The influence of *Mycobacterium tuberculosis* on B cells during disease and treatment response

Jacques Du Plessis

Classically, tuberculosis (TB) research is T-cell dominated because of the pathogens intracellular nature. It has come to our attention that B cells have possible function as mediators in cellular immunity in a non-antibody fashion. We hypothesize that B cells play an early role during TB infection/disease and that TB treatment also have an effect on its frequency/maturation. With this study we aim to determine the B cell immune profile of newly diagnosed and treated TB patients. To address these questions, fresh whole blood was collected from patients and stained with an appropriate panel of antibodies for flow cytometric analysis. By comparing median values of the observed B cell phenotypes, we demonstrate that there is a difference between B cell population regarding healthy controls, TB patients at diagnosis and end-of-treatment (week 24) TB patients. These results show that there is a prominent difference between the various B cell phenotypes during disease and treatment response. Further investigation regarding the functional capabilities of these phenotypes is warranted to elucidate their role during the disease state.

**SAMRC researcher:** Jacques Du Plessis - jdup@sun.ac.za

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Implementation of IPT among health care workers living with HIV in KwaZulu-Natal, South Africa

Tudor C, Van der Walt M, Golub JE

Carrie Tudor, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; Martie Van der Walt, South African Medical Research Council, Pretoria, South Africa; Jonathan E. Golub, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Health care workers (HCWs) are at greater risk of TB than the general population – this risk is greater for HIV-positive HCWs. Isoniazid preventive therapy (IPT) is recommended to prevent TB among people living with HIV. A retrospective operational research study to assess IPT implementation among HIV-positive HCWs in 15 hospital occupational health (OH) clinics in KwaZulu-Natal.

865 HIV-positive HCWs were identified via OH clinic chart review. 275 (32%) HCWs were offered IPT and 198 (72%) accepted. 28% (168/590) were ineligible due to either current TB treatment (n=43) or treatment for TB within two years (n=125). Of the 198 who took IPT, 160 (81%) completed therapy, 21 (11%) stopped IPT, and 17 (9%) had no outcome recorded. Among those who stopped IPT 10 (5%) defaulted, 8 (4%) were diagnosed with TB (2 MDR-TB), and 1 (5%) due to side effects. 6 (3%) HCWs were diagnosed with TB (2 MDR-TB) after completing IPT. The proportion of HCWs who accepted IPT did not differ by age, sex, or occupation compared with those who did not accept. While a small proportion of HCWs were offered IPT, the majority accepted IPT with low rates of default. However, several HCWs had to stop IPT because they were diagnosed with TB or MDR-TB while on therapy.

**SAMRC researcher:** M Van der Walt - martie.VanderWalt@mrc.ac.za
Development and evaluation of a nurse case management model to address MDR-TB and HIV in South Africa

Farley, J.E.1, Kelly, A.M.2, Reiser, K.1, Brown, M.1, Kub, J.1, Davis, J.G.1, Walshe, L.3, Van der Walt, M.4

1School of Nursing, Johns Hopkins University, Baltimore, MD, USA; 2College of Nursing, Michigan State University, East Lansing, MI, USA; 3Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; 4Tuberculosis Epidemiology and Intervention Research Unit, Medical Research Council, South Africa

Corresponding Author: Jason E. Farley, PhD, MPH, CRNP, FAAN. 525 N. Wolfe Street, Room 450, Johns Hopkins University School of Nursing, Baltimore, MD 21205, USA. Tel: (+1) 410 502 7563. Fax: (+1) 410 955 7733. e-mail: jfarley@son.jhmi.edu

To develop and evaluate a nurse case management model and intervention using the tenets of the Chronic Care Model to manage treatment for MDR-TB patients with a high prevalence of human immunodeficiency virus (HIV) co-infection. A quasi-experimental pilot programme utilizing a nurse case manager to manage care for 40 hospitalized MDR-TB patients, 70% HIV co-infected, during the intensive phase of MDR-TB treatment. Patients were followed for six months to compare proximal outcomes identified in the model between the pre- and post-intervention period. The greatest per cent differences between baseline and six-month MDR-TB proximal outcomes were seen in the following three areas: baseline symptom evaluation on treatment initiation (95% improvement), baseline and monthly laboratory evaluations completed per guidelines (75% improvement), and adverse drug reactions acted upon by medical and/or nursing intervention (75% improvement). Discharge planning was newly implemented for all patients. The nurse case management model provided improvements in guideline-based treatment, monitoring adverse drug reactions and utilizing discharge readiness tools across a six-month period. Further study is required to determine if the intervention introduced in this model will ultimately result in improvements in final MDR-TB treatment outcomes.

SAMRC researcher: M Van der Walt- martie.VanderWalt@mrc.ac.za

WGS pipeline applications to interrogate genetic diversity among clinical strains of Mycobacterium tuberculosis

Ruben van der Merwe

Mycobacterium tuberculosis, the causative agent for Tuberculosis disease, is a global epidemic with a mortality rate of over 1.6 million annual lives. The rampant spread of drug resistance drastically exacerbates drug treatment outcomes and logistics. Investigation of Mycobacterium tuberculosis on a genomic level has provided several tools to help combat and understand the organism by providing key information for use in drug development, molecular diagnostics and epidemiological investigations. The genetic diversity among clinical strains of M. tb primarily consist of those originating from evolutionary changes between strains families. Further genomic differences are those that correspond to phenotypic differences such as drug resistance and strain fitness. Additionally, it is increasing becoming clear that synergistic genomic mutations are at play that determine more complex strain attributes such those that play a role in transmission, persistence, reactivation and relapse. WGS is increasingly encroaching mainstream research and medical diagnostics as costs plummet which allow us to address these important research questions. However, there is no standardized pipeline for NGS data processing and analysis specific for Mycobacterium tuberculosis. Here we discuss the development, complexities and applications of mycobacterium tuberculosis specific WGS pipelines applied to clinically relevant strains.

SAMRC researcher: Ruben van der Merwe - rvdm@sun.ac.za
Using next generation sequencing to unravel the molecular epidemiology of the drug-resistant TB epidemic in the Eastern Cape

M Klopper1*, G Hill-Cawthorne2, RM Warren3, EM Streicher1, PD van Helden1, AP Trollip1, AM Abdallah2, Arnab Pain2, TC Victor1

1Stellenbosch University, Faculty of Health Science, Department of Biomedical Science, South Africa; 2King Abdullah University of Science and Technology, Saudi Arabia; *Presenting author

The Tuberculosis (TB) prevalence, and in particular drug-resistance, is of great concern in the Eastern Cape Province of South Africa with high incidence and dismal outcomes for XDR-TB patients.

Objectiver: To investigate the molecular basis of the drug-resistant TB epidemic in the Eastern Cape.

Hypothesis: Molecular evolution of M. tuberculosis strains gave rise to specific features which are responsible for the success of highly drug-resistant strains occurring in this region.

Molecular techniques, including spoligotyping, targeted gene sequencing, whole genome sequencing, RNA sequencing and phenotyping in conjunction with bioinformatics analyses were used to characterise TB strains from the Eastern Cape.

A specific strain type is overrepresented among pre-XDR and XDR-TB cases, when compared to MDR and sensitive TB cases. These strains are genetically highly similar with certain features that are unique to the group of strains. Furthermore, they are resistant to nearly all available drugs in the TB treatment regimen.

The high degree of similarity among pre- and XDR-TB isolates indicates that these strains are highly transmissible. Further evolution results in acquisition of specific mutations in certain genes associated with resistance. These strains are virtually untreatable and indicates the emergence of ‘totally drug-resistant TB (‘TDR’-TB). Four reports of highly resistant clinical isolates have been published in recent years from sites across the globe. It has, however been swept under the carpet under the auspices that outcomes are no worse than for XDR-TB. This work for the first time shows that ‘TDR’-TB is highly transmissible. A monster is lurking in the shadows of neglect and ignorance and calls for immediate action by TB control worldwide to prevent untreatable TB from becoming a catastrophe.

Acknowledgement of the situation is vital in order to protect the efficacy of old and new available drugs.

SAMRC researcher: M Klopper - marisat@sun.ac.za
Effect of Diabetes Mellitus (DM) in the human host immune response against Mycobacterium tuberculosis (TB)
Katiso Mgadi

Phagocytes function at the interface between innate and acquired immune responses and are important for efficient eradication of infectious pathogens. Receptor-ligand interactions that mediate phagocytosis in monocytes depends largely on the complement and Fcγ receptors. Efficient killing of mycobacteria by host monocytes depends on a number of mechanisms including production of reactive oxygen species (ROS) by the phagosomal NADPH oxidase, NOX2. People living with Diabetes Mellitus (DM) patients have a higher risk of developing Tuberculosis (TB) after encounter with Mycobacterium tuberculosis. We investigate the phagocytic function and microbicidal activity of monocytes from DM, DM/TB, and TB patients compared to normal healthy controls. Whole blood monocyte phenotypes expressing complement and Fcγ receptors will be analysed using multiparameter flow cytometry and compared between groups with and without DM. Mycobacterium bovis BCG will be used for the infection studies to investigate monocyte phagocytic ability and microbicidal activity in cell culture. Induced ROS production will also be analysed. The work will shed new light on the mechanisms by which DM affects phagocytic function and influences susceptibility to TB.

SAMRC researcher: Katiso Mgadi - kmgadi@sun.ac.za

Evaluation of anyplex plus assay for rapid detection of rifampicin and isoniazid resistance in mycobacterium tuberculosis isolates
Lesibana Malinga

Novel Anyplex assay allows rapid detection of target sequences for Mycobacterium Tuberculosis (MTB), non-MTB, concomitant with the amplification of rifampicin (RIF) and isoniazid (INH) resistance. It permits screening of 15 and 6 mutations for RIF and INH drugs respectively. The aim of the study was to compare Anyplex plus assay and MTBDRplus with culture DST Materials We analysed 107 MTB culture isolates (68 RIFR/IHNR, 2 RIF & 25 INH Mono-resistant and 9 susceptible) stored at our Biobank. RIF and INH drugs susceptibility was previously tested on culture. MTBDRplus and Anyplex assay were done on the same extracted DNA. Results Of 107, Anyplex assay had 96% while MTBDRplus had 79% MTB detection rate respectively. The sensitivity of RIF for both assays was comparable at 88%. Anyplex assay had a lower sensitivity to INH of 27% compared to 62.0% of MTBDRplus. The overall detection of RIFR/INHR was higher in MDRTBplus with 69% as compared to 22% in Anyplex assay. Anyplex assay resolved 20% of MTBDRplus inconclusive results with 18 MTB (8 RIFR & 1 INHR) and 4 with non-MTB. Conclusion Genotype MTBDRplus is highly accurate for detection of RIFR/INHR. The Anyplex assay was able to resolve inconclusive results with high accuracy. Anyplex assay increases the diagnostic yield of molecular assays.

SAMRC researcher: Lesibana Malinga – lesibana.malinga@mrc.ac.za
The effect of efflux pump inhibitors on first and second-line tuberculosis drugs differs in rifampicin mono-resistant clinical isolates of mycobacterium tuberculosis
M.C Pule1, G.E Louw1, R.M Warren1, P.D Van Helden1, T.C Victor1
DST/NRF Centre of Excellence in Biomedical Tuberculosis Research/MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, Stellenbosch University, South Africa

Present reports suggest that specific rpoB mutations are associated with altered intrinsic resistance to other first and second-line anti-tuberculosis drugs.

Objective: To determine the effect of efflux pump inhibitors (EPIs) on the susceptibility of rifampicin mono-resistant Mycobacterium tuberculosis clinical isolates to first and second-line anti-tuberculosis.

Rifampicin mono-resistant clinical isolates (n=8) from different strain families with the same rpoB mutation (Ser531Leu) were selected. The effect of the EPIs (verapamil, CCCP) to first-line (isoniazid, pyrazinamide, ethambutol, streptomycin) and second-line (ciprofloxacin, moxifloxacin, ofloxacin, amikacin, capreomycin and ethionamide) drugs was determined by the MGIT 960 in combination with EpiCenter Technology. The isolates were exposed to the above drugs at MIC in the presence/absence of EPIs. Thereafter, the fractional inhibitory concentration (FIC) was calculated and the interaction values were classified as synergistic when FIC index ≤ 0.5-0.9, indifference/additive when FIC index = 1-1.9 and antagonistic when FIC index ≥ 2.

The FIC values for first-line drugs in combination with verapamil and CCCP respectively were as follows: isoniazid (1.0;0.4), streptomycin (0.4;1.2), ethambutol (1.4;1.5) and pyrazinamide (0.7;0.2), then for second line drugs amikacin (0.7;1.7), capreomycin (0.8;0.7), ciprofloxacin (0.8;2.6), ofloxacin (1.1;1.8), moxifloxacin (1.0;1.1) and ethionamide (0.2;0.9). Verapamil had an effect on the anti-TB drugs MICs with p-value= 0.0001 and in contrast to CCCP (p= 0.01196) in rifampicin mono-resistant strains with rpoB mutation 531.

Our findings demonstrated that EPIs enhanced the susceptibility to certain first- and second-line anti-TB drugs in rifampicin mono-resistant strains. Thus suggests the involvement of efflux pumps activities in defining the level of intrinsic resistance.

SAMRC researcher: M Pule - cpule@sun.ac.za

Circulating serum microRNAs as potential markers of early treatment response in pulmonary tuberculosis
Lance Andrew Lucas

Tuberculosis (TB) remains a key universal health concern. New biomarkers for TB diagnosis are urgently needed. MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a major part in the post-transcriptional regulation and circulate freely in body fluids in a stable and robust form. Recently, circulating miRNAs have been proposed as TB diagnostic markers. However, various technical aspects cripple their detection and analysis. RNA from plasma/serum was extracted using the Qiagen miRNeasy Serum/Plasma Kit. RNA quantity, purity and integrity were evaluated using the NanoDrop® ND-1000 and Agilent 2100 Bioanalyser. Real-Time PCR (qRT-PCR) and TaqMan® MicroRNA Assays were used for the detection and quantification of miRNA. Selected miRNAs will be analysed as potential early treatment response markers. Conventional methods for RNA quality control seem inadequate for serum/plasma miRNA. Spike-in RNA controls are necessary to determine small RNA extraction efficiency. qRT-PCR detects serum miRNAs, but at minute levels. Serum/plasma miRNA are attractive potential markers but technical difficulties, as well as cost hamper rapid progress.

SAMRC researcher: Lance Lucas - lalucas@sun.ac.za
To evaluate the accuracy, sensitivity and specificity of the Loop-mediated Isothermal Amplification (LAMP) assay as compared to microscopy and culture in a Primary Healthcare clinic (PHC) on the detection of M. tuberculosis (TB)

S. Reddy¹, S. Ntoyanto¹, M. Fosi¹, Z. Mkhalipi¹, B. Spooner¹, P. Nabeta⁴, M. Dlamini², T. Reddy³, G. Ramjee¹, K. Mlisana² and P. Kiepiela¹

¹HIV Prevention Research Unit, Medical Research Council, Durban, South Africa; ²Medical Microbiology, National Health Laboratory Services, Durban, South Africa; ³Biostatistics Unit, Medical Research Council, Durban, South Africa; ⁴FIND Diagnostics, Genève, Switzerland

South Africa accounts for approximately 25% of the global burden of TB-HIV while approximately 1% of the population develops TB each year. WHO reported a >3 fold increase in the number of TB cases in South Africa since 1990. KwaZulu-Natal bears 31% of the burden of TB in South Africa, and is the epi-centre of the HIV epidemic resulting in high HIV/TB co-infection rates. The confirmatory test used in countries where TB is endemic is smear microscopy; however this has low sensitivity. The GeneXpert Rif TB test has been shown to have >84% sensitivity and 100% specificity in smear negative TB, and 99% sensitivity in smear positive TB. The South African government has implemented the GeneXpert as a diagnostic test in conjunction with smear microscopy which is performed in centralised laboratories. The novel Loop-mediated isothermal Amplification (“LAMP”), has been developed by FIND. The speed of the reaction (40 minutes), absence of need for a thermo-cycler, and visual readout make LAMP an excellent platform for the development of a simple and sensitive tool for molecular detection of TB in developing country settings. We present preliminary data. METHOD: TB suspected individuals who attended the two eThekwini Clinics in Durban, South Africa were enrolled into the study after informed consent was obtained. Sixty microliters of sputum sample was used to extract DNA using the LoopampTM PURE DNA Extraction kit and results were compared to smear microscopy and culture on the same sputum sample. RESULTS: Sixty four/230 samples yielded a positive TB culture result. The sensitivity of smear microscopy compared to culture, was 43% with a specificity of 98.8% while the sensitivity of the TB LAMP was 73.8% and specificity 98.2% respectively. The TB LAMP is a suitable diagnostic platform which appears to be a promising point-of-care test for the molecular detection of pulmonary TB in developing countries.

SAMRC researcher: S Reddy – shabashini.reddy@mrc.ac.za
**Ion Torrent Next-Generation Deep Sequencing compared to other phenotypic and genotypic methods for detection of pyrazinamide resistance in Mycobacterium tuberculosis**


1Department of Medical Microbiology, University of Pretoria, South Africa; 2TB Epidemiology and Intervention Research Unit, Medical Research Council, Pretoria, South Africa; 3Longhorn Vaccines & Diagnostics, San Antonio, Texas, U.S.A.; 4University of Texas at San Antonio, San Antonio, Texas, U.S.A.

Pyrazinamide (PZA) is the only anti-tuberculosis compound used for first-line and second-line therapy of TB. However, PZA inclusion in second-line regimens for MDR-TB cases is rarely based on PZA susceptibility of the isolates. We used Ion Torrent next-generation sequencing (NGS) to identify known PZA resistance mutations in the pncA gene and compared these results to BACTEC 960 and other PZA susceptibility assays.

We successfully re-cultured 94 MDR isolates from a 2001 archived panel. Ion Torrent NGS was performed for genotypic analysis of a 606 bp fragment to detect mutations known to be associated with PZA resistance. For all isolates, PZA susceptibility was previously determined by BACTEC 460, Wayne’s Test, and Sanger sequencing.

Ion Torrent showed 100% concordance to phenotypic data obtained by BACTEC 960/460. The Ion Torrent detected hetero-resistance that resolved discordance in results from other PZA resistance assay.

Culture cannot identify hetero-resistance. Ion Torrent sequencing, in contrast to traditional allows depth of coverage, i.e., deep sequencing of mixed strains within an isolate. Ion Torrent NGS may provide an important new tool in fight against TB.

**SAMRC researcher: Nontuthuko Maningi – nontuthuko.maningi@mrc.ac.za**

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**Xpert MTB/RIF reduces treatment initiation delay and morbidity in MDR-TB patients co-infected with HIV**

Nesri Padayatchi, Marian Loveday, Naressa Naidu, Kumeren Govender, Garth Osburn, Kogie Naidoo, Max O’Donnell

The Xpert MTB/RIF RIF assay has been rapidly introduced in public health facilities in South Africa. This retrospective study assesses the impact of Xpert MTB/RIF in reducing time to treatment initiation and morbidity in MDR-TB patients co-infected with HIV.

In this expedited record review, between 2008 and 2010 813 MDR-TB patients diagnosed with traditional culture methods were referred to a specialist TB hospital. In 2011, 197 patients found to be rifampicin resistant by Xpert MTB/RIF were referred to the same hospital. 79% of all the patients were HIV co-infected, with most receiving ART.

The median time from diagnosis to MDR-TB treatment initiation was 92 days (IQR 69-120) with traditional culture testing, compared to 21 days (IQR 14-33) using Xpert MTB/RIF. A review of the chest radiographs of patients referred with traditional culture testing compared with Xpert MTB/RIF result showed that 88.7% and 22.1% respectively had bilateral disease or cavities greater than 4cm.

Conclusion: The Xpert MTB/RIF assay limits the presentation of advanced disease. We believe this will improve treatment success, which, together with reduced time to treatment initiation will accelerate control of the disease.

**SAMRC researcher: Marian Loveday – marian.loveday@mrc.ac.za**
Decentralised vs. centralised care for MDR-TB patients: A prospective cohort study comparing final treatment outcomes in KwaZulu-Natal, South Africa
Marian Loveday, Kristina Wallengren, James CM Brust, Jacquelin Roberts, Anna Voce, Bruce Margot, Jacquelin Ngozo, Iqbal Master, Gail Cassell, Nesri Padayatchi

To address the MDR-TB epidemic the KwaZulu-Natal Department of Health introduced a novel model of decentralised care. However, the effectiveness of decentralised care in a setting with a high burden of HIV and TB is unknown.

In an observational, prospective cohort study of adult MDR-TB patients with and without HIV co-infection we compare treatment outcomes and examine predictors of treatment success in patients managed closer to home in 4 decentralised rural sites with those in traditional care at a central, specialised hospital.

Of 1549 patients treated, 736 were at 4 decentralised sites and 813 at a central hospital. Overall, 75% were HIV co-infected and 86% received ART.

In multivariate analysis MDR-TB patients were more likely to have a successful treatment outcome if they were treated at a decentralised site (adjusted OR=1.43, p=0.01). Although there was heterogeneity in outcomes at the 4 decentralised sites, Site 1 demonstrated that treatment success of 72% can be attained in a decentralised setting.

Decentralised care for patients with MDR-TB was more effective than care in a central, specialised hospital.

SAMRC researcher: Marian Loveday – marian.loveday@mrc.ac.za