Detection and treatment of subclinical tuberculosis

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S U M M A R Y

Reduction of active disease by preventive therapy has the potential to make an important contribution towards the goal of tuberculosis (TB) elimination. This report summarises discussions amongst a Working Group convened to consider areas of research that will be important in optimising the design and delivery of preventative therapies. The Working Group met in Cape Town on 26th February 2012, following presentation of results from the GC11 Grand Challenges in Global Health project to discover drugs for latent TB.

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1. The 2050 elimination goal

The Stop TB Partnership has set the goal of tuberculosis elimination (defined as an annual incidence of fewer than one case per million individuals) by 2050. Although this is an ambitious goal in the context of global trends over recent decades, the required rate of decline is broadly in line with successful TB control achieved in post-second world war Europe and North America. The current global control strategy is guided by Millennium Development Goal 6, which is to reduce the incidence of cases by 2015, and the Stop TB Partnership target of halving 1990 prevalence and mortality rates by 2015. Historically, control efforts have prioritised identification and treatment of all cases of active TB, but this has been insufficient to interrupt transmission and there is a need to combine the existing approach with new interventions that will reduce the development of infectious cases by vaccination and preventive therapy. The framework chosen by the United Nations to replace the Millennium Development Goals after 2015 will be critical for TB control.
Research is an essential driver towards TB elimination, and the aim of the present document is to outline a research strategy that will optimise the impact of therapeutic intervention during the phase of infection prior to development of clinical TB.

Latent tuberculosis infection (LTBI) is currently defined by immunological sensitisation to Mycobacterium tuberculosis antigens in the absence of clinical symptoms of disease. The relationship of the immune response to the continued presence of live bacilli is unclear. This description is applicable to one third of the global population and encompasses a diverse spectrum of individuals at widely differing risk of developing active TB. While the broad concept of a clinically “latent” phase of infection provides the rationale underlying preventive therapy, effective implementation will depend on stratification of individuals on the basis of disease risk rather than the presence or absence of an immune response. We propose to use the term “subclinical tuberculosis” to denote at-risk populations who would receive the greatest benefit from preventive therapy.

There is extensive evidence that preventive therapy can reduce the incidence of future disease, and current WHO guidelines strongly recommend that HIV-infected persons in whom active TB has been excluded should receive isoniazid for at least 6 months, and preferably 9 months, as part of a comprehensive package of HIV care. Addition of rifapentine allows an important reduction to a three-month treatment schedule in HIV-negative individuals, though the risk of reinfection imposes an obvious limitation on the utility of preventive therapy in areas with high rates of transmission. Current preventive therapy involves prolonged treatment of large populations in order to prevent a relatively small number of cases. For tractable integration into global strategies for TB control, we need short, safe preventive treatments targeted at high-risk populations.

The overall strategic requirements are:

- Strategies to identify groups with subclinical TB at high risk of developing symptomatic infectious disease
- Short, simple therapeutic regimens to treat subclinical TB and prevent the development of active disease
- Integration of preventive therapy with pre- and post-exposure vaccination strategies

### 2. Widening the diagnostic net

The effectiveness of current control efforts is limited by incomplete recruitment of infectious cases, thereby limiting their impact on TB transmission. Prevalence surveys have shown that an important proportion of infectious individuals with bacteriological positive sputum do not experience symptoms of sufficient severity to prompt health-seeking behaviour. In the context of HIV co-infection in high burden settings, the prevalence of asymptomatic culture-positive TB can be as high as 8.5%. Improved recruitment of prevalent cases will require expansion of the diagnostic net by increased social awareness of early symptoms as well as the incorporation of more flexible diagnostic tests into health care programmes.

Three broad criteria can be proposed as potential early markers of disease risk. The first is bacterial load. Potential biomarkers include detection of bacterial components in systemic fluids, detection of antibodies, and detection of M. tuberculosis-specific T cell responses based on novel phenotypic markers. These strategies are being explored in the context of definitive diagnosis of active disease, but may have alternative application as indicators of future risk. A second approach could be based on molecular markers of early pathology, for example, metabolic or proteomic disturbances related to lung damage. Blood-based transcriptional profiling offers a novel approach with potential application in this context. The third could be novel imaging modalities that reflect bacterial load and early disease activity, which may provide important tools to track host-pathogen interactions and to discover biomarkers for different stages of infection. With an increasing knowledge of systems immunology, it may be possible to identify immunological biomarkers that are precursors of future pathology. In order to achieve the desired levels of specificity it may be necessary to combine multiple biomarkers, using mathematical modelling approaches to determine the potential utility of combining tests with lower levels of sensitivity and specificity in point-of-care tests. Finally, we should anticipate that, as the prevalence of infection declines as TB control moves towards the elimination goal, we may need to translate increasingly sophisticated diagnostic tools from research into practical application.

In summary, in order to identify individuals who would receive maximum benefit from preventive therapy, we need to identify novel biomarkers, or combinations thereof, that provide information about:

- Bacterial load
- Early pathology
- Phenotypic state of antigen-specific T cells

### 3. Bacterial physiology

An intuitive expectation is that clearance of the small numbers of bacteria present prior to disease onset should require less extensive treatment than active disease. The need for prolonged preventive therapy is thought to reflect either sequestration of bacteria in sites of low drug penetration, or transient periods of persistence involving a physiological state that confers phenotypic tolerance. Phenotypic tolerance can be induced in a variety of *in vitro* culture systems in which bacterial replication is inhibited by starvation, oxygen deprivation, or other means, and it is likely that such systems are broadly reflective of the *in vivo* situation. Stochastic persisters that express a drug-tolerant phenotype independent of environmental signals have also been observed within an otherwise logarithmically-growing population *in vitro*, although their relevance to *in vivo* infection is unclear. Non-replicating persistence under oxygen-starved conditions has been well-characterised, and the observation that metronidazole—a drug that has *in vitro* activity only under hypoxic conditions—can be applied successfully as preventive therapy in a nonhuman primate model provides compelling evidence that hypoxia contributes to latent TB. Phenotypic tolerance is also observed during active disease, and the distinction between subclinical infection and active TB is best viewed as a quantitative difference in distribution of bacterial phenotype and load rather than as a strict qualitative difference between replicating and non-replicating states. A consequence of this line of reasoning is that the targeting of phenotypically tolerant populations is of equal importance for improved preventive therapy and for shortening regimens applied in the treatment of active disease.

While technical advances, particularly in the area of transcriptomics, have allowed characterisation of *M. tuberculosis* in mouse tissues and in human sputum, there is very little information about the physiological state of bacteria within human lesions. Studies of mycobacteria isolated directly from human sputum surprisingly revealed that the organisms are phenotypically and transcriptionally similar to bacteria adapted to hypoxia *in vitro*. This conflicts with the conventional view of the origin of sputum-borne bacilli as emerging from rapidly replicating bacterial populations at the cavity surface. Also contrary to conventional wisdom, in sputum (and lesions including cavities) the bacilli are...
not replicating within macrophages but are primarily extracellular or within neutrophils. The extensive heterogeneity of cell types and architecture that make up human TB lesions suggests a diversity of microenvironments that are likely to induce a corresponding heterogeneity in bacterial phenotypes. Understanding of mycobacterial physiology in vivo will depend on our ability to interpret sparse experimental data using systems biology models that integrate transcriptomic, proteomic, lipidomic and metabolomic networks. This should be complemented by the use of appropriate experimental models in which methods not easily applied to humans, such as reporter bacteria and tool compounds to assess drug penetration and lesion targeting, can be exploited.

A characteristic of bacteria in the non-replicating hypoxia model is that they survive with a marked reduction in their energy reserves. Further reduction of low ATP levels by targeting of ATP synthase provides an attractive rationale for the ability of TMC207 to kill non-replicating M. tuberculosis. Recent evidence suggests that pyrazinamide, another drug with activity against non-replicating populations, disrupts processes involved in translation, highlighting this as another potential area for drug discovery.

Improved understanding of the physiology of M. tuberculosis in human tissues will provide an important foundation for the rational design of novel drugs and regimens for preventive therapy and for treatment of active TB. This will depend on:

- Technologies that provide direct experimental information about bacteria in lesions from patients
- The development of experimental models that accurately recapitulate the clinical efficacy of drugs
- Integrative systems biology models that allow reconstruction of bacterial physiology from partial datasets

4. Drug discovery

Renewed efforts to discover novel anti-mycobacterial drugs over the last decade have followed two approaches. The first is based on the screening of small molecule libraries for compounds with the ability to kill mycobacteria, followed by identification of molecular targets using genetic tools such as re-sequencing of resistant mutants. The second approach involves the initial application of genetic tools to identify essential enzymes that are then used as targets in high-throughput biochemical screens. The first approach has been more successful, with TMC207 and novel nitroimidazole derivatives in early stage clinical development for drug-resistant and drug-susceptible TB, and both showing promising activity against non-replicating phenotypes. However, whole cell screens generate only a small number of potent hits, along with a large number of hits with moderate potency. To exploit the latter category of compounds, bacterial cell death surrogates are needed which are more tractable and informative than measurement of colony forming units, and which can be applied in screens that better model in vivo mycobacterial physiology to prioritize compounds for follow-up by medicinal chemistry.

The failure of target-based drug discovery can be rationalised in part by the difficulty of moving from hits with potent activity against purified enzymes to molecules that efficiently penetrate the mycobacterial cell envelope. A second limitation is that the conventional model, based on the assumption that inhibition of an individual metabolic reaction will cause cell death, may not be a complete description of the mode of action of effective anti-bacterials. Bactericidal activity is likely to depend on the downstream consequences of an initial drug-target interaction which results in the generation of toxic intermediates that act as the true effector molecules. The selection of targets based on an understanding of downstream consequences, rather than on simple genetic evidence of essentiality, is likely to increase the productivity of target-based discovery. The availability of medicinal chemistry resources and the early use of ‘proof of concept’ or tool compounds in vitro and in appropriate animal models, will be key in elucidating issues associated with permeability, efflux and target cell metabolism.

In the mouse model, combined administration of novel combinations such as TMC207 and pyrazinamide have a striking synergy that allows a significant reduction in the treatment time required for sterilisation. These empirical findings highlight the importance of screening for positive and negative interactions between old and new drugs in novel combinations and dosages. Understanding which model systems or in vitro tests predict synergies that might translate into therapies for human disease will require significantly more effort. Consideration of combined activities and synthetic lethality could usefully be incorporated into drug discovery strategies, and early stage screening assays should be developed that mimic in vivo conditions to identify candidates such as pro-drugs with poor in vitro activity.

To accelerate the drug discovery pipeline, there is a need to develop:

- Strategies for target selection based on an improved understanding of the cascade of biochemical events triggered by the initial drug–target interaction
- Molecular assays for mycobacterial cell death that can be used to monitor real-time activity of drugs and prioritize hits
- Structure-based understanding of penetration and accumulation of small molecules in tissues, lesions and bacteria
- Understanding of drug–drug interactions and mechanisms

5. Moving drugs to the clinic

The clinical development of new antimycobacterials typically involves evaluation in a mouse model followed by assessment of early bactericidal activity in humans. In the conventional mouse model, disease is associated predominantly with the presence of intracellular bacteria in loosely structured lesions. This represents only a portion of human pathology; in particular, it does not recapitulate the heterogeneous microenvironments and bacterial phenotypes characteristic of human granulomas or cavities. Early bactericidal activity (EBA) measures the impact of drugs on bacteria that contribute to sputum positivity and transmission, but provides no information concerning their effect on closed lesions that are likely to contribute to the requirement for prolonged therapy; measuring EBA over extended times may help to address this limitation. Thus, while it can be anticipated that mainstream drug discovery efforts will generate new compounds with potential application to preventive therapy, many opportunities will be missed without alternative late preclinical and early clinical screens.

The low-dose non-human primate model reproduces key features of latent TB in humans (reactivation by anti-TNF antibodies, for example) and has been used to compare drug activities during preventive therapy. The model is expensive but, by incorporating live imaging readouts, provides high value data that closely parallel clinical studies. Extension from cynomolgus macaques to smaller primates, such as marmosets, would make the model more tractable by reducing the amount of drug that is required for testing.

Serial live imaging by combined computed tomography (CT) and positron emission tomography (PET) provides a comprehensive analysis of the dynamics of TB lesions in patients and experimental
animals. Monitoring changes to individual lesions in response to drug exposure complements early bactericidal activity by distinguishing lesions that resolve quickly or slowly in response to conventional or novel treatment regimens. Evidence from preliminary studies shows that lesions can be imaged in individuals with latent TB, suggesting that PET/CT could be used to define rapid endpoints for early clinical trials of preventive therapy regimens. Host markers may be a simple way to infer bacterial load as the host responds to this assault, and to monitor the decline in load during effective therapy; imaging studies may aid in the discovery of such markers.

To evaluate the potential role of novel drug regimens in preventive therapy, it will be necessary:

- To extend the use of experimental models that mimic human subclinical TB
- To exploit live imaging technologies to monitor effectiveness of drugs in preclinical models and in clinical studies
- To identify biomarkers that reflect the declining bacterial load in response to effective therapy

6. Immune modulation

Modulation of the host response presents an important adjunct or alternative alongside preventive therapy targeting the pathogen. While there are well-characterised examples of immune modulation that enhance progression to active disease – the use of anti-TNF antibodies, HIV-mediated depletion of CD4 cells – there is no clear proof-of-concept for beneficial immune modulation during subclinical TB. If protective immunity requires a balanced regulation of multiple immune functions, it would be misleading to assume that a negative impact associated with reduction in one component should necessarily imply a positive outcome were this component enhanced. For example, while the absence of IFNγ is detrimental to protection, increasing amounts of IFNγ will not necessarily correlate with increased protection. There is some evidence that combining antiretroviral therapy and isoniazid preventative therapy has synergistic benefits in preventing TB in HIV-infected people.

Immune reconstitution in HIV-TB patients with a higher bacterial load demonstrates the pathogenic potential of such an increase in immune response, in the form of immune reconstitution inflammatory syndrome (IRIS). Therefore, the study of pathological and protective immune restoration in TB-infected patients during antiretroviral therapy provides fertile ground to understand the difference between protective and pathological immunity.

In principle, improved control of subclinical TB could be achieved by induction of an enhanced immune response to clear persisting bacteria, or by boosting the existing immune response to maintain its containment function. Clearance of persisting infection might be achieved by transient stimulation of innate effector functions, using small molecules to modulate intracellular pathways or by blocking of regulatory cytokines or co-stimulatory molecules. An obvious concern with such an approach is the risk that systemic activation of innate immune mechanisms may result in collateral pathological damage. In contrast, it may be possible to induce targeted dampening of innate immune cell function to reactivate quiescent lesions by transient immune modulation.

This “wake ‘em and whack ‘em” strategy would have to be carefully coordinated with delivery of anti-mycobacterial drugs, and may have deleterious consequences in individuals with high bacterial load or harbouring a drug resistant strain.

The rational design of strategies to boost existing immune cover is frustrated by a lack of understanding of pathways (other than HIV co-infection) that lead to immune breakdown and reactivation. Potential mechanisms include senescence or exhaustion of key T cell populations, or an imbalance between activating and regulatory circuits that control immune homeostasis and the balance between inflammation and antimicrobial mechanisms. A series of booster vaccines that enhance the CD4 and CD8 T cell responses that are dominant in the natural response to infection are currently in clinical trials to assess their prophylactic efficacy in combination with BCG. Initial safety trials have found no detrimental impact associated with delivery of these vaccines to individuals with evidence of prior immune sensitisation, and plans are underway to test their ability to reduce reactivation or reinfection disease. Given the likely complexity of the regulatory networks associated with existing immune responses, an intriguing alternative vaccine strategy would be to induce a de novo immune response, for example by targeting antigens that are not well-recognised during the natural immune response; improved phenotypic characterisation of mycobacterial subpopulations in human lesions has the potential to contribute to such an approach. The application of mathematical modelling to these immunological systems may provide insights that can assist in the prioritization of proposed interventions.

Opportunities to combat subclinical TB by immune modulation include:

- Evaluation of current booster vaccines in populations with subclinical TB
- Development of vaccines targeting novel antigens and/or immune mechanisms
- Development of immunomodulatory vaccines that alter pathology, prevent disease progression, or achieve sterile pathogen eradication
- Molecular definition of immune mechanisms of mycobacterial killing
- Development of robust, well characterised preclinical models of subclinical TB for comparative assessment of vaccines and treatment modalities

7. Coordination and networking

Despite the substantial global population of individuals with subclinical TB, there are disturbingly few experimental techniques that generate interpretable data concerning their immune status or the physiological condition of the bacteria that they are harbouring. There is very limited access to material from subclinical TB lesions, and experimental models that reflect the human condition are expensive and available only in highly specialised laboratories. To promote effective research in this area, there is a need to support research networks that link availability of samples from clinical and experimental infection with expertise in novel technologies, and to enhance partnerships between clinicians, experimentalists and epidemiologists. Clinical trial sites need baseline support to maintain their operational capacity, and long-term cohorts to support surveillance and related studies, such as biomarker discovery and validation. Addressing the logistic constraints associated with implementation of disease control programmes in often poorly resourced societies will be critical to the success of any new intervention to combat subclinical TB; notably, this offers an important opportunity to improve social aspects of TB control such as access to drugs and health care, as well as facilitating operational research. The reduction of disease by preventive therapy has the potential to make a significant contribution to the goal of TB elimination. This is not a simple undertaking, and will require a vigorous response to the challenge of implementing a short, sharp intervention that is targeted to at-risk groups and can be integrated within the TB elimination strategy.
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