Fighting TUBERCULOSIS through Research
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THE DISEASE THAT LIVES AMONGST US

NO END IN SIGHT FOR THE EPIDEMIC

“THE TB EPIDEMIC IS LARGER THAN PREVIOUSLY ESTIMATED... IN 2015, THERE WERE AN ESTIMATED 10.4 MILLION NEW (INCIDENT) TB CASES WORLDWIDE.”

WHO Global TB Report 2016

IN SINGAPORE

THERE ARE 2,500 TB CASES/YEAR IN SINGAPORE (INCIDENCE OF 44 PER 100,000 PEOPLE: A MEDIUM TB BURDEN COUNTRY). SINGAPORE IS SURROUNDED BY MANY HIGH-BURDEN COUNTRIES IN ASIA.

Based on WHO Global TB Report 2016

IN THE WORLD

1.4 MILLION LIVES WERE LOST TO TB IN 2015

IT REMAINS ONE OF THE TOP 10 CAUSES OF DEATH WORLDWIDE.

From WHO Global TB Report 2016
VISION

By 2019, to become a leading international academic research programme focused on delivering new treatments for tuberculosis.

MISSION

To discover, develop and deliver new treatments that ultimately save lives of people infected with tuberculosis.

AIMS

By 2019...

♦ Discover at least two new genes essential for dormancy in mycobacteria
♦ Discover at least two new chemically-validated dormancy targets
♦ Deliver one preclinical development compound or one clinical candidate with activity against TB
♦ Identify two or more innovations in the standard TB drug development process (preclinical or clinical) that accelerate or improve the evaluation of new compounds or combination regimens
♦ Establish an Asian TB trials and clinical research network
♦ Complete the evaluation of at least one new or repurposed antibacterial drug (phase 2 or phase 2/3) with potential for regimen shortening
♦ Complete the evaluation of at least one new or repurposed immune-modulatory drug (phase 2 or phase 2/3) with potential for regimen shortening
♦ Identify an effective 2 month regimen for drug-sensitive TB
♦ Identify at least one improvement to treatment delivery systems that improves adherence and/or clinical outcome from TB treatment

THROUGH OUR INNOVATIVE RESEARCH, WE ARE WORKING TOWARDS A WORLD FREE OF TB.
With just over 2 years since its foundation, SPRINT-TB has grown to become a major contributor to the international research effort to improve the treatment of TB. Our comprehensive programme of research, spanning basic science, drug development, clinical trials and health systems research, all focused on delivering improved TB treatment, anchored in a single academic campus (National University of Singapore (NUS) and nearby Agency for Science, Technology and Research (A*STAR) institutes), is globally unique. Currently over 150 staff and collaborators are working on projects funded under the programme.

SPRINT-TB has a number of differentiating strengths. It is based in Singapore, in the heart of Asia – a region with many high TB burden countries and containing nearly 60% of the world’s TB cases. SPRINT-TB has succeeded in building extensive intra-Asian collaborations. The programme is supported by the extraordinary scientific facilities in Singapore – such as state-of-the-art imaging at the A*STAR-NUS Clinical Imaging Research Centre (p. 31), the NUS BSL-3 Core Facility (p. 30), technology platforms at the A*STAR Experimental Therapeutics Centre (p. 15) and the A*STAR Genome Institute of Singapore (p. 22). The greatest strength of SPRINT-TB is that it truly spans the continuum of research from bench to bedside and beyond, including the essential part of implementing research findings in affected populations – all aligned to a common goal of delivering new treatments for TB.

Research highlights of the past year have been the promising new compounds identified with dihydrofolate reductase inhibitory activity in Theme 1 (p. 10) and the medicinal chemistry work in Theme 2 that has identified several derivatives of bortezomib – found to be active against a new target on the mycobacterium – that have greater selectivity in killing bacteria with reduced toxicity (p. 15). Our TB animal models have been established and a comprehensive programme of drug testing ready have begun in 2016. In the clinical trials area (Theme 3), our platform for early phase clinical testing of new/repurposed TB drugs in humans – the whole blood bactericidal activity assay (p. 19) – was successfully applied to test a number of repurposed drugs and combinations, including immune-based therapies. We also achieved a major milestone in completing our innovative trial of anti-IL-4 monoclonal antibody as an adjunct to TB treatment, with analysis underway and results expected in mid-2017 (p. 20). Our flagship trial of a two-month TB treatment regimen – the TRUNCATE-TB trial – with sites in Singapore, Indonesia, Thailand and Philippines is now ready to start recruitment after nearly 3 years of preparation (p. 21). In the treatment delivery part of the programme (Theme 4), we have now
SPRINT-TB truly spans the continuum of research from bench to bedside and beyond, including the essential part of addressing the necessary steps of implementing research findings in affected populations – all aligned to a common goal of delivering new treatments for tuberculosis.

Optimized our mobile phone app that permits remote supervised therapy and this is now moving into field trials in several countries in the region (p. 27).

Scientifically, we continue to explore new research opportunities with the potential to transform both the approach to TB research as well as yield innovations in treatment. One of the other highlights of the last year was the on-site visit by our Scientific Advisory Board (p. 36) in November 2016. Several days of intense discussions and scrutiny of our research provided both confidence in the scientific value and potential for clinical impact of our existing research agenda, as well as yielded important new directions to strengthen our research portfolio in ways that are consistent with our scientific mission.

Looking forward to the coming year, as well as building on the existing successful research areas, we will seek to identify new opportunities particularly in the treatment delivery area (Theme 4), with new proposals for TB screening/treatment outcome monitoring tests that are feasible at a community level. Our regional collaborations – one of the great strengths of the programme – have now grown to such an extent that in 2017 we will launch a regional collaborative research network for TB research, catalyzed by initial funding support from Asia Pacific Economic Cooperation (APEC).

For such an ambitious programme to succeed, it is essential that it has sound foundations within the host institution. One of the most encouraging developments in the last year has been the strengthening of support from the National University of Singapore, that has selected SPRINT-TB as one of its five Summit Research Programmes – translational research programmes that are assessed to have the potential to be internationally-leading. This support will enable us to further strengthen our academic links across the university campus, as well as train the next generation of clinician scientists who can bring new ideas and energy to the programme, to secure the future of SPRINT-TB and ensure that it can make an ever increasing contribution to the field until the task of eradicating TB is achieved.

I hope you will enjoy reading this report and welcome any new opportunity to work together with you in the global effort to fight TB through research.

Nicholas Paton
SPRINT-TB Director
SPRINT-TB research connects four bench-to-bedside themes.

**TARGET DISCOVERY**

**Lead:** Assoc. Prof. Thomas Dick  
*Department of Microbiology and Immunology*  
*National University of Singapore*

Using genetic and chemical approaches to identify new mycobacterial targets.

**CLINICAL TRIALS**

**Lead:** Prof. Nicholas Paton  
*Department of Medicine*  
*National University of Singapore*

Conducting clinical trials to evaluate safety and efficacy of new drugs and combination regimens for TB.
The four research themes of SPRINT-TB reflect sequential stages in the drug discovery, development and delivery pathway. The themes work closely together towards new TB treatment approaches by exploiting identified synergies to maximize the programme’s output and translational impact.

Find out more about SPRINT-TB research at www.sprinttb.org/research.
Research under this theme is focused on delivering chemically and genetically validated targets with bactericidal potential. The theme evaluates existing candidate targets and enables lead finding and optimization carried out under Theme 2. Research under Theme 1 also furthers the understanding of the biology of mycobacteria and host response to infection.
Dr. Carolyn Mulu Wu, mycobacterial dormancy project lead.

Mycobacteria are well known to survive starvation in saline for extended periods of time in a non-replicating state without any apparent morphological changes and are generally believed to be non-sporulating.

Theme 1 discovered 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. Meanwhile, saline shock-starved large resting cells (LARCs) remodeled their internal structure to septated, multi-nucleoided cells, similar to the intermediates seen during differentiation to SMRCs. These findings suggest that mycobacteria harbor a 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 mononucleoided SMRCs. Both SMRCs and LARCs were found to exhibit extreme antibiotic tolerance.

The full study was published in Frontiers in Microbiology in June and August 2016, as well as BMC genomics in October 2016.

Scanning electron microscopy images of log-phase and 14 days starved *M. smegmatis*.
Dihydrofolate reductase (DHFR) is a well known clinically-validated drug target, which remains attractive due to its essentiality for growth and structural differences between the bacterial and the human enzyme. However, there are no DHFR inhibitors used in the current standard TB chemotherapy.

Theme 1 embarked on a mission of finding novel lead compounds with activity against \textit{M. tuberculosis} DHFR. Their screening approach uses a target-focused library of compounds with varying side chains at the triazine scaffold, which is the bioactive moiety in a broad range of known DHFR inhibitors.

The project is conducted in collaboration with Assoc. Prof. Chui Wai Keung’s team (Department of Pharmacy, National University of Singapore), which carries out the design of the derivatives via \textit{in silico} structure-based modelling.

This approach helps to achieve selectivity towards the mycobacterial DHFR over the human ortholog by exploiting key chemical differences within the binding pocket of the enzymes.

The synthesized compounds are further evaluated for their whole cell activity at single point concentrations against \textit{M. bovis} BCG. The most potent derivatives are further confirmed and tested in \textit{M. tuberculosis} and assessed for toxicity in selected cell lines.

To date, \textit{in vitro} enzymatic activity assays of both \textit{M. tuberculosis} DHFR and human DHFR have revealed promising selectivity towards the inhibition of the former, supporting the evidence for selectivity from the \textit{in silico} structural modelling study. Theme 1 further carries out target activity tests, as well as isolation and characterization of spontaneous resistant mutants for mechanism of action studies.
Ms. Annanya Shetty, mycobacterial cell envelope inhibitors project lead.

**CELLS UNDER STRESS**

Antibiotic-mediated cell death is a complex process that begins with primary drug target modulation, which triggers intracellular events leading to the death of bacilli.

Theme 1 undertook a project to correlate the exposure to various cell envelope targeting drugs with the induction of specific cell envelope transmembrane proteins and changes in the intracellular ATP concentration in mycobacteria.

To date, a correlation between increased ATP levels and induction of a cell envelope stress-responding operon has been observed, indicating that ATP may drive activation of the stress response. Preliminary results show that bedaquiline, an ATP synthase inhibitor, may be capable of abolishing the stress response induction, which also causes drastic reduction in the cidal activity of tested cell envelope targeting antibiotics. This suggests that ATP elevation may act as a trigger for activating the stress response.

The mechanistic basis of these phenomena are currently under investigation.
Ms. Dinah Binte Aziz works on the boromycin project with Dr. Wilfried Moreira.

Boromycin is a boron-containing polyether macrolide antibiotic. It is active against Gram-positive bacteria and is an ionophore for potassium ions. However, the antibiotic is ineffective against Gram-negative bacteria where the outer membrane appears to block access of the molecule to the cytoplasmic membrane.

Theme 1 has asked whether boromycin is active against *M. tuberculosis* which, similar to Gram negative bacteria, possesses an outer membrane. Their experiments have shown that boromycin is a potent inhibitor of mycobacterial growth with strong bactericidal activity against drug tolerant persisters. Exposure of mycobacteria to boromycin resulted in a rapid loss of membrane potential, reduction of the intracellular ATP levels and leakage of cytoplasmic proteins. The results suggest that targeting the mycobacterial membrane and ion gradient may be an attractive chemotherapeutic intervention to kill otherwise drug-tolerant persister bacilli.

The study was published in Frontiers in Microbiology in February 2016.

Pyrazinamide (PZA) is a critical drug in TB treatment, yet its mechanism of action largely remained an enigma. Theme 1 researchers carried out a genetic screen to isolate *M. bovis* BCG mutants resistant to pyrazinoic acid (POA), the bioactive derivative of PZA, followed by whole genome sequencing of the resistant strains. Resistance conferring mutations were found in two pathways: aspartate decarboxylase *panD*, involved in synthesis of the essential acyl carrier coenzyme A (CoA), and in non-essential polyketide synthase genes *mas* and *ppsA-E*, involved in the synthesis of the virulence factor phthiocerol dimycocerosate (PDIM). Sequencing POA resistant *M. tuberculosis* H37Rv isolates confirmed the presence of at least two distinct mechanisms of resistance to the drug. Emergence of resistance through the loss of a virulence factor *in vitro* may explain the lack of clear molecular patterns in PZA resistant clinical isolates, other than mutations in the prodrug converting enzyme. The apparent interference of POA with virulence pathways may contribute to the drug’s excellent *in vivo* efficacy compared to its modest *in vitro* potency. To further explore resistance mechanisms, Theme 1 isolated POA-resistant *M. tuberculosis* H37Rv mutants and subjected *panD* wild-type strains to whole-genome sequencing. Eight of the nine resistant strains harbored missense mutations in the unfoldase ClpC1 associated with the caseinolytic protease complex. This approach unveiled another novel mechanism of resistance to pyrazinamide.

These studies were published in ACS Infectious Diseases in September 2016 and Antimicrobial Agents and Chemotherapy in November 2016.
Many TB drugs (isoniazid, pyrazinamide, ethionamide) are low molecular weight compounds that are bioactivated to reactive metabolites. By today’s standards, these drugs would be considered fragments due to their low molecular weight, a property which would generally exclude them from most libraries used in high-throughput screens.

Theme 1 looked at fragment libraries as a rich source of untapped chemical matter for the identification of new antitubercular agents. A thousand compound library was screened and at the concentration of 1 mM, the growth inhibition of *M. tuberculosis* H37Rv delivered 8.4% primary hits at 70% cut off. Of the hits, 33 compounds showed inhibition levels comparable to pyrazinamide, a first line TB drug. Further profiling of the hits for membrane toxicity and cytotoxicity identified 19 compounds with acceptable hemolytic activity and selectivity.

These findings suggest that fragment libraries may be valuable resources for antimycobacterial drug discovery. Further investigation of the identified hits is in progress.
Research under this theme is focused on characterizing compounds discovered by Theme 1 and by external sources. Theme 2 conducts high-throughput screening, medicinal chemistry and pharmacology for lead finding, optimization and preclinical development to deliver new TB drug candidates for clinical development. The theme also develops imaging biomarkers that can provide an early indication of activity of drugs or combination regimens against TB bacilli and works on TB models to conduct preclinical development studies in order to support investigational product use in humans.

Bortezomib analogues chemistry team: Dr. Sridhar Santhanakrishnan and Assoc. Prof. Brian Dymock at Medicinal Chemistry Laboratories, Department of Pharmacy, National University of Singapore.
Indoles chemistry team: Assoc. Prof. Mei Lin Go (principal investigator), Mr. Samuel Nyantakyi Agyei, Mr. Marcus Phua.

**INDOLES**

**HITTING THE MEMBRANE**

Damaging the membrane as a therapeutic strategy raises concerns of selectivity and perturbation of mammalian plasma membranes. Yet, the availability of potent and selective membrane targeting agents would offer an effective means to eradicate persistent dormant mycobacteria.

In collaboration with Assoc. Prof. Mei Lin Go (Department of Pharmacy, National University of Singapore), Theme 2 explored indoles bearing a cationic amphiphilic motif, which exhibited potent and selective killing of mycobacteria. Mechanism of action investigations revealed cell membrane permeabilization and depolarization, which preceded cell death in *M. bovis* BCG. Several potent analogs showed monophasic kill kinetics and demonstrated a low spontaneous resistance mutation frequency. Further lead optimization work for the compounds is in progress.

**INHIBITING BACTERIAL PROTEASE**

In 2015 Theme 1 reported the identification of the anti-cancer drug, bortezomib, as a promising anti-TB compound inhibiting the mycobacterial caseinolytic protease ClpP1P2. However, as a human proteasome inhibitor, bortezomib exhibits significant toxicity, which would not justify its use in TB treatment.

To address this issue, Theme 2 researchers have produced a series of bortezomib analogues that more selectively target the bacterial protease. The top compounds were up to a 100-fold less active against the human proteasome, but retained ClpP1P2 inhibition, as well as bactericidal potency.

This work demonstrated that selectivity over the human proteasome can be achieved without sacrificing the efficacy against *M. tuberculosis*.

The most promising lead compounds have been jointly patented by the Experimental Therapeutics Centre, A*STAR and National University of Singapore. The leads are currently undergoing further collaborative testing and optimization process.

Dr. Anders Poulsen (bortezomib analogues project leader—Experimental Therapeutics Centre, A*STAR) and Ms. Li Ming (National University of Singapore), who oversees biology aspect of the bortezomib analogues project.
NEW PET IMAGING TRACERS

One of the focus areas for Theme 2 is the development of new tracers for positron emission tomography (PET) imaging of pulmonary TB. The new tracers will enter the pipeline of imaging PET probes tested by Theme 3 in clinical studies (p. 23).

Last year we reported the launch of a proof-of-concept study with $^{89}$Zr-labelled infliximab, one of the clinically licensed monoclonal antibodies against tumour necrosis factor-α (TNF-α). TNF-α is a critical cytokine in the immune response and clinical symptomatology of TB. The tracer doses of labeled infliximab used in PET imaging are too small to have any notable biological effect, but will enable the localization and quantification of TNF-α secretion. Having a method to measure TNF-α secretion in situ could provide valuable new insights into the disease pathogenesis, as well as act as a useful indicator of drug activity. The developed method may have further applications to other pathologies characterized by high local TNF-α levels (for instance, rheumatoid arthritis, inflammatory bowel disease). This approach may also be extended to label other monoclonal antibodies for use in TB imaging and treatment or relapse monitoring.

The study is conducted in collaboration with the Singapore Bioimaging Consortium, A*STAR, using the humanized mouse model developed at the Institute of Molecular and Cell Biology, A*STAR. Several zirconium (Zr) chelation approaches have been tested in vivo. Upon the completion of the animal studies, the GMP-grade $^{89}$Zr-infliximab is planned for progression to clinical studies.

New PET imaging tracer development collaborative team: Dr. Chen Qingfeng (inventor of the humanized mouse—Institute of Molecular and Cell Biology, A*STAR), Dr. Claire Naftalin (lead of new PET tracer project—Department of Medicine, National University of Singapore), Dr. Edward Robins (head of PET radiochemistry—Singapore Bioimaging Consortium, A*STAR (SBIC)), Dr. Julian Goggi (PET biology team leader—SBIC).
TB relapse model development team: Dr. Martin Gengenbacher (head of SPRINT-TB Laboratory and Deputy Director (Research) of National University of Singapore BSL-3 Core Facility), Dr. Rupangi Verma (project lead), Mr. Peh Jih Hou, Mr. Mark Tan.

**MODELING OF TB RELAPSE**

Human-like granuloma from the lungs of a *M. tuberculosis* infected C3HeB/FeJ mouse. A central necrotic core is surrounded by a layer of foamy macrophages and enclosed in a fibrous cuff.

Theme 2 has undertaken the task to develop novel TB relapse models with relapse rates equivalent to those seen in TB clinical trials. Such a tool would be useful in prioritizing drug regimens for clinical studies.

The researchers are focusing on adapting and developing the C3HeB/FeJ (Kramnik) model, characterized by lesions more representative of the TB pathology seen in humans, and a novel humanized mouse model developed at the Institute of Molecular and Cell Biology, A*STAR. The project will introduce further refinements to the relapse model: preclinical PET/CT imaging, which will enable longitudinal monitoring, decrease sample size, improve the precision of outcome detection, and more closely resemble the relapse assessment methods used in clinical trials.

The team has evaluated several infection routes in order to develop the most optimal approach for the establishment of human-like infection. The models will be further refined to mimic relapse rates seen in humans.
Research under this theme focuses on clinical trials of new drugs and treatment regimens for drug-sensitive TB, as well as trials of new or repurposed anti-mycobacterial or immune-modulatory drugs and combination regimens to shorten treatment duration for TB. Theme 3 also aims to improve trial methodology and to establish collaborations between Asian research centres to form a network to conduct high standard TB clinical trials.

Lead: Professor Nicholas Paton
Department of Medicine
National University of Singapore

Theme 3 team: Ms. Raihimah Binte Hamid, Mr. Benjamin Yeo Chaik Meng, Dr. Lin Wenwei, Dr. Padmasayee Papineni, Ms. Pang Yan, Dr. Meera Gurumurthy, Dr. Claire Naftalin, Ms. Celina Suresh, Mr. Mark Tan, Assoc. Prof. Lawrence Lee, Ms. Sheminaaz Akbar, Dr. James Molton, Prof. Nicholas Paton (Theme 3 Lead & Programme Director).
Last year we reported the establishment of the whole blood bactericidal activity assay (WBA) platform by Theme 3. WBA is an *ex vivo* model for measuring drug effects on mycobacterial sterilization. Healthy volunteers take the study drug and their blood, taken at predetermined time points and infected in the laboratory with TB bacteria strains, is cultured to assess bacterial killing by circulating drugs and host factors.

Theme 3 has successfully completed and is preparing for publishing a range of WBA studies. Further work using this paradigm focuses not only on antimicrobial, but also on host-directed therapy approaches and modulation of immune factors in blood by selective cell depletion.

The studies are performed with healthy volunteers, who are recruited and dosed at the Investigational Medicine Unit, National University Hospital. The assay work is done at the BSL-3 Core Facility, National University of Singapore.

Mr. Benjamin Yeo Chaik Meng setting up liquid cultures of *M. tuberculosis* for WBA assays at the BSL-3 Core Facility, National University of Singapore.
Theme 3 has now successfully completed recruitment for the pascolizumab trial, which tested safety and efficacy of blocking interleukin-4 (IL-4) using a monoclonal antibody in patients receiving standard combination therapy for pulmonary TB. A total of 32 patients were recruited at sites in Singapore, Malaysia and the Philippines. The trial was based on a strong immunological rationale for targeting IL-4, which may accelerate clearance of mycobacteria.

Data analysis for this randomized, double-blind, placebo-controlled proof-of-concept trial is currently in progress. Besides standard clinical and microbiological data, end points in host and bacterial transcriptomics, host immune profile and lung PET/MRI or PET/CT imaging are also being assessed. These analyses are conducted in collaboration with Genome Institute of Singapore and Institute of Molecular and Cell Biology, A*STAR and A*STAR-NUS Clinical Research Imaging Centre (CIRC), Singapore.

The humanized anti-IL-4 monoclonal GMP-grade antibody, pascolizumab, was made available to SPRINT-TB by GlaxoSmithKline, which has also partially supported the trial. Trial monitoring, data management and biostatistics support was provided by Singapore Clinical Research Institute (SCRI).

Site initiation visit at Quezon Institute, Philippines: Mr. Arnold Sarto (Quezon Institute (QI)), Dr. Meera Gurumurthy (project lead—National University of Singapore (NUS)), Ms. Megan Tadeo (QI), Dr. Claire Naftalin (NUS), Ms. Bianca Austria (INC Research), Prof. Nicholas Paton (principal investigator—NUS), Dr. Lalaine Mortera (site principal investigator—QI), Mr. Harry De Mesa (QI).
Theme 3 flagship project, TRUNCATE-TB (Two-month Regimens Using Novel Combinations to Augment Treatment Effectiveness for drug-sensitive Tuberculosis), the ground-breaking phase 2/3 TB regimen-shortening trial, continued its preparations for patient recruitment.

Site engagement work has been underway in multiple sites in Thailand, Indonesia, the Philippines and Singapore. To date, ethics approvals have been received in UK and at 11 of the Asian regional sites. Singapore and Philippines authorities have also given regulatory approvals for the trial, with further approvals in process in other trial site countries.

Patient recruitment will commence in 2017. The trial will recruit up to 900 patients until its completion in 2019.

The study is conducted with Singapore Clinical Research Institute (SCRI) and University College London, UK.
Recent studies have shown that measuring the host transcriptome response to TB infection and its treatment or the bacterial transcriptomic response to TB treatment can provide important insights into the nature of TB disease and effects of treatment. This approach, so far applied only in observational studies, also holds promise for application to clinical trials both as a marker of treatment efficacy and cure as well as a way for shedding light on the mechanisms by which treatments exert their beneficial effects.

Theme 3 is working with the GIS Efficient Rapid Microbial Sequencing (GERMS) at Genome Institute of Singapore, A*STAR, which houses state-of-the-art transcriptomics platforms and bioinformatics expertise, to assess host and bacterial transcriptome responses to treatments in a variety of TB clinical trials.

Host transcriptomics is done using standard RNA sequencing. For the mycobacterial transcriptome the team is using standard microarrays and also developing experimental approaches in order to improve the resolution of bacterial transcriptomes from biological samples that often contain an extremely low proportion of microbial biomass.
PET/MRI AND PET/CT IMAGING:
TB IN COLOURS

In collaboration with the A*STAR-NUS Clinical Imaging Research Centre (CIRC), Theme 3 uses various imaging platforms to measure the extent and activity of pulmonary TB and evaluate the relationship between the imaging features and clinical, microbiological and immunological markers of TB disease in the course of standard and experimental chemotherapy.

To date, Theme 3 has completed a PET/MRI versus PET/CT comparison study in active TB patients, as well as comparison of active pulmonary TB versus non-TB bacterial pneumonia using the fludeoxyglucose (FDG) tracer. A study in TB-exposed contacts seeking to differentiate between patients with completely quiescent latent TB and those with subclinical latent disease has also been successfully completed using the same tracer.

Theme 3 is now advancing a range of other TB imaging studies, with the current focus on using novel or repurposed tracers ($^{18}$Ga-DOTANOC, $^{11}$C-acetate; $^{89}$Zr-infliximab (p. 16) in the pipeline) in both active pulmonary TB patients and TB-exposed contacts.

Dr. Benjamin A. Thomas (A*STAR-NUS Clinical Imaging Research Centre) led the imaging and image analysis aspects of the PET/MRI vs PET/CT comparison in TB patients project.
Theme 4 studies factors that hinder provision of TB therapy and develops solutions to these issues. It conducts observational studies to define individual or systematic barriers that impair the success of effective combination therapy and drives interventional studies of new approaches to address these problems. The theme also focuses on TB treatment delivery, such as novel models of observed therapy and inducing behavioral changes to enhance adherence to treatment.

TREATMENT DELIVERY BARRIERS: THE CASCADE OF CARE

The importance of barriers to treatment delivery is illustrated by a recent publication of the cascade of TB care in India (image on p. 25), which depicts a situation common to many high-burden countries in Asia – even though we have some effective treatments available, only a small proportion of the patients who have active TB ever reach the stage of being cured. The situation is even worse for patients with drug-resistant TB. WHO estimates that 4.3 million TB patients were missed by health systems and remained either untreated or unreported to national TB programmes in 2015. This situation severely undermines global efforts to combat TB. Developed new drugs and treatment regimens may not therefore have the desired effect on TB incidence if they cannot reach every TB patient.

Theme 4 projects seek to prevent this attrition of good outcomes at each stage of the cascade. The goal is to prevent these patients from going missing from the pathway of being diagnosed, treated and cured of their disease.

The cascade of care for all forms of tuberculosis in India’s Revised National Tuberculosis Control Programme (RNTCP) in India, 2013. Error bars depict 95% confidence intervals. Arrows indicate areas for intervention being explored in SPRINT-TB Theme 4. Modified from: Subbaraman R et al. PLoS Med 201612 (Open Access, under Creative Commons License).
FINDING CASES: HEALTH SYSTEM BARRIERS

Theme 4 conducted a study in Yangon, Myanmar to assess differences in case finding efficiency among genders. The researchers collected data on all new adult smear-positive cases in ten township health centres across Yangon and conducted a descriptive cross-sectional analysis of sex differences in TB diagnoses. National prevalence survey data was also analysed. Only 30% of the new smear-positive TB patients were female. The sex ratio of newly diagnosed cases varied by age group, month of diagnosis and township of diagnosis. Potential health system barriers were identified.

This results of this study were published in BMC Infectious Diseases in March 2016.

A township in Myanmar— one of Theme 4 health systems research sites.

IMPROVING DIAGNOSIS: GENETIC TESTS FOR MDR-TB

The findings in the cascade of care are even more alarming when it comes to patients with MDR-TB, with even greater rates of attrition at the stages of diagnosing drug resistance and in accessing and completing appropriate treatment.

The main barrier to diagnosis of MDR-TB is that methods for identification of multidrug resistance are slow and expensive. In recent years, the development of the commercial GeneXpert test that uses molecular approaches to identify genetic mutations underlying resistance to rifampicin has made a major contribution improving rapid diagnosis of TB drug resistance. However, some patients have resistance to many drugs and developing new genetic approaches that could rapidly identify patients with more complex resistance patterns is also urgently needed.

Theme 4 undertook the task of creating a comprehensive genomic database of clinical *M. tuberculosis* isolates from countries across Asia. Led by principal investigator Assoc. Prof. Teo Yik Ying and project lead Assist. Prof. Rick Ong Twee Hee at Saw Swee Hock School of Public Health, National University of Singapore, this project is conducted in strategic partnership with other worldwide initiatives such as the Relational Sequencing TB Data Platform (ReSeqTB) and CRyPTIC. The collaboration seeks to create a relational database containing genetic and drug resistance phenotype information that can ultimately function as a tool that clinicians could use to choose the optimal individual treatment regimen based on the whole genome sequence from a patient.
One of the main problems with TB treatment is that it takes a long time. This has been especially challenging for MDR-TB, where the usual course of treatment lasts 18-24 months. In 2016, WHO launched a new shorter treatment combination for drug-resistant TB requiring just 9 months of treatment. This could have a major positive impact on the ability of national TB programmes to provide treatment, as well as enhance treatment completion rates. In addition, for people in whom the 9-month combination is not suitable, WHO made changes to their recommended combinations of drugs for use in the 18-24 month treatment course. Although observational data indicate that cure rates are likely to be higher with these newer combination regimens, there is very limited information about the potential for evolution of resistance on these regimens. Such information is desperately needed.

Using new technology developed for detailed testing of resistance to multiple drugs using a microtitre drug susceptibility platform, SPRINT-TB is initiating a project to assess outcomes with these new treatment combinations and to relate these to the evolution of drug resistance during the prolonged treatment with these new regimens.

This will be one of the inaugural projects for the A-TRACTION network (the Asian TB Research and Clinical Trials Integrated Organisational Network) (p. 31) – a network of multiple sites across Asia that is being set up by SPRINT-TB in collaboration with the International Union Against TB and Lung Disease and with financial support from the Asia Pacific Economic Cooperation (APEC).
In last year’s report we introduced MIST (Mobile Interactive Supervised Therapy), a smartphone platform to monitor TB treatment regimen adherence. The system enables patients to submit videos of themselves taking pills, with the aim of reducing the need for patients to attend a directly observed therapy (DOT) clinic in person. MIST pilot studies with healthy volunteers were published in BMJ Open in December 2016.

After raising further funding from National Health Innovation Centre Singapore and Singapore’s National Medical Research Council, the system is being further developed to include advanced features such as face recognition and automated detection of pill intake. The platform will be tested with TB patients in selected countries in the region in 2017.

MIST Project Team: Ms. Pang Yan (National University Hospital (NUH)), Mr. Teck Sin Lim (I3 Precision Pte Ltd (I3P)), Prof. Nicholas Paton (Department of Medicine, National University of Singapore (NUS)), Dr. James Molton (principal investigator—NUH), Assoc. Prof. Wei Tsang Ooi (School of Computing, NUS), Mr. Modukuru Naga Kishore (National Health Innovation Centre Singapore), Mr. Jianan Wang (I3P).
SPRINT-TB NEWS
The Summit Research Programme (SRP) concept was launched in 2016 by National University of Singapore (NUS) Yong Loo Lin School of Medicine in order to differentiate research programmes with established high-caliber investigators, high international impact and potential to achieve significant improvements and innovations in disease understanding, clinical practice, policy, products and population health.

After an extensive review process, SPRINT-TB was selected as one of the five SRP programmes at NUS in October 2016.
SPRINT-TB LABORATORY

SPRINT-TB Laboratory is at the core of SPRINT-TB research, accommodating staff and students from all four research themes. It is situated at the top level of National University of Singapore’s Centre for Translational Medicine—adjacent to the BSL-3 Core Facility (below) and in the same building as the Clinical Imaging Research Centre and Investigational Medicine Unit (next page). This set up allows to efficiently streamline and integrate the programme’s translational research workflow.

BSL-3 CORE FACILITY

The Biosafety Level-3 (BSL-3) Core Facility at National University of Singapore (NUS) is a vital part of the SPRINT-TB infrastructure, with the majority of SPRINT-TB projects fully or partially conducted at the facility.
The Investigational Medicine Unit (IMU) at National University Hospital helps to conduct multiple SPRINT-TB clinical trials or studies that require the participation of healthy volunteers or TB patients. IMU research is conducted in compliance with Good Clinical Practice standards by highly trained clinicians and research coordinators.

Regional TB Trials Network

Most clinical trials of SPRINT-TB are conducted as regional multi-centre collaborations. With the facilitation of SPRINT-TB, the clinical sites in Singapore and overseas are forming into the Asian TB Research and Clinical Trials Integrated Organisational Network (A-TRACTION), which will provide a platform for standardizing laboratory and data management, as well as conducting high quality multicenter collaborative TB studies and facilitating intra-Asian capacity building.

A large range of SPRINT-TB clinical imaging studies and trials with imaging end points are conducted in collaboration with the A*STAR-NUS Clinical Imaging Research Centre (CIRC), which is equipped with state-of-the-art set up such as PET/CT and PET/MRI scanners and the GMP Cyclotron Facility.
Training of future generations of researchers is one of the key mandates of SPRINT-TB, which continued its efforts in the area in 2016. To date, SPRINT-TB enrolled eleven graduate and twelve undergraduate students. Ten postdoctoral fellows and six clinician-scientists work under the programme.

**STUDENTS**

Kenneth Neo Zheng Wei is an undergraduate student in Life Sciences at National University of Singapore, who joined SPRINT-TB for his summer break attachment in 2016. He contributed to a range of projects across all Themes and has set up a mycobacterial strain verification protocol at the SPRINT-TB laboratory. Kenneth went on to participating in the student exchange programme at the KTH Royal Institute of Technology in Sweden, where he focused on bacteriology and immunology courses.

Benedict Lee and Cleon Kho are undergraduate medical students at National University of Singapore Yong Loo Lin School of Medicine. They have joined SPRINT-TB Theme 1 for their research attachment in Assoc. Prof. Thomas Dick’s laboratory at Department of Microbiology and Immunology. Benedict’s and Cleon’s research work will contribute to their skill set in their pursuit of a medical degree, igniting interest in and providing a solid basis for further research after their graduation.
CLINICIAN-SCIENTISTS

Dr. Gail Cross received her medical degree from Monash University, Australia and underwent advanced training in infectious diseases across multiple hospitals in Australia. She currently holds the position of an associate consultant at the Division of Infectious Diseases, National University Hospital. Dr. Cross has research interests in TB immunology and host-directed therapy for TB. She leads SPRINT-TB projects investigating the role of immune system factors via WBA assays (p. 19) and will be assuming the project lead role in one of the new host-directed therapy trials under Theme 3.

Dr. James Molton received his undergraduate medical training in the UK and postgraduate Infectious Diseases specialist training in Australia. Currently he works as a consultant at National University Hospital (NUH). As Clinical Director, he is in charge of clinical matters within the NUH Division of Infectious Diseases. Dr. Molton’s research under SPRINT-TB includes a range of TB imaging studies (p. 23) and development of approaches to enhance treatment adherence (p. 27).

Dr. Lim Hui Fang is a consultant at the Division of Respiratory and Critical Care Medicine of National University Hospital. She is a recipient of Singapore Ministry of Health’s National Medical Excellence Award (team) and holds several research awards. Dr. Lim is interested in breath analysis as an approach to TB point-of-care diagnostics and will be launching her research under SPRINT-TB Theme 4 in 2017.
EVENTS AND VISITORS

Since its start in 2014, SPRINT-TB welcomed a number of distinguished guests and renowned international experts in the field of TB, who visited the programme for collaboration and experience sharing. Seminar talks given by the visitors in the field of their expertise are organised and hosted by SPRINT-TB and are open to all researchers, scientists and clinicians in Singapore.

Besides its seminar series, SPRINT-TB hosts bi-weekly journal clubs run by the programme’s graduate students and postdoctoral fellows. The most prominent of SPRINT-TB regular public events are its annual symposiums in TB research (next page), which started in 2015 and have been attracting multidisciplinary attendees from Singapore and overseas.

SPRINT-TB hosted Dr. Toshihiro Nukiwa, Executive Director of Japan Anti-Tuberculosis Association (JATA) in October 2016.

SEMINARS

Dr. Geraint Davies
University of Liverpool, UK

Prof. Stefan H. E. Kaufmann
Max Planck Institute for Infection Biology, Germany

Dr. Richard Hafner
National Institutes of Health, USA

Prof. Veronique Dartois
Rutgers-New Jersey Medical School, USA

Assoc. Prof. Jun Zheng
University of Macau, China

Prof. Robert Wallis
The Aurum Institute, South Africa

Prof. Barry R. Bloom
Harvard University, USA

Dr. Ashlee Earl
The Broad Institute of MIT & Harvard, USA

Prof. Daniela Cirillo
San Raffaele Scientific Institute, Italy

Prof. Richard K. Haynes
North-West University, South Africa; The Hong Kong University of Science and Technology, Hong Kong

Dr. Toshihiro Nukiwa
Japan Anti-Tuberculosis Association, Japan
In 2016, SPRINT-TB continued its TB symposium series by holding its 2nd Annual Symposium at National University of Singapore on November 10, 2016. The symposium programme included presentations on mycobacterial targets, development of new TB drug candidates, clinical trials and new approaches to treatment delivery.

Since its inauguration, the symposium series has welcomed repeat attendees and hosted international speakers such as Prof. Eric Rubin (Harvard T. H. Chan School of Public Health, USA), Dr. I. D. Rusen (International Union Against Tuberculosis and Lung Disease), Dr. Davide Manissero (Qiagen, UK), Prof. David Sherman (Center for Infectious Diseases Research, USA), Prof. Sabine Ehrt (Weill Cornell Medical College, USA), Prof. Brian Angus (University of Oxford, UK) and Dr. Kiyoyasu Fukushima (Nagasaki Genbaku Isahaya Hospital, Japan).

More information about SPRINT-TB’s next annual symposium will be available at www.tuberculosis.sg.
Since its inception in 2014, SPRINT-TB has been overseen by the Scientific Advisory Board (SAB), which meets annually—by teleconference or on site—to review the progress of the programme, make phase transition decisions, and prioritize activities for allocation of funding. The SPRINT-TB SAB consists of three members: Prof. Brian Angus (SAB Chair; University of Oxford, UK), Prof. Sabine Ehrt (Weill Cornell Medical College, USA) and Prof. David Sherman (Center for Infectious Diseases Research, USA).

The first on-site review by the SAB took place in November 2016. During the review, which took place over three days, SPRINT-TB projects were presented to the SAB. The SAB members met the programme team and toured its facilities. The SAB members commended SPRINT-TB achievements in its first two years of operations and made valuable suggestions for directions of the key research projects. The following SAB on-site review will be held in 2017.

“SPRINT–TB IS AN IMPRESSIVE COLLECTION OF TALENTS THAT HAS COME TOGETHER AS AN INNOVATIVE BENCH TO BEDSIDE PROGRAMME IN TUBERCULOSIS RESEARCH. IT POSITIONS SINGAPORE AS A LEADER IN ADDRESSING A MAJOR PUBLIC HEALTH CHALLENGE IN ASIA AND THROUGHOUT THE WORLD.”

From SAB Review Report 2016
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RESEARCH OUTPUT 2016

PAPERS


ABSTRACTS


Khan MS, Ning Y, Chen J, Xu L, Coker RJ. Prolonged delay to diagnosis despite availability of free health care services. A study of 76, 486 tuberculosis patients in Yunnan, China. The 47th Union World Conference on Lung Health, Liverpool, United Kingdom, 26-29 Oct 2016.


TALKS


Nicholas Paton. Approaches to Shortening Treatment for TB. Department of Medicine, Saiful Anwar Hospital, Malang, Indonesia, 16 November 2016.

PATENTS


Dipeptidyl Boronates As Inhibitors Of Mycobacterial Caseinolytic Protease (Clp) For The Treatment Of Tuberculosis. Inventors: Brian Dymock, Anders Poulsen, Thomas Dick, Wilfried Moreira, Sridhar Santhanakrishnan. IPOS No.: 10201609299R; Filing Date: November 7, 2016.
HOW YOU CAN HELP FIGHT TB

WHY WE FUNDRAISE

SPRINT-TB research is primarily supported by grants from Singapore’s National Medical Research Council and other public funding sources. These awards are a testament to the scientific quality and clinical impact of the work we do. However, the process of securing public sector funding for new projects requires substantial time — usually at least 18 months.

SPRINT-TB is at the cutting edge of TB research, with our research team brimming with innovation and novel ideas. Currently the only limit on the growth of the programme is the funding available to develop these new ideas into initial studies. We rely on donations and other discretionary funding to kick-start these new projects, thereby maximising the contribution that Singapore and SPRINT-TB can make to the global fight against tuberculosis.

HOW YOU CAN HELP

We welcome each and every donation, which will be held by National University of Singapore and used exclusively for SPRINT-TB research activities.

Get in touch with us at any time. Here is an easy way to contact us:

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Thank you for your support of the fight against TB!
PURCHASE OF LABORATORY EQUIPMENT AND MATERIALS

SYNTHESIS OF COMPOUNDS AND DRUG CANDIDATE MOLECULES

SUPPORT OF PATIENTS’ TEST COSTS IN CLINICAL RESEARCH
ACKNOWLEDGEMENTS

Cover art: Colonies of mycobacteria—photography by Michelle Yee Mei Kheng. Michelle is a research assistant working under SPRINT-TB Theme 1. Read about Michelle’s research on pages 12-13.

Many photos in this report were taken by SPRINT-TB undergraduate researcher Kenneth Neo Zheng Wei. Read Kenneth’s story on page 32.

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