy. This underpins an urgent need to develop new anti-TB drugs and exploration of other treatment strategies to control the disease. One such strategy is to develop host-directed drug therapies (HDTs) with the aim of boosting the host's innate ability to fight the infection and also limit the deleterious tissue pathology[9]. Although this field is still in its infancy, it holds huge potential as adjunctive therapy for TB in clinical settings with a high disease burden.

Associate Prof Reto Guler discussed in detail their published pre-clinical data on the use of statins as a potential host-directed therapy for TB in mice[10,11]. He then spoke about the translation of this work in a proof-of-concept phase IIB, double-blind, randomized, placebo-controlled trial launched in Khayelitsha township and funded by the European & Developing Countries Clinical Trials Partnership ((EDCTP), RIA2017T-2004). The coordinator of this consortium is Reto Guler (University of Cape Town, Division of Immunology). Chief principal investigator of the clinical trial is Friedrich Thienemann (University of Zürich), local PI in Cape Town is Sandra Mukasa (University of Cape Town). Other project partners include Robert J. Wilkinson (Imperial College London), Claudia Schacht (LINQ Management GmbH, Germany), Gunar Günther and Emmanuel Nepolo (University of Namibia). The aim of the clinical trial is to investigate the use of statins to prevent chronic lung inflammation and potentially TB relapse in patients post completion of a standard TB treatment regimen. He also talked about other potential candidate targets for HDTs such as the transcriptional factor BATF2[12] and microRNA-143 and microRNA-365[13].

Another speaker on this topic was Dr Suraj Parihar, a Senior Research Officer and contributing investigator at CIDRI-Africa in the Institute of Infectious Diseases and Molecular Medicine (IDM). His talk focused mainly on preclinical studies that investigated the efficacy of repurposed drug (barberine) generally used to reduce blood glucose and cholesterol as HDTs for TB. He found that this drug was able to reduce lung inflammation in

pre-clinical *in vitro* and *in vivo* models of TB. He then went on to speak about how growth factors can also be repurposed to enhance the killing ability of human and mouse macrophages. Although some of these studies are still at the pre-clinical stage, they hold a great promise and may pave way for alternative therapies and new clinical trials supported by strong pre-clinical data.

Transmission of Mtb

Mtb is transmitted through the inhalation of Mtb-containing aerosols from person to person. Transmission of the bacteria is high in places such as schools, churches, mines, hospitals and heavily congested neighbourhoods of Cape Town such as informal settlements and townships[14,15]. In places with high prevalence of HIV, such as Khayelitsha, the rate of new Mtb infections accounts for more than half of TB cases. In 2006, it was reported that the incidence was as high as 1500/100,000 in some townships, exceeding that of national average[16]. There are many challenges when it comes to measuring Mtb transmission via aerosol such as the low numbers of bacilli that can be captured, contamination by other bacterial or fungal particles in patients and other airborne particulate matter[17]. New aerosol methods for capturing Mtb such as the respiratory aerosol sampling chamber (RASC) hold promise in quantifying rate of transmission especially in high endemic areas. The capacity to measure the rate of transmission and the type of bacilli strain circulating becomes even more critical in the era of high antimicrobial resistance[18].

Dr Anastasia Koch, a Carnegie Developing the Next African Leaders (DEAL) early career fellow, mentored by Prof Helen Cox and Prof Digby Warner, started off her talk by mentioning the potential of WGS technology in identifying Mtb genotypes resistant to the first line TB drugs[19]. She discussed the major differences in genetic diversity observed in broth cultured Mtb populations and those derived directly from sputum, and moreover how simple culturing could result in loss of some of key genotypes. Anastasia then moved on to discussing the importance of getting a sample as close to that being transmitted by an infected person as possible in order to accurately study the strains that are being transmitted and driving disease in a community. She gave an example of how colleagues from the MMRU and the Desmond Tutu HIV Centre, have been able to capture and isolate Mtb strains from RASC bio-aerosols. She was able to culture these samples and compare whole genome of those strains with sputum induced strains. The ability to isolate Mtb from bio-aerosols and combining that with whole genome sequencing could greatly inform our understanding of TB transmission and treatment, particularly of emerging drug or multi-drug resistant strains.

TB/HIV co-infection

More than 36.7 million people live with HIV/AIDS globally and most of these people live in sub-Saharan Africa. In 2017, it was estimated that more than 350 000 people died due to HIV/TB co-infection, making the TB the highest contributor to death in people living with HIV[20]. HIV targets and depletes CD4 T cells at later stages of disease, including protective TB specific CD4 T cells[21,22]. Early antiretroviral (ARV) drug treatment is associated with improved outcomes and helps restore CD4 T cell count, including protective TB-specific CD4 T cells. However, there are complications associated with early ARV treatment in people who are also starting TB treatment. Some of these people develop TB-associated immune reconstitution syndrome (TB-IRIS), which can be fatal, unless controlled by host-directed immune suppressants such as corticosteroids[23].

To set the scene, Mohau Makatsa, a PhD candidate in the laboratory of Prof Wendy Burgers in the Division of Medical Virology, talked about a particular subset of CD4 T helper cells expressing IL-22, or "Th22 cells", which are targeted by HIV and are implicated as a key player in natural resistance to TB[24]. He showed how these cells can be stimulated *ex-vivo* by Mtb antigens and express different surface molecules compared to Th1 cells. There is a similar magnitude of these cells compared to Th1 cells in people with latent TB, but they are depleted in the peripheral blood in active TB disease and HIV co-infection. IL-22 has been shown to be important for the control of Mtb in mice[25]. It is unclear how these cells play a role in the pathogenesis of TB in humans.

Dr Muki Shey, a Senior Research Officer & Wellcome Intermediate Fellow at CIDRI-Africa in the IDM followed and talked about identification of predictive immunological biomarkers associated with mortality in people who died of severe HIV-associated TB (HIV-TB). The study identified molecules such as interleukin-1 receptor agonist (IL-1Ra), IL-6, IL-8, macrophage inflammatory protein-1 beta (MIP-1B), and interferon gamma induced-protein 10 (IP-10) to be immune mediators that segregated patients who died from those who survived[26]. The identification of these predictive markers could lead to better management of patients in the clinics, and also potentially lead to development of host-directed therapies.

Engineering tools for TB

The international guest speaker at the Nanosymposium was Assistant Prof Bryan Bryson from the Massachusetts Institute of Technology. Bryan spoke about using cutting-edge single cell RNA sequencing technology to dissect activation states of macrophages infected with Mtb. He identified key regulatory proteins that are differentially expressed in granulocyte-macrophage colony-stimulating factor (GM-CSF) versus M-CSF differentiated macrophages and how these heterogenous macrophages play a role in the control of Mtb infection[27]. He also described a new method they have developed to summarize transcriptomic heterogeneity within a dataset using geometric sketching[28]. This method allows for greater understanding of macrophage heterogeneity and spatiotemporal localisation within granulomas. He also talked about phagosomics, a new way of measuring phagosome maturation and identifying new genes/proteins associated with phagosome formation during Mtb infection. He also talked about how to build a phagosome *de novo*, which enables for large scale testing of Mtb host stresses such as drugs. This uses tagged Mtb strains in combination with gene expression data to allow for better understanding of phagosome transcriptional changes in the presence of multiple Mtb stresses.

Way forward

Prof Valerie Mizrahi, the director of the IDM gave the closing speech at the end of the symposium. In her concluding remarks she said, "It's with incredible passion that people are progressing TB research, and that is because we're living with it. It's incumbent on all of us to think about why we're doing what we're doing and to remember that at the end of the day, it's about the TB patients. Ultimately, one of the things we want to do is put ourselves out of business."

Conclusion

It is evident from the talks given by the various speakers that important research is being done to combat TB at the IDM in the Faculty of Health Sciences at the University of Cape Town. The research ranges from the identification of new candidate drug targets, investigating the utility of repurposed drugs as host-directed drug therapies, understanding the transmission of the bacteria in high burden communities, investigating the immunology of TB/HIV co-infection and identification of biomarkers for TB disease progression. An important highlight of this year's World TB Day Nanosymposium is that a bulk of this research is being undertaken and led by early career research; thus, demonstrating the depth and breadth of talented TB researchers at the IDM.

Data availability

No data are associated with this article.

Acknowledgements

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Version 3

Reviewer Report 20 May 2020

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✔ **Graham H. Bothamley**

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I think we will not make much more progress regarding the unanswered sections, but I hope the authors found the comments helpful when they write up manuscripts and theses.

The remaining comments are details, apart from swapping references 2 and 3:

1. References 2 should be reference 3 and vice versa to make sense with the text.

2. Page 4, paragraph 5, line 15: “that pathogenic mycobacteria survive…” not “survives”.

3. Page 5, paragraph 5, line 4: “making TB the highest contributor” instead of “making the TB a highest contributor”.

4. Page 5, paragraph 5, line 11: “who are also starting…” instead of “who also are starting…”

5. Page 6, paragraph 3, line 2: Perhaps replace “a lot of research” with “important research”?

I do not need to see the revisions above.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 21 May 2020

Sabelo Hadebe, Institute of Infectious Diseases and Molecular Medicine (IDM), Cape Town,

South Africa

Response to reviewer:

The remaining comments are details, apart from swapping references 2 and 3:
1. Reference 2 should be reference 3 and vice versa to make sense with the text.
Responses: *We thank the reviewer for highlighting this oversight, we have changed references accordingly in the revised manuscript.*

2. Page 4, paragraph 5, line 15: “that pathogenic mycobacteria survive…” not “survives”.
Responses: *We thank the reviewer for highlighting this oversight, we have changed survives to “survive” in the revised manuscript.*

3. Page 5, paragraph 5, line 4: “making TB the highest contributor” instead of “making the TB a highest contributor”.
Responses: *We thank the reviewer for highlighting this oversight, we have changed “making TB a highest contributor” to “making TB the highest contributor” in the revised manuscript.*

4. Page 5, paragraph 5, line 11: “who are also starting…” instead of “who also are starting…”
Responses: *We thank the reviewer for highlighting this oversight, we have changed “who also are starting…” to “who are also starting…” in the revised manuscript.*

5. Page 6, paragraph 3, line 2: Perhaps replace “a lot of research” with “important research”?
Responses: *We thank the reviewer for the comment, we have changed “a lot of research" to “important research” in the revised manuscript.*

Competing Interests: No competing interests

Version 2

Reviewer Report 23 October 2019

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- This Open Letter describes a meeting held on World TB Day at the Institute of Infectious

Diseases and Molecular Medicine at the University of Cape Town, South Africa.

- The abstract does not well describe the meeting. The material up to "The theme of the nanosymposium was..." could be deleted.

- A symposium consists of both presentations and discussion. The paper does not describe the discussion.

- The meeting took place in 2019. The WHO Global Report on Tuberculosis 2018 was published 6 months before the meeting and reflects data gained in 2017. The reference should therefore be corrected.

- The statement in the abstract (which would be better in the preamble) that "In South Africa, 322,000 people were infected with TB in 2017" is actually the incidence of disease rather than infection and the wide confidence intervals (230,000-428,000) should be given.

- Reference 2 is not a primary source estimate for either the prevalence of latent tuberculosis infection (see Behr *et al.*, 2018[1]) nor the prevalence of an immune response to tuberculin or in an interferon-gamma release assay (see Houben and Dodd, 2016[2]).

- Although the WHO 2018 report records that there were 160,684 cases of MDR/RR-TB detected and notified in 2017, the same paragraph gives an estimated incidence of 558,000 cases.

- The statement that "This underpins an urgent need to develop better and efficacious vaccines than the currently available BCG and to develop novel drugs for TB" misses several steps in logical argument from the previous sentences regarding epidemiology to this conclusion.

- When describing the speakers' contributions, it is helpful to present a brief statement indicating where their research fits into the TB research agenda. For instance, reference 3 indicates how tryptophan protects Mtb from macrophages stimulated by CD4+ T cells and reference 4 describes how arginine deprivation makes Mtb susceptible to killing via reactive oxygen intermediates. However, it is then unclear whether these essential amino acids were the "elements" sequestered by Mtb and were, in a different way, responsible for evading host recognition (not mentioned in either of the two references). How was riboflavin related to these references? My reading of the literature is that riboflavin metabolites might be recognized by MAIT cells.

- The section on Dr Nakedi's work is more understandable, although its positioning within New TB drugs rather than TB pathogenesis is difficult to fathom.

- Under "Host-directed drug therapies", statins should be mentioned in the first sentence and an explanation given as to how they might affect a successful response to respiratory pathogens, especially Mtb. The final sentence does not give the reasons why BATF2 and miR-143 and 365 might be relevant to the topic of host directed drug therapies.

- The section on Dr Purihar's work on barbarine is understandable.

- The section on “Transmission of Mtb” appeared to deal with whole genome sequencing rather than transmission. It was not clear how these might be related. The need to obtain early samples would seem to relate more to the transcriptome (and proteome) of such bacilli and perhaps to epigenetic changes.

- The section on IL-22 could usefully have referred to Ronacher *et al.* (2018[3]) as a summary of the position at the time of the symposium. New data (Ardain *et al.*, 2019[4]) make a more interesting role regarding group 3 innate lymphoid cells in mice in combination with IL-17.

- The paragraph on the talk by Dr Shey would benefit from a listing of “predictive immunological biomarkers” which distinguish severe illness from likely death from tuberculosis.

- The paragraph “Engineering tools for TB” covers a range of topics and bullet points for the different parts of the talk would be helpful. The difference in the macrophage populations needs an introductory sentence. The term “parametric stitching” is a neologism – presumably sequences are aligned and differences noted?

- This sounds an interesting symposium, but needs context and discussion to realise its value.

References

1. Behr M, Edelstein P, Ramakrishnan L: Revisiting the timetable of tuberculosis. *BMJ*. 2018. Publisher Full Text
2. Houben RM, Dodd PJ: The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling.*PLoS Med*. 2016; **13** (10): e1002152 PubMed Abstract | Publisher Full Text
3. Ronacher K, Sinha R, Cestari M: IL-22: An Underestimated Player in Natural Resistance to Tuberculosis?. *Front Immunol*. 2018; **9**: 2209 PubMed Abstract | Publisher Full Text
4. Ardain A, Domingo-Gonzalez R, Das S, Kazer S, et al.: Group 3 innate lymphoid cells mediate early protective immunity against tuberculosis. *Nature*. 2019; **570** (7762): 528-532 Publisher Full Text

Is the rationale for the Open Letter provided in sufficient detail?

Partly

Does the article adequately reference differing views and opinions?

No

Are all factual statements correct, and are statements and arguments made adequately supported by citations?

Partly

Is the Open Letter written in accessible language?

Partly

Where applicable, are recommendations and next steps explained clearly for others to

follow?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Tuberculosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 12 Nov 2019

Sabelo Hadebe, Institute of Infectious Diseases and Molecular Medicine (IDM), Cape Town, South Africa

Response to reviewer

· This Open Letter describes a meeting held on World TB Day at the Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town, South Africa.

· The abstract does not well describe the meeting. The material up to "The theme of the nanosymposium was..." could be deleted.
Authors response: We note the comment from the reviewer. We have modified the abstract to address this comment by the reviewer.

· A symposium consists of both presentations and discussion. The paper does not describe the discussion.
Authors response: We note this comment by the reviewer. However, due to time constraints, we could not set aside time dedicated to discussion. However, there was an opportunity for engagement between speakers and guests during the poster session. Also, people were given time to ask question to speakers during the question and answer session. However, these questions and responses were not captured in this meeting report. We will look to implement this in future meeting reports.

· The meeting took place in 2019. The WHO Global Report on Tuberculosis 2018 was published 6 months before the meeting and reflects data gained in 2017. The reference should therefore be corrected.
Authors response: We have corrected the reference.

· The statement in the abstract (which would be better in the preamble) that "In South Africa, 322,000 people were infected with TB in 2017" is actually the incidence of disease rather than infection and the wide confidence intervals (230,000-428,000) should be given.
Authors response: We have given the wide confidence interval as suggested by the reviewer.

· Reference 2 is not a primary source estimate for either the prevalence of latent tuberculosis infection (see Behr *et al.*, 2018[1]) nor the prevalence of an immune response to tuberculin or in an interferon-gamma release assay (see Houben and Dodd, 2016[2]).

Authors response: We thank the reviewer for bringing this to our attention. We have cited the review Behr et al., 2018 study by Houben and Dodd, 2019.

· Although the WHO 2018 report records that there were 160,684 cases of MDR/RR-TB detected and notified in 2017, the same paragraph gives an estimated incidence of 558,000 cases.
Authors response: We have amended the sentence to reflect the estimated range by the WHO. The sentence now reads "In 2017, the WHO estimated that 558 000 (range 483,000-639,000) people developed resistance to rifampicin (RR-TB), and 82% of these people had multi-drug resistant TB (MDR-TB) [1]".

· The statement that "This underpins an urgent need to develop better and efficacious vaccines than the currently available BCG and to develop novel drugs for TB" misses several steps in logical argument from the previous sentences regarding epidemiology to this conclusion.
Authors respone: The sentence is drawn from all the presented epidemiology presented in the paragraph. The aim of it is to chart a way forward in the fight to combat the scourge of TB.

· When describing the speakers' contributions, it is helpful to present a brief statement indicating where their research fits into the TB research agenda. For instance, reference 3 indicates how tryptophan protects Mtb from macrophages stimulated by CD4+ T cells and reference 4 describes how arginine deprivation makes Mtb susceptible to killing via reactive oxygen intermediates. However, it is then unclear whether these essential amino acids were the "elements" sequestered by Mtb and were, in a different way, responsible for evading host recognition (not mentioned in either of the two references). How was riboflavin related to these references? My reading of the literature is that riboflavin metabolites might be recognized by MAIT cells.
Authors response: The two references show that Mtb requires some essential amino acids and if we disable its ability to sequester these amino acids, it becomes susceptible to immune mediated killing. Dr Chengalroyen described her research on nutrients required for Mtb survival and focus specifically on the metabolism of riboflavin and explored the draggability of this pathway.

· The section on Dr Nakedi's work is more understandable, although its positioning within New TB drugs rather than TB pathogenesis is difficult to fathom.
Authors response: We thank the reviewer for this comment. The candidate proteins identified by mass spectrometry can be investigated as potential candidate drug targets, thus, leading to the development of new drugs. We do however take the point of the reviewer but we think that the talk is still well placed in the topic of New TB drugs.

· Under "Host-directed drug therapies", statins should be mentioned in the first sentence and an explanation given as to how they might affect a successful response to respiratory pathogens, especially Mtb. The final sentence does not give the reasons why BATF2 and miR-143 and 365 might be relevant to the topic of host directed drug therapies.
Authors response: The statins are mentioned in the first sentence of the second paragraph and we provide a citation of the work published by Prof Guler and colleagues. The citation is

provided for the readers who would like to know more about the role of statins in response to Mtb infection. The last sentence is stating the other molecular targets Prof Guler is investigating as HDT for TB. Citations are also provided for the readers that would want to know more about these targets and why they are important for TB.

· The section on Dr Purihar's work on barbarine is understandable.
Authors response: Noted.

· The section on "Transmission of Mtb" appeared to deal with whole genome sequencing rather than transmission. It was not clear how these might be related. The need to obtain early samples would seem to relate more to the transcriptome (and proteome) of such bacilli and perhaps to epigenetic changes.
Authors response: Whole genome sequencing has been suggested as a tool to investigate and track transmission of Mtb (Hatherill et al., 2016. BMC Medicine), however, little work has been done in high burden settings to understand how genetically related Mtb strains change during transmission. The need to obtain early samples is not related to the transcriptome or proteome of such bacteria but related to capturing the strains that might be driving transmission in communities .

· The section on IL-22 could usefully have referred to Ronacher *et al.* (2018[3]) as a summary of the position at the time of the symposium. New data (Ardain *et al.*, 2019[4]) make a more interesting role regarding group 3 innate lymphoid cells in mice in combination with IL-17.
Authors response: We thank the reviewer for bringing these references to our attention. We have cited Ronacher et al., 2018.

· The paragraph on the talk by Dr Shey would benefit from a listing of "predictive immunological biomarkers" which distinguish severe illness from likely death from tuberculosis.
Authors Response: We thank the reviewer for this comment. We have provided a list of predictive biomarkers and cited the recently published manuscript for readers with keen interest on the topic.

· The paragraph "Engineering tools for TB" covers a range of topics and bullet points for the different parts of the talk would be helpful. The difference in the macrophage populations needs an introductory sentence. The term "parametric stitching" is a neologism – presumably sequences are aligned and differences noted?
Authors response: We have cited the recent article by Bryson et al.,2019 describing heterogenous macrophages and their contribution to the control of Mtb infection. We have also cited the article by Hie et al., 2019 that describes geometric sketching as a tool for summarizing single cell RNA sequencing data.

Competing Interests: No competing interests

Version 1

Reviewer Report 02 July 2019

https://doi.org/10.21956/gatesopenres.14148.r27372

Larry S. Schlesinger
Texas Biomedical Research Institute (Texas Biomed), San Antonio, TX, USA

This Open Letter is a nicely constructed report out of a meeting held in March 2019 around World TB day for the Institute of Infectious Disease and Molecular Medicine at the University of Cape Town in South Africa. The letter consists of a broad overview of relevant areas in tuberculosis (TB) research, brief summaries of the speakers at the meeting and a comment regarding the road forward. The program was by and large for local participants. However, there was participation from KwaZulu Natal, the University College London and MIT/Harvard.

New information is provided that will be of interest to the field. However, the nature of this format necessitated that information was brief and not really detailed so that the reader only gets some insight into the general topics and way forward.

My comments are mostly editorial:

- Page 3, first paragraph after the disclaimer: “Interestingly, almost one-third of the world population that is exposed to Mtb…control infection…latently infected.” Given current thoughts in the field, I would suggest changing the beginning of the sentence to something like: “Realizing the limitations of the TST and IGRA, it has been estimated (or it is assumed) that almost one-third…”.
- Page 3, third paragraph: “…mechanisms mycobacteria interferes with” should be “…mechanisms by which mycobacteria interfere with…”.
- Page 3, regarding the paragraph about PknG: Has it ever been proven that this enzyme enters the cytosol? It would be important to know regarding its presumed functions.
- The manuscript uses both tuberculosis and TB…should stick with TB once defined.
- Page 3, last sentence paragraph 5: “candidates targets” should be “candidate targets”.
- Page 3, paragraph 6: Is it repurposed drug or drugs? Also which drug is “this drug”?
- Page 4, first paragraph: “capturing Mtb such as respiratory….” Would add “the” after as.
- Page 4, third paragraph: “TB a highest contributor…” Would change “a” to “the”.
- Page 4, 5th paragraph: “…after presenting to hospital…” Would add “the” after to.

- Page 4, 6th paragraph: “allows” appears twice and should be followed by “for”.

Is the rationale for the Open Letter provided in sufficient detail?
Yes

Does the article adequately reference differing views and opinions?
Partly

Are all factual statements correct, and are statements and arguments made adequately supported by citations?
Yes

Is the Open Letter written in accessible language?
Yes

Where applicable, are recommendations and next steps explained clearly for others to follow?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: TB research, innate immunity, lung cellular immunity

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 22 Jul 2019

Sabelo Hadebe, Institute of Infectious Diseases and Molecular Medicine (IDM), Cape Town, South Africa

- Page 3, first paragraph after the disclaimer: “Interestingly, almost one-third of the world population that is exposed to Mtb…control infection…latently infected.” Given current thoughts in the field, I would suggest changing the beginning of the sentence to something like: “Realizing the limitations of the TST and IGRA, it has been estimated (or it is assumed) that almost one-third…”.
Response: Thank you for this comment, this is absolutely true, we have changed the sentence as suggested.

- Page 3, third paragraph: “…mechanisms mycobacteria interferes with” should be “…mechanisms by which mycobacteria interfere with…”.
Response: changed as suggested.

- Page 3, regarding the para graph about PknG: Has it ever been proven that this enzyme enters the cytosol? It would be important to know regarding its presumed functions.
Response: The enzyme is predicted to be secreted into the cytosol, but there is no biochemical proof that has definitely shown that it is in the cytosol.

- The manuscript uses both tuberculosis and TB...should stick with TB once defined.
Response: changed, Tuberculosis has been used once in Abstract followed by abbreviation TB in brackets, TB is then used throughout.

- Page 3, last sentence paragraph 5: “candidates targets” should be “candidate targets”.
- Page 3, paragraph 6: Is it repurposed drug or drugs? Also which drug is “this drug”?
Response: barberine, this has been added in main article now.

- Page 4, first paragraph: “capturing Mtb such as respiratory....” Would add “the” after as.
Response: changed as suggested.

- Page 4, third paragraph: “TB a highest contributor...” Would change “a” to “the”.
Response: changed as suggested

- Page 4, 5th paragraph: “...after presenting to hospital...” Would add “the” after to.
Response: changed as suggested.

- Page 4, 6th paragraph: “allows” appears twice and should be followed by “for”.
Response: changed as suggested.

Competing Interests: No competing interests were disclosed.