CONTENTS

Event Info

Programme

Speakers & Talks

Posters

Contact
SPRINT-TB is a translational bench-to-bedside research programme uniting clinicians and scientists working in tuberculosis (TB) research, with the aim of improving medicines and approaches to treat and eradicate this high-priority infectious disease. SPRINT-TB started in 2014 at National University of Singapore and is supported by the National Research Foundation Singapore under its Translational and Clinical Research (TCR) Flagship Programme administered by the Singapore Ministry of Health’s National Medical Research Council.

We are pleased to welcome you at the SPRINT-TB Inaugural Annual Symposium—the first in the series of events that SPRINT-TB will host annually, each year featuring renowned scientists, clinicians and key opinion leaders in TB.

**SPRINT-TB Inaugural Annual Symposium:**
Advances in Tuberculosis Therapy Research

**Date**
September 18, 2015

**Venue**
CeLS Auditorium, Level 1, Centre for Life Sciences, National University of Singapore, 28 Medical Drive, Singapore 117456

**Website**
www.tuberculosis.sg
# PROGRAMME

**September 18, 2015**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.30 am</td>
<td>Arrival &amp; Registration</td>
</tr>
<tr>
<td>9.00 am</td>
<td>Welcome Address</td>
</tr>
<tr>
<td></td>
<td><strong>Prof. Nicholas Paton</strong>, SPRINT-TB Director, NUS, Singapore</td>
</tr>
<tr>
<td>9.10 am</td>
<td><strong>Daring to Be Different - Metabolic Heterogeneity in Mycobacteria and Their Hosts</strong></td>
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<tr>
<td></td>
<td><strong>Prof. Eric Rubin</strong>, Harvard School of Public Health, USA</td>
</tr>
<tr>
<td>9.40 am</td>
<td>TB Drug Discovery: Challenges and Opportunities in Phenotypic Screening</td>
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<tr>
<td></td>
<td><strong>Dr. Manjunatha Ujjini</strong>, Senior Investigator, Novartis Institute for Tropical Diseases, Singapore</td>
</tr>
<tr>
<td>10.10 am</td>
<td>SPRINT-TB Drug Discovery: New Approaches, New Targets</td>
</tr>
<tr>
<td></td>
<td><strong>A/Prof. Thomas Dick</strong>, Department of Microbiology, NUS, Singapore</td>
</tr>
<tr>
<td>10.30 am</td>
<td>Tea Break</td>
</tr>
<tr>
<td>10.50 am</td>
<td>From phenotypic screens to target identification: the role of structural biology in TB drug discovery</td>
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<tr>
<td></td>
<td><strong>Dr. Christian Noble</strong>, Investigator, Novartis Institute for Tropical Diseases, Singapore</td>
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<tr>
<td>11.20 am</td>
<td>Identification of Mycobacterial Growth Inhibitors by High-Throughput Screening</td>
</tr>
<tr>
<td></td>
<td><strong>Dr. Umayal Lakshmanan</strong>, Experimental Therapeutics Centre, A*STAR, Singapore</td>
</tr>
<tr>
<td>11.50 pm</td>
<td>Selected SPRINT-TB Young Investigator Talk</td>
</tr>
<tr>
<td>12.05 pm</td>
<td>Lunch &amp; Poster Session</td>
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<td>Time</td>
<td>Session</td>
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<tr>
<td>1.05 pm</td>
<td>Union Research Support for Shortened MDR-TB Treatment Regimens</td>
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<td></td>
<td><strong>Dr. I.D. Rusen</strong>, Senior Vice President, Research and Development, the International Union Against Tuberculosis &amp; Lung Disease – North America</td>
</tr>
<tr>
<td>1.35 pm</td>
<td>TRUNCATE-TB: Shortening Standard TB Treatment Regimens</td>
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<tr>
<td></td>
<td><strong>Prof. Nicholas Paton</strong>, Department of Medicine, NUS, Singapore</td>
</tr>
<tr>
<td>2.05 pm</td>
<td>TB Biomarkers: Tackling the Need for a Surrogate of Treatment Outcome</td>
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<tr>
<td></td>
<td><strong>Dr. Davide Manissero</strong>, Senior Director, Medical and Scientific Affairs, Qiagen, UK</td>
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<tr>
<td>2.35 pm</td>
<td>TB Health Systems Research in Myanmar</td>
</tr>
<tr>
<td></td>
<td><strong>A/Prof. Mishal Khan</strong>, NUS Saw Swee Hock School of Public Health, Singapore</td>
</tr>
<tr>
<td>3.05 pm</td>
<td>Selected SPRINT-TB Young Investigator Talk</td>
</tr>
<tr>
<td>3.20 pm</td>
<td>Panel Discussion: TB Research Overseas – Why Bother?</td>
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<td><strong>Prof. Richard Coker</strong>, NUS Saw Swee Hock School of Public Health, Singapore</td>
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<td><strong>Dr. I.D. Rusen</strong>, Senior Vice President, Research and Development, the International Union Against Tuberculosis &amp; Lung Disease – North America</td>
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<td><strong>Dr. Jeffery Cutter</strong>, Director, Communicable Diseases Division, Ministry of Health, Singapore</td>
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<tr>
<td></td>
<td><strong>Prof. Nicholas Paton</strong>, Department of Medicine, NUS, Singapore</td>
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<tr>
<td>4.00 pm</td>
<td>Networking Reception</td>
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<tr>
<td>5.00 pm</td>
<td>End of Symposium</td>
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Eric Rubin is a Professor of Immunology and Infectious Diseases at the Harvard School of Public Health and a Senior Associate Member of the Broad Institute. His research focuses on developing new tools for studying *M. tuberculosis* and related mycobacteria in an effort to identify genes required for growth, survival and virulence in mycobacteria. Prof. Rubin received his MD and PhD from Tufts University, USA.

**Daring to Be Different: Metabolic Heterogeneity in Mycobacteria and Their Hosts**

*Mycobacterium tuberculosis*, which is the aetiological agent of TB, owes much of its success as a pathogen to its unique cell wall and unusual mechanism of growth, which facilitate its adaptation to the human host and could have a role in clinical latency. Asymmetric growth and division increase population heterogeneity, which may promote antibiotic tolerance and the fitness of single cells. Unusual mechanisms of mycobacterial growth, cell wall biogenesis and division might affect the survival of *M. tuberculosis in vivo* and contribute to the persistence of infection.
Dr. Ujjini received his PhD from the Indian Institute of Science, Bangalore in 2001. Upon award of a five-year John E. Fogarty International Visiting Post-doctoral research fellowship at the National Institutes of Allergy and Infectious Diseases, NIH, USA, he worked on elucidating the mechanism of action of TB drug Protomanid. Dr. Ujjini joined NITD in 2007, where he has led a multi-disciplinary scientific team to identify a novel class of anti-TB candidates including indolcarboxamides and hydroxyl-pyridones. In 2009 he was appointed as an honorary adjunct assistant professor at Yong Loo Lin School of Medicine, National University Singapore. He has published more than 40 research articles and also has a number of patents.

**TB Drug Discovery: Challenges and Opportunities in Phenotypic Screening**

TB poses a major global health problem and multi-drug resistant strains are increasingly prevalent. Hence there is an urgent need for new TB drugs. Cell based phenotypic screening represents a powerful approach to identify anti-mycobacterial compounds and elucidate novel targets. Three high-throughput phenotypic screens at NITD against mycobacterium identified hits and chemical series were selected for optimisation. This produced compounds with good *in vitro* anti-mycobacterial activity and pharmacokinetic properties. Some compounds displayed oral activity in mouse efficacy models of TB. The presentation will review NITD efforts in TB discovery and share experiences in optimisation of phenotypic hits, including our recent efforts in identifying two promising novel scaffolds, indolcarboxamides and 4-hydroxy pyridines against TB.
A/Prof. Dick leads Theme 1 of SPRINT-TB and the Antibacterial Drug Discovery Laboratory at the Department of Microbiology, National University of Singapore (NUS). The goal of his group is to identify new targets and lead compounds for the development of more effective chemotherapies. Thomas is also the Director of NUS BSL-3 Core Facility, which houses the largest TB research BSL-3 lab in Singapore. Prior to his current position at NUS, he led the TB drug unit at Novartis Institute of Tropical Diseases, Singapore, where he established and managed the discovery portfolio from target identification to preclinical development. He also headed the Mycobacterium Biology Laboratory at Institute of Molecular and Cell Biology, A*STAR. A/Prof. Dick obtained his PhD in molecular bacteriology at the University of Heidelberg, Germany.

SPRINT-TB Drug Discovery: New Approaches, New Targets

Antibacterial drug discovery suffers from a whole series of issues. Target-based approaches do not deliver. Whole cell approaches are not effective. We do not know how to kill persister bacteria effectively and how to slow down resistance development. This presentation will discuss various novel approaches and new therapeutic intervention levels we are using in the SPRINT-TB program to tackle those issues.
CHRISTIAN NOBLE

Investigator
Novartis Institute for Tropical Diseases
Singapore

Christian received his Ph.D. from the John Innes Centre, Norwich, UK, where he investigated the mechanism of the bacterial drug target, DNA gyrase, and the mode of action of the antibacterial drugs fluoroquinolones. Christian completed post-doctoral training at the National Institute for Medical Research, London, UK and the Institute of Molecular and Cell Biology, Singapore, focusing on protein biophysics and structural biology. In 2008 Christian joined the Novartis Institute for Tropical Diseases (NITD) in Singapore as a principal investigator. Christian has worked on drug-discovery projects against several infectious-disease targets including TB, dengue fever, malaria and African trypanosomiasis. He currently leads the protein chemistry and biophysics platform in the Drug Discovery unit at NITD.

From Phenotypic Screens to Target Identification: the Role of Structural Biology in TB Drug Discovery

Multi-drug resistant TB remains an urgent unmet medical need and discovery of novel anti-TB treatments is extremely challenging. This Phenotypic screens can be used to identify novel compounds from which the specific targets have been identified. Once the target is known, it is possible to use modern biophysical techniques to understand how molecules are interacting with their target and to rationally design better inhibitors. This presentation will discuss two compound series that were identified from high-throughput screen—the natural product cyclomarin, which targets ClpC1, and a synthetic pyridone compound that targets InhA, a clinically-validated anti-TB target.
One of the major barriers to developing new drugs for TB is the lack of attractive whole cell-active lead compounds for target-based lead optimization. The goal of our project is to identify such target-lead couples through High Throughput Screening (HTS) for the development of new clinical TB drug candidates. Our HTS design is based on phenotypic screening, where the whole cell is treated with various compounds in order to select for compounds that inhibit or kill the whole cell. This phenotypic screening in comparison to target based screens is specially advantageous in TB drug discovery as the hits will have already overcome some of the important criteria like solubility and cell permeability that is a major issue with the almost impermeable mycobacterial cell wall. With this HTS screening of ETC’s synthetic compound libraries, we are aiming to find novel compounds that will provide starting points for various drug development activities.
CAROLYN MULU WU
PhD Student
National University of Singapore
Singapore

Carolyn is pursuing her PhD at National University of Singapore under the guidance of A/Prof Thomas Dick. She received her Bachelor’s degree in Pharmaceutical Science from Fudan University, China. Her current research focuses on nutrient-starvation induced dormancy in mycobacteria and identification of the key regulators of the adaptation process to this nutrient-starvation induced non-replicating state in an effort to find new drug targets against TB.

Mild nutrient starvation triggers the development of a small-cell survival form in Mycobacteria

Mycobacteria, generally believed to be non-sporulating, are known to survive shock starvation in saline by shifting to a non-replicating state without apparent morphological changes. We uncovered that mycobacteria can undergo cellular differentiation by exposing *M. smegmatis* to mild starvation conditions. Traces of carbon sources in saline triggered the development of a novel small resting cell (SMRC) morphotype. Saline shock-starved large resting cells (LARCs) remodeled their internal structure to the septated, multi-nucleoided cells seen during differentiation to SMRCs. Further characterization of LARCs and SMRCs revealed a mycobacterial starvation-induced differentiation program in which at first septated, multi-nucleoided cells are generated. Under zero-nutrient conditions bacteria terminate development at this stage as LARCs. In the presence of traces of a carbon source, these multi-nucleoided cells continue differentiation into mono-nucleoided SMRCs. We are employing reverse genetics approach to identify the key regulators in this starvation-induced differentiation program.
I. D. RUSEN

Senior Vice President
Research and Development,
International Union Against Tuberculosis & Lung Disease
North America

Dr. Rusen received his medical degree from the University of Manitoba, Canada and completed a Community Medicine Residency and a Masters degree in Epidemiology at the University of Toronto. He has over 15 years of experience as a technical advisor for TB in low-income countries with donor agencies and non-governmental organisations.

Dr. Rusen has been with The Union since 2004 in many capacities, including Coordinator of the FIDELIS Initiative and the Director of the TB Department for three years. He is currently leading the TREAT TB Initiative, which is spearheading the coordination and sponsorship of the STREAM clinical trial, a landmark trial for shortened MDR-TB treatment.

Union Research Support for Shortened MDR-TB Treatment Regimens

The currently recommended treatment for MDR-TB is both lengthy and difficult to tolerate. Consequently, treatment success rates for the WHO standard treatment are disappointingly low at less than 50%. The International Union Against Tuberculosis and Lung Disease (The Union) has long recognized the need for research and evaluation of shorter, more tolerable regimens for MDR-TB. This presentation will provide a historical perspective of The Union’s efforts in this field, as well as promising results of more recent research efforts.
NICHOLAS PATON

Professor
National University of Singapore
Singapore

Prof. Paton has extensive experience in clinical trials and translational research in HIV and TB, and is the Director of the NMRC-funded TCR Flagship SPRINT-TB programme. He led multiple large scale trials, including EARNEST and PIVOT, and is the Chief Investigator of the multinational TRUNCATE-TB trial, which aims to shorten the standard TB treatment regimen to 2 months. He has also been involved in building a pan-European network of HIV clinical research sites (NEAT) and has been the Principal Investigator for the UK CTU responsible for 8 European countries in the NIH-funded INSIGHT research network.

Prof. Paton has obtained his medical degrees from University of Cambridge, UK, and has held numerous clinical and research positions in UK and Singapore.

TRUNCATE-TB: Shortening Standard TB Treatment Regimen

TRUNCATE-TB (Two-month Regimens Using Novel Combinations to Augment Treatment Effectiveness for drug-sensitive Tuberculosis) is a ground-breaking Phase 3 TB treatment regimen shortening trial, which will recruit over 1,000 patients in multiple Asian countries. The aim of this study is to determine whether a strategy of treating drug-sensitive TB for 2 months with novel combination regimens and re-treating relapses with a 6 month course of standard treatment will be non-inferior to the standard 6 month treatment / 6-month re-treatment approach.
Dr. Manissero holds a medical degree from the Universita’ Cattolica del Sacro Cuore, Italy, and a Master’s degree and a clinical diploma in tropical medicine and international health from the London School of Hygiene and Tropical Medicine, UK. He served as Head of TB Programme and Respiratory Infections Section at the European Centre for Disease Prevention and Control, as a Medical Officer for the WHO, Indonesia, and as a Director of Public Health Programme for Otsuka SA, Switzerland. Dr. Manissero provides expert input to a number of international TB task forces and groups under the Stop TB Partnership, the WHO and the European Union. His key areas of TB expertise include TB elimination, childhood TB, new TB tools, TB monitoring and surveillance, BCG vaccination, impact measurement and programme management.

TB Biomarkers: Tackling the Need for a Surrogate of Treatment Outcome

Interferon Gamma Release Assays (IGRAs) have brought significant advantages to the diagnosis of TB infection, particularly in improving specificity over the Tuberculin Skin Test (TST). IGRAs have shown limitations as biomarkers able to distinguish active from latent TB. However, an increasing amount of evidence is demonstrating a crucial role of *M. tuberculosis* specific CD8+ T-cell responses. The presentation will focus on reviewing the literature on CD8/CD4 flow cytometry studies, introduce relevant proof of concept studies for IGRA assays and hypothesize on the potential study of the novel IGRA tests as a multi-faceted TB biomarker.
MISHAL KHAN

Associate Professor
National University of Singapore
Singapore
London School of Hygiene and Tropical Medicine
United Kingdom

Mishal Khan is an Associate Professor at SSHPH, National University of Singapore and a Lecturer in Public Health at the London School of Hygiene and Tropical Medicine. She is experienced in epidemiological and operational research and policy analysis. Mishal’s main research areas include gender inequalities, health systems strengthening, public-private partnerships and tuberculosis control and she has led large studies in Pakistan, Bangladesh, China, Myanmar and Cambodia. Her interest is in developing locally appropriate, sustainable interventions and policy measures to improve health.

TB Health Systems Research in Myanmar

While it is widely recognised that the health system needs to be strengthened in order to control the tuberculosis epidemic in Myanmar, and resources are being mobilised, there is limited evidence to inform control strategies. Our group is among the first to have collaborated with the National TB Control programme to conduct a series of operational and epidemiological studies investigating drivers of the TB and MDR-TB epidemic in Myanmar, and present our findings during this presentation.
Safety and Efficacy of Blocking IL-4 with Pascolizumab in Patients Receiving Standard Therapy for Pulmonary Tuberculosis

New approaches are needed to achieve more rapid elimination of dormant mycobacteria and shorten treatment for drug-sensitive and drug-resistant TB. Approaches that enhance immune clearance have the potential to be more effective. Interleukin-4 (IL-4) is a key cytokine in the immune response to TB that may impair the clearance of mycobacteria. Pascolizumab, an anti-IL-4 monoclonal antibody, might be of value as an adjunct to standard TB treatment. The primary aim of this trial is to determine whether administration of pascolizumab with standard treatment for TB produces changes bacterial or host response that may indicate potential for enhanced sterilization. This trial involves is innovative not only in the therapeutic approach but also in the outcomes assessment, with new microbiological, immunological and imaging endpoints. It presents a unique opportunity to intervene against a specific component of the immune response and has the potential to generate a wealth of scientific data that could lead to improved understanding of the host immune response to TB.
Panel Discussion: Research Overseas—Why Bother?

**RICHARD COKER**
Professor
National University of Singapore
Singapore

Prof. Coker heads Infectious Diseases Programme at Saw Swee Hock School of Public Health, National University of Singapore. His research interests include emerging infectious diseases, tuberculosis, health systems analysis and strategic planning, policy analysis, development and ranking of indicators to assess performance, and the development of models to support health system functioning.

**JEFFERY CUTTER**
Director
Communicable Diseases Division
Ministry of Health, Singapore

Dr Cutter is Director, Communicable Diseases Division at the Ministry of Health, Singapore, where he oversees the prevention and control of communicable diseases, including pandemic preparedness. He obtained his MBBS and Masters in Medicine in Public Health from the National University of Singapore and MSc in Epidemiology from the London School of Hygiene and Tropical Medicine. He is a Fellow of the Academy of Medicine Singapore.
**I.D. RUSEN**

Senior Vice President  
*Research and Development, International Union Against Tuberculosis & Lung Disease, North America*

Dr. Rusen has over 15 years of experience as a technical advisor for TB in low-income countries with donor agencies and non-governmental organisations. He has been with The Union since 2004 in many capacities, including Coordinator of the FIDELIS Initiative and the Director of the TB Department for three years, currently leading the TREAT TB Initiative.

**NICHOLAS PATON**

Professor  
*National University of Singapore Singapore*

Prof. Paton has extensive experience in clinical trials and translational research in HIV and TB, and is the Director of the NMRC-funded TCR Flagship SPRINT-TB programme. He led multiple large scale trials, including EARNEST and PIVOT, and is the Chief Investigator of the multinational TRUNCATE-TB trial, which aims to shorten the standard TB treatment regimen to 2 months.
A Target Mechanism-Based Whole Cell Screen Identifies Bortezomib as an Inhibitor of Caseinolytic Protease in Mycobacteria

Combining assets of whole-cell and target-based screens, we developed a reporter strain that allows the identification of inhibitors of mycobacterial caseinolytic protease (Clp) in a target-based whole cell screen. Clp constitute a key degradative proteolytic machine involved in central proteome homeostasis. We report here for the first time the identification of a whole-cell active Clp inhibitor: Bortezomib (Velcade®), a clinically approved human proteasome inhibitor. Several lines of evidence demonstrate that Bortezomib exerts its antimicrobial activity indeed via inhibition of Clp. These include genetic manipulation of Clp protein level, potentiation of drugs acting upstream of Clp’s cellular function, structure activity relationship of Bortezomib derivatives, Clp-specific substrate accumulation upon Bortezomib treatment and molecular modelling of Bortezomib into Clp catalytic sites.

The identification of Bortezomib as Clp inhibitor in mycobacteria is an important finding for at least two reasons: i. It represents a valuable chemical tool readily available to decipher the complex role of Clp proteolytic activity in mycobacterial cell physiology. ii. Bortezomib, as a clinically approved drug with orally available derivatives, represents a promising starting point for the initiation of a lead optimization program.
Devika Mukherjee¹, Jun Jie Koh², Zou Hanxun², Shouping Liu²,³, Roger Beuerman²,³, Thomas Dick¹

¹National University of Singapore, Singapore; ²Singapore Eye Research Institute (SERI), Singapore; ³Duke-NUS, Singapore

Membrane Targeting Xanthones—the Road to Eliminating Mycobacterial Persisters

We have demonstrated the ability of membrane targeting Xanthones to kill M. tuberculosis complex and Non-tuberculous mycobacteria. The lead compound in our study, AM16, exhibits attractive potency features. Not only does it kill mycobacteria rapidly and completely (up to the limit of detection) but it is also active against non replicating mycobacteria. In addition, mycobacteria show a very low spontaneous resistant mutation frequency against AM16. Our mechanism of action studies includes, structural investigations via Scanning Electron Microscopy and membrane characterization using biochemical assays. Both studies indicate that the membrane of the mycobacteria is being affected by AM16. Furthermore we demonstrated a dynamic Structure Activity Relationship and identified compounds with better potency and selectivity. In parallel we plan to carry out in vivo studies in the mouse model to determine the efficacy of the compounds which show good activity in vitro.

Our aim is to develop a new antibiotic class against mycobacterial infections that has the potential to reduce treatment time and has a low rate of resistance development.
Pyrazinamide Acts Independent of pH in vitro

Pyrazinamide (PZA) is a critical component of the first line TB combination therapy known for its sterilizing activity in shortening the duration of chemotherapy from 9-12 months to 6 months. Although discovered due to its activity against TB in mice, PZA is known to possess no activity against growing bacilli in vitro under physiological pH (6.5). The current paradigm is that it is active in vitro only at an acidic pH (5.5), which was hypothesized to correlate with acid production by inflammatory cells at the site of lesions. However, an early German study published in 1934 and more recent measurements of pH in necrotic mouse lung lesions (A. Lenaerts, unpublished data) show that mature TB lesions are not acidic. Hence, we revisited the existing model of the pH-dependent activity of PZA which is based on studies by McDermott et al. in 1954, suggesting in fact that PZA is just more active at acidic pH but not that it is inactive at physiological pH.

The only accepted consensus is that PZA needs to be converted into Pyrazinoic Acid (POA) within the cell and it is POA which is the active component in the activity of Pyrazinamide. Here, we re-evaluate the dependence of pH on the potency of both PZA and POA in susceptible mycobacteria. Preliminary results indicate that both PZA and POA exert significant activity at neutral pH which upon closer analysis is found to be similar to their inhibitory activity at acidic pH.
Fishing for Bortezomib Mode of Action and Mechanism of Resistance in Mycobacteria

We demonstrated previously a novel type of antibacterial screening method, a target mechanism based whole-cell screening method that delivered bortezomib (BZ), a human 26S proteasome drug, as a potent inhibitor of ClpP1P2 activity and bacterial growth in mycobacteria. Previous work validated ClpP1P2 as a druggable target, and delivered BZ as lead compound for tuberculosis therapy. In our current study, we aim to elucidate the mode of action (MoA) and mechanism of resistance (MoR) of BZ in mycobacteria.
Tuberculosis (TB) is a highly infectious disease caused by Mycobacterium tuberculosis (M. tuberculosis). Despite its known etiology, it remains one of the leading causes of mortality and morbidity, with nearly a third of the world’s population harboring the latent bacteria. Increasing reports of multidrug and extensively drug resistant tuberculosis in recent years have been a source of concern. Resistance and persistence are the major unresolved issues in current TB chemotherapy and there is an urgent medical need for new drugs acting via novel mechanistic routes that could address these pressing concerns. An in-house library of functionalized indoles was screened for activity against M. smegmatis and M bovis BCG Pasteur. We have found promising activity in a class of N-octylindoles, members of which demonstrated low micromolar minimum inhibitory concentrations on the mycobacterial strains (M.smegmatis and M bovis). Structural elaboration provided evidence of a dynamic structure-activity relationship in which the n-octyl substituent was identified as a critical structural feature. Preliminary evaluation of cytotoxicity and solubility are promising but highlighted areas that should be rectified in subsequent design and synthesis. Taken together, we have identified functionalized indole scaffolds that could form a springboard for the discovery of promising antituberculosis agents.
Bridging the Gap between Need and Reality for New TB Drugs

The current tuberculosis (TB) epidemic remains unabated due to two important factors: i) co-infection with HIV and ii) protracted treatment period coupled with non-compliance, which has resulted in the rapid emergence of drug-resistant TB. In this context, there is a pressing need for new anti-tubercular drugs that can shorten the treatment duration and be effective against multidrug resistant TB. We aim to probe new chemotypes that have potent anti-mycobacterial sterilizing activity and an unconventional mode of action to address the underlying issue. In our study design, we have combined the assets of both whole cell and target-based screening to identify mycobacterial cell wall inhibitors. The primary reason we are focussing on the cell envelope of *M. tuberculosis* is because it is a highly vulnerable and druggable target. Secondly, the most efficient first line drugs, isoniazid and ethambutol work by disruption of mycolic acids biosynthesis. We will employ a ‘compound first’ approach by performing a high-throughput screen of half a million chemical scaffolds (done by Experimental Therapeutics Centre) to select growth inhibitors of BCG. The hits will then undergo a target-based secondary screen, using two strains of transformed BCG, one harbouring the P-iniB-luc reporter plasmid and the other carrying a P-iniB-wasabi green reporter plasmid. P-iniB is the promoter region of the iniBAC operon that gets specifically induced only by cell wall inhibitors (pre-established and preliminary data has confirmed the same). In addition to the screens, hit-lead optimization studies (like demonstration of dynamic structure-activity relationship (SAR), in-vitro pharmacokinetic and toxicological profiling) will be done to determine attractive tool compounds, that can enter preclinical development.
There is an urgent medical need for new drugs with new mechanism of action to keep multi-drug resistant TB away, and to deliver shorter, more effective treatments of TB. An unbiased whole cell approach which identifies growth inhibitory compounds has several advantages. It can screen complete set of anti-mycobacterial compounds regardless of their bacterial target. As we demonstrated in the past, subsequent target deconvolution can be done by using the approach of spontaneous drug-resistant strain recovery followed by the whole genome sequencing. Our goal in this project is to identify new attractive target-lead couples and place it on the TB drug discovery pipeline.

To identify growth inhibitory compounds, a simple turbidity-based assay by using M. bovis BCG was selected and established, because we observed that high-sensitive, indirect readouts such as a total ATP amount generates higher-rate of false positives/negatives by the influence of glycerol in the media and particularly in the presence of cell wall synthesis inhibitors, which causes high level of ATP accumulation within mycobacteria. Whole cell screen for BCG growth inhibitors is ongoing at Experimental Therapeutic Centre. The hits delivered from the first compounds library are currently being characterized under our batteries of secondary screen, including mechanism-based phenotypic reporter systems to identify inhibitors for clp, cell wall inhibitors and DNA gyrase, respectively. We also developed a reporter BCG strains to identify the types of bacterial stress followed by the exposure to anti-mycobacterial compounds and also to do real-time monitoring of bacterial physiological condition.
Mycobacteria, generally believed to be non-sporulating, are well known to survive shock starvation in saline for extended periods of time by shifting to a non-replicating state without any apparent morphological changes. Here we uncover that mycobacteria can undergo cellular differentiation by exposing *M. smegmatis* to mild starvation conditions. Traces of various carbon sources in saline triggered the development of a novel small resting cell (SMRC) morphotype. Fluorescence microscopic analyses showed that development of SMRCs progresses via septated, multi-nucleoided cell intermediates, which divide to generate mono-nucleoided SMRCs. Intriguingly, saline shock-starved large resting cells (LARCs), which did not show cell size or surface changes when observed by scanning electron microscopy, remodeled their internal structure to the septated, multi-nucleoided cells seen during differentiation to SMRCs. Comparative transcriptome analysis of SMRC and LARC development revealed largely overlapping sets of differentially expressed regulatory and metabolic genes. These transcriptome data are consistent with a mycobacterial starvation-induced differentiation program in which at first septated, multi-nucleoided cells are generated. Under zero-nutrient conditions bacteria terminate development at this stage as LARCs. In the presence of traces of a carbon source, these multi-nucleoided cells continue differentiation into mono-nucleoided SMRCs. Currently, we are employing reverse genetics approach to determine the key regulators in this starvation-induced differentiation program.
Whole Cell Screening of Compounds against Mycobacteria: Targeting Caseinolytic Protease (Clp) and the SOS Response Network

The rapid increase in bacterial resistance to the standard anti-tuberculosis drugs is a compelling reason to explore alternative drugs that are potent against active and dormant mycobacteria. The major barrier to developing new drugs for TB is the lack of attractive targets with their associated whole cell active lead compounds. We employ a combination of target based-whole cell screening approach to identify novel antimycobacterial drug candidates. Compounds with growth inhibition activity against *M. bovis* BCG will be identified from a library of 500,000 synthetic chemicals through high throughput screening process. Hits selected from the primary screen will be subjected to secondary screening approaches to assess cytotoxicity and characterize activity against active and dormant Mycobacteria. Resistant mutant selected in this approach will be subjected to whole genome sequencing to elucidate the molecular mechanism of action of the candidate compounds. Parallel to the whole cell screening strategy, a target based approach will be applied to find new chemotypes that inhibit caseinolytic protease (Clp), a family of proteases essential for the survival of mycobacteria. The study will also explore the mycobacterial SOS response network as a potential drug target. DNA repair is a crucial process to bacterial survival. This repair and recombination event is controlled by a repertoire of genes under the SOS regulon, a regulatory network present in most bacteria. For this, we employ a recA promoter based pathway screening approach to identify chemotypes that interfere with the activity of SOS proteins including DNA Gyrase.
Reprogrammed tRNAs Read a Code of Codons to Regulate Mycobacterial Persistence

Codon usage bias and RNA modifications are ubiquitous features of life; yet, the manner in which these genetic and epigenetic traits interact to influence gene expression remains elusive. For members of the Mycobacterium tuberculosis Complex especially, persistence in the presence of cell-mediated immunity is dependent upon the timely regulation of dormancy programs within hypoxic granulomas. Seeking to understand the mechanism behind the selective translation of stress response genes when M. bovis BCG is exposed to hypoxia, we examined the molecular factors contributing to translation efficiency. Of the 40 modified ribonucleosides in BCG tRNA, levels of wobble 5-oxyacetyl-uridine (cmo$^5$U) in tRNA$^{Thr(UGU)}$, herald changes in DosR abundance – the transcription factor critical to the establishment of hypoxic dormancy. Translation of $dosR$ and other transcripts biased in Thr$^{ACG}$ usage are synchronously enhanced during hypoxia. Recombinant BCG with $dosR$ coded by other threonine codons showed varied fitness during and after hypoxic exposures in accordance with the availability of modified tRNAs. Our results connect previous observations on mycobacterial dormancy with mechanistic insights as to how synonymous substitutions affect protein abundances and cellular phenotype.
TRUNCATE-TB: an Innovative Trial Design for Drug-Sensitive Tuberculosis

The number of potential regimens of drug treatment for TB is vast, meaning that evaluating each new treatment against a control in separate two-arm trials requires a huge amount of resources. There is, therefore, a need for innovative trial designs that can evaluate drug regimens simultaneously.

TRUNCATE-TB (two-month regimens using novel combinations to augment treatment effectiveness for drug-sensitive tuberculosis (DS-TB)) is a randomised, open-label, multi-arm, multi-stage (MAMS), parallel group strategy trial. The primary aim of the trial is to determine whether a strategy of treating DS-TB for 2 months with one of a number of novel combination regimens and re-treating relapse with a 6 month course of standard treatment will be non-inferior to the standard WHO-recommended 6 month treatment/8-month re-treatment approach in terms of TB sputum culture status at 2 years after randomisation. The secondary aims are to determine whether there are advantages of a 2-month initial treatment strategy for preventing the emergence of drug resistance, improving quality of life, and decreasing programme resource costs.

Adult patients with pulmonary TB will be randomised at equal probability to receive 6 months’ standard treatment as a control, or to one of a number of boosted regimens (these regimens are combinations of standard drugs with new or repurposed drugs including: high-dose rifampicin, linezolid, clofazimine, bedaquiline, delamanid, rifapentine, or levofloxacin).
Investigating novel biomarkers for tuberculosis using multi-modality imaging

The existing end points used in clinical trials to assess the efficacy of tuberculosis (TB) drugs are imprecise. As a result, larger patient cohorts are required to evaluate potential pharmaceuticals. We are currently investigating the utility of multi-modal imaging in tracking treatment response to TB drugs. Patients undergo Positron Emission Tomography (PET) imaging while Magnetic Resonance Imaging (MRI) data are simultaneously acquired. The combination of the high sensitivity of PET with the higher spatial resolution of MRI, potentially provides disease biomarkers that are more accurate than existing techniques. The ability to more accurately assess TB in-vivo will facilitate further research into treatment and drug development.
Lipid-Specific Immune Response for Tuberculosis Diagnosis

Treatment of tuberculosis (TB) remains difficult due to the low specificity of clinical findings and poor performances of diagnostic methods (the gold standard diagnostic method, sputum smear microscopy, has only 35-70% sensitivity). During *Mycobacterium tuberculosis* (*Mt*b) infection, changes in the human plasma lipidome may constitute useful biomarkers for diagnostic purposes. The aim of this project is to detect a specific immune response to *Mycobacterium tuberculosis* infection. 288 plasma samples were obtained from 3 different groups of donors: active TB patients, latent TB patients and healthy controls. After lipid extraction optimisation, analyses were performed by liquid chromatography mass spectrometry (LC/MS) using targeted analysis with Agilent 1290 UHPLC 6460 Triple Quadrupole (QQQ) mass spectrometer for the major classes of lipids. Potential biomarkers were identified. Future work would include validation of these potential disease biomarkers in longitudinal studies with human plasma samples from active TB patients who were on established TB drugs treatment over a period of 6 months. Using the untargeted LC/MS analysis with Agilent 1290 ChipLC 6550 Quadrupole Time of Flight (Qtof) mass spectrometer and appropriate software and databases, we will also search for lipid markers in the plasma which might be of bacterial origin.
SPRINTing to TB Drug Discovery

SPRINT-TB (Singapore Programme of Research Investigating New Approaches to Treatment of TB) focuses on discovering, developing, and delivering new improved therapeutics for tuberculosis (TB). SPRINT-TB runs over thirty research projects, which span bench-to-bedside in the TB drug discovery and development process.

In SPRINT-TB preclinical studies, bortezomib has been demonstrated to inhibit Mtb Clp complex thereby exhibiting potent whole cell activity, while several xanthone compounds have been shown to be cidal against several mycobacterial strains. The drug perchlozone was found to share the mechanism of action with thiosemicarbazone, both drugs thus using EthA as activating enzyme and HadABC as their principal target in Mtb. Further, ongoing whole cell screens are in the process of identifying whole cell-active target-lead couples for development of new clinical TB drug candidates over the next several years.

SPRINT-TB’s clinical stage projects, among others, investigate pascolizumab, an anti-IL-4 monoclonal antibody, in a proof-of-concept study seeking to demonstrate that inactivation of IL-4 may facilitate the clearance of dormant mycobacteria. Faropenem is being tested in an early bactericidal activity trial in regimens entailing faropenem plus augmentin, isoniazid or pyrazinamide. The clinical development part of the programme also runs an array of whole blood bactericidal activity studies testing Mtb sterilization efficacy of several compounds and drug regimens.
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