

Mycobacterium tuberculosis... Can we beat it?

A Euroscicon conference 2013

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The rise of drug-resistant strains of *Mycobacterium tuberculosis* in a world where at least one-third of the entire population is infected with the tuberculosis-causing infectious bacterial pathogen is a serious threat to human health, well-being, and global economy, as is the devastating effect of this dreadful disease on communities with high incidence rates of HIV infections. Integration of interdisciplinary approaches by academia and industry can herald a generation that can aim to be free from tuberculosis (TB).¹ The challenges faced are varied and range from developing quicker, cheaper and more accurate diagnostic tools to establishing new effective strategies for therapy and identifying novel anti-tubercular agents.

The conference, “*Mycobacterium tuberculosis*... can we beat it?” organized by Euroscicon, aimed to pool together cutting-edge research performed in the fields of TB diagnostics and treatment from across the world. Scientists from various disciplines discussed their research and exchanged technical knowledge. The Royal College of Pathologists, located at heart of the cosmopolitan city of London, where TB incidences have been reported to be higher than HIV infections in the recent past, created the perfect environment to host the international meeting, which took place on 21 March 2013, commemorating World TB Day.^{2,3}

Issues Regarding TB Control and Management

The World Health Organization (WHO) declared TB as a global health emergency in 1993. In 2011, 8.7 million reported incidences and 1.4 million deaths due to TB validate the fact that, irrespective of concerted efforts in the last two decades, the disease remains a serious threat to the wellbeing of mankind.⁴

The guest speakers of the day belonged to varied backgrounds—from research scientists to clinicians, from academics to industrial partners—and yet they all concurred on the challenges that need to be addressed to overcome the menace of TB. In this report, we have made an attempt to record these and the possible solutions that arose from a number of interactive discussions throughout the day.

One of the major roadblocks in the control and management of the disease is to develop ways to tackle the different physiological states in which the causative organism, *Mycobacterium tuberculosis* (*M. tuberculosis*) can exist in vivo.⁵

In most cases, the host's natural immune responses contain the pathogen as an asymptomatic infection. However, about 10% of the infected individuals develop active disease, which can then be treated and cured by a directly-observed combination therapy currently in use, provided the microorganism is susceptible to it. However, years of inadequate point-of-care diagnostics followed by a personalized treatment and patients' non-adherence to complex treatment regimens have allowed selection of bacteria resistant to a wide array of currently available antibiotics. The rise of these multi-, extensively-, as well as total drug-resistant TB (MDR/XDR/TDR-TB) cases are a cause for alarm as any chemotherapeutic combination is either lengthy, unreliable, has toxic side effects, or is ineffective on patients, most of whom are co-infected with HIV.⁶ Discovery of new drugs that shorten the treatment period and do not interfere with highly active anti-retroviral treatment (HAART) is therefore essential.

M. tuberculosis can also lie dormant in the host only to be “awakened” unpredictably at a later stage to cause active disease.^{7,8} This population is commonly referred to as the “persisters” and the current preventive treatment regimens call for administration of isoniazid or rifampin or a combination of the two for multiple months to clear this subset of the organism from the HIV infected host. Regimens involving rifapentine are also being studied in predominantly HIV-uninfected individuals.⁶ These latent TB infections (LTBI) have an estimated 10% chance to convert to active infection and spread the contagion. This large pool of LTBI needs to be addressed along with active infections as the bacilli can survive indefinitely in the human host.

TB incidences are enormously high in resource-limited areas that lack the necessary infrastructure for appropriate diagnosis of the disease. As there are no rapid and accurate point-of-care diagnostic tests yet available, multiple samples need to be collected and sent to distant laboratories, risking contamination and unreliable results. LTBI detection tests, such as the tuberculin skin test (TST), commonly referred to as the Mantoux test, suffers from cross-reactivity, as previous vaccinations with BCG or exposure to non-tuberculous mycobacteria (NTM) might result in a false-positive result for TB.⁹ Based on patient background this

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test may be offered in combination with an interferon gamma (IFN γ) release assay (IGRA) test to validate infection, increasing the costs incurred.

The 100-year-old live attenuated whole-cell vaccine, BCG, is the only existing vaccine meant to prevent incidence of TB in children. It offers good protection against the more severe forms of TB, but lacks effectiveness in preventing respiratory disease, the more common form in adults.¹⁰ The strains used for the vaccine vary with geography which translates to varying efficacies of protection offered.¹⁰ An effective vaccine that can protect adults as well as children would greatly benefit economically challenged areas where the burden of TB is the highest.

Critical gaps in the unmet need, available research support, and advocacy for TB research reflect upon the lack of political commitment to address the challenges in TB control strategies.¹¹ Pharmaceutical companies are not incentivized to invest large capital on antibiotic discovery. It is of paramount importance that, as borders become increasingly permeable, countries unite to follow common treatment regimens that have been proven to be effective. There are regimens followed in the Russian Federation that are found severely lacking by the WHO-approved guidelines. A survey of drug availability as shown by Professor Graham Bothamley (Homerton University Hospital) revealed a number of the second-line antibiotics and injectable drugs to be unavailable in many countries in the Eurozone. The ills of tuberculosis have to be fought with technological advancements and strong public policy and administration, all attuned to the common goal they share.

Developments in Diagnostics

Though X-ray, smear microscopy, and culture tests are the prevalent methods to detect TB infection, they are time-consuming, labor-intensive, and require skilled personnel. Xpert-MTB/RIF, approved by WHO for detection of TB and drug resistance, is a remarkable breakthrough in TB diagnostics; however, adapting the technology for use in resource-poor settings is under implementation and the need for simpler assays has been expressed.¹² Ngoc A Dang (University of Amsterdam) is working on using unique cell wall lipids as biomarkers and developing sophisticated instrumentation for rapid diagnosis of TB. It uses thermally assisted hydrolysis and methylation followed by gas chromatography and mass spectrometry to determine the biomarkers.¹³ The advantages of this approach are that it is fully automated, sensitive and can distinguish between *M. tuberculosis* and non-tuberculous mycobacteria. Viscosity of sputum, however, causes problems in processing and decontaminating samples prior to testing is required.

Dr Jayne Sutherland from the MRC Unit in The Gambia pressed strongly upon the need to develop rapid, sensitive, point-of-care diagnostic tools to detect active TB in adults and children infected with HIV. Her field studies include monitoring active TB patients and their household members to identify valid biomarkers found in the blood of those infected. Her team has found that, once stimulated, the cytokine levels in blood are easily detectable, thus can be used as valid biomarkers for diagnostic tests. Pleural fluid, though difficult to obtain, shows high levels

of cytokine without any stimulation whatsoever. Development of IP-10 lateral flow strips is underway and once completed these will yield results as easily as other dip-stick tests such as the pregnancy test kits readily available today. Exposure to the less virulent *M. africanum* and co-infection with HIV, both having potential to affect the sensitivity of the test, were shown to have no significant effect on the results.

Among other tentative diagnostic methods, exhaled breath was discussed as a future possibility for easy diagnostics. The use of mobile phone technology, which could allow clinics in remote areas instant access to skilled personnel in laboratories, also inspired some interest from the speakers.¹⁴

Detection of dormant bacilli. Professor Mike Barer (University of Leicester) reported an interesting finding that could have far-reaching effects in latent TB detection. His group has recently discovered that TB bacilli in sputum when stained appropriately show the presence of lipid bodies within the cells.¹⁵ Microarray analyses showed that the formation of these lipid bodies coincide with the cells expressing dormancy factors.¹⁶ This could mean that sputum analysis, usually used to detect active infection, could give an insight to the level of persister population among the patients.

Due to the unreliability of the tuberculin skin test (TST), guidelines in various countries call for an interferon gamma release assay (IGRA). A simple but sensitive and robust IGRA test based on ELISpot called the T-Spot TB, currently being provided by Oxford Immunotech, is one of the four IGRA-based tests to detect LTBI as approved by the US Food and Drug Administration.¹⁷ As explained by Dr Christopher Granger (Director of Global Professional Relations, Oxford Immunotech), white blood cells (WBC) are extracted from the patient's blood sample and diluted to give a monolayer of cells on a micro well plate. TB-specific antigens, ESAT-6, and CFP-10 are then added to the wells containing the cells, which get stimulated and release IFN γ . The IFN γ is then bound to a secondary antibody that produces a positive result that can be detected visually or with UV. A minimum of 6 spots on a well is required for a positive result. Each spot is accepted to be the footprint of one WBC. IGRA, other than being more specific than TST, also provides the logistical advantage of one patient visit only.

Drug Discovery and Development

Development of new treatment regimen can be broadly classified into two categories: (a) immediate, using the drugs that are already in various phases of clinical trials, and (b) longer-term, which involves identification of novel therapeutic hits or leads against the TB pathogen.

Three strategies usually employed for developing anti-tubercular drugs are: (a) whole cell screening, (b) target based drug development, and (c) repurposing old drugs.

Whole cell phenotypic screening involves testing existing drugs, novel synthetic or natural compounds with potential anti-tubercular activity against the organism. There are a number of whole cell phenotypic assay models for determining minimum

inhibitory concentrations (MIC) and/or minimum bactericidal concentrations (MBC) of drugs or inhibitors against mycobacteria from an in vitro culture to an intracellular macrophage environment.¹⁸⁻²²

Target-based discovery involves detection of novel endogenous mechanisms or pathways that can be targeted to hinder intracellular viability of the TB pathogen. The peptidoglycan (PG) layer of the mycobacterial cell wall is one of its essential constituents, the loss of which results in cell death. The enzymes involved in the earlier stages of peptidoglycan biosynthesis, especially the Mur enzymes have attracted a lot of attention of the scientific community as they are essential and specific to bacteria, making them ideal drug targets.²³⁻²⁸

Immediate solutions toward tackling TB. In addition to the drugs that target *M. tuberculosis* specifically, non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and oxyphenbutazone, used for fever, pain, and inflammation, have shown specific anti-tubercular activity.^{22,29} Reinvestigation of known drugs or compounds for new indications, known as drug repositioning or repurposing, could save on the time and investment required for new antibiotic development programs.³⁰

Professor Stephen Gillespie (University of St Andrews) discussed clinical trials of anti-tubercular drugs past and present. After the first controlled clinical trial was performed on streptomycin—the first affective antibiotic against TB—in 1948, a multidrug regimen was set up in 1952, which was subsequently altered before the final regimen was set in the 1980s and has been followed to date. After a hiatus of 40 years, accelerated drug development promises a new portfolio of drugs active against TB. Several new drugs are in various phases of clinical trials. A placebo-controlled double-blinded study of moxifloxacin in 1931 patients recruited over 32 sites across the world, and due to be completed in mid-2013, shows promise to reduce the current 6 mo drug treatment period to 4 mo. Other drugs such as gatifloxacin, an ethambutol substitute, and SQ109, a novel anti-TB drug also show promise. Phase II trials for delamanid have been completed and it appears to be useful in MDR-TB indications, but no future for this drug is seen against susceptible disease.³¹ Professor Gillespie also pointed out that, as more natural or synthetic novel chemical entities come to light, it becomes essential to develop predictive models that can ascertain future treatment regimens. Developing and selecting regimens is challenging and effective sites for evaluation of regimens will remain a barrier. However, there is hope that new, shorter treatment regimens for drug-susceptible TB will be on the market within the next couple of years.

Longer term solution toward tackling TB. Professor Edith Sim (Oxford University and Kingston University) has long believed in using targets that are essential for intracellular survival of mycobacteria, such as those that enable it to use lipids/cholesterol as a fuel for survival inside the macrophages.

Arylamine *N*-acetyltransferase (NAT) was first identified in humans to metabolize a front line anti-TB drug, isoniazid. Homology studies later identified the homologous gene in several mycobacterial species and its product garnered interest as a

potential drug target.³² Professor Sim's team noted that the Δnat mutant grew slower than usual, differed in their cell morphology and lipid profiles, and failed to survive inside macrophages. A selection of potent NAT inhibitors, evaluated against the whole cell mycobacteria, resulted in phenotypes that were very similar to the Δnat mutant. The authors therefore claimed that the NAT inhibitory properties have the potential for developing novel anti-tubercular treatment.³²⁻³⁴

In addition, HsaD, a hydrolase that catalyzes the cleavage of carbon chains in cholesterol, has also attracted much attention as a potential therapeutic target. HsaD deleted mutants do not grow on cholesterol or inside macrophages. Professor Sim indicated that, using the molecular structure of HsaD and fragment-based drug discovery, a new class of compounds that bind to the active site of the enzyme has been identified.

The mycobacterial cell wall, as mentioned earlier, is an attractive target for developing anti-tubercular drugs. However, the enzymes of its biosynthetic machinery are largely uncharacterized. Dr Luke Alderwick (University of Birmingham) explained the mode of action of benzothiazinone (BTZ) inhibitors. BTZ targets FAD-containing oxidoreductase, DprE1, which, in combination with DprE2, epimerizes decaprenylphosphoryl- β -D-ribose (DPR) to decaprenylphosphoryl- β -D-arabinofuranose (DPA), a cell wall precursor.³⁵ Genetic modification studies revealed DprE1 to be essential, and biochemical and structural analyses enabled determination of the mode of action of BTZ on DprE1. It is interesting to note that, in the absence of the flippase UbiA, which transports DPR through the cell membrane, BTZ lacks any bactericidal activities.

Another area of interest is that of proteins with more than one unique biological activity, also known as moonlighting proteins, being worked on by Professor Brian Henderson (Eastman Dental Institute, University College London). Studies have shown that several of these moonlighting proteins are responsible for virulence in various bacterial species. In mycobacteria, these proteins usually serve as adhesins, enabling uptake by macrophages or as macrophage activators. Inhibition of these proteins usually results in cells that show fewer granuloma formations, and thus lowered virulence. The most common of these is a chaperone, Hsp 60.2 which is involved in macrophage uptake and as a cytokine-inducing factor.³⁶ The probability of resuscitation-promoting factors (Rpfs) acting as moonlighting proteins was also taken into consideration. Various host proteins are also known to behave as moonlighting proteins and can aid infection by serving as receptors for the bacterial moieties.³⁷

Monitoring Response to TB Drug Treatment

A biomarker is an indicator of a biological state—in this case, infection. A biomarker can be of host or bacterial origin and can give an insight into the effectiveness of treatment. Monitoring early response to treatment in clinical trials is of prime importance as it can help optimize therapy, minimize adverse side effects, and predict the likely outcome of the treatment.³⁸

Use of bacterial rather than host biomarkers provides a more direct means of observation of infection. Bacterial biomarkers currently in use are sputum smears and early bactericidal assay (EBA) studies. A speculative biomarker assay being developed under the leadership of Professor Timothy McHugh (Centre for Clinical Microbiology, Royal Free Hospital, University College London) uses the concept of viral load to measure the bacterial load in TB infection. Bacterial loads can be measured using Lowenstein–Jensen (LJ) slopes, serially diluted sputum colony counts or in mycobacteria growth indicator tube (MGIT). The rate of decline of bacterial load in the first three days of treatment is indicative of the likely outcome of the treatment. Patients with a high bacterial load were found to be more likely to relapse, thus validating bacterial load as a reliable biomarker.

Professor McHugh also mentioned other biomarkers of bacterial origin, namely, the nucleic acids; DNA, however, is not reliable as it persists in the host tissues long after treatment is complete. mRNA molecules are difficult to isolate; 16S rRNA molecules, on the other hand provide a good alternative as they have shorter half-lives, do not persist after bacterial cell death, and are abundant.

An initial burst of dead cells upon the start of an effective treatment could also enable detection and study of the early kinetics of response to treatment. This “treat to test” strategy could have implications in detection of resistant forms of infection and is being investigated by Dr RM Anthony (Royal Tropical Institute, KIT Biomedical Research).³⁹

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Conclusion

In summary, the conference was highly informative, inspiring, and thought-provoking. It brought together pioneering research from various parts of the UK and abroad and provided an opportunity for colleagues to meet, discuss their work and propose future collaborations. The question-and-answer round provided even the internet audience a chance to put forth their questions to the speakers of the day. The interactive session was directed along the lines of the current issues regarding point of care diagnostics and novel approaches to treatment; topics ranged from the necessity of dip-stick diagnostic tests to chemical scaffolds that might be indicative of the effectiveness of anti-TB molecules. The conference also featured several excellent posters in two separate sessions, some of which were selected for short talks as well. Finally, Euroscicon must be recognized for the important role they played in convening the conference; the success of the conference is reflective of their commitment to help advance science.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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