DST-NRF CENTRE OF EXCELLENCE

ANNUAL PROGRESS REPORT

Reporting Period

1 January 2010 – 31 December 2010

CONTENTS **Page**

Executive Summary……2
Progress Report……5
Human Resources……44
Outputs……46
Finances……57

Identification

Names of Co-Directors	:	Professor Paul D. van Helden Professor Valerie Mizrahi
Name of CoE	:	DST/NRF Centre of Excellence for Biomedical TB Research
Abbreviated CoE Name	:	CBTBR
Host institutions	:	University of Stellenbosch and University of the Witwatersrand
Date completed	:	31/03/2011

EXECUTIVE SUMMARY

1. Financial Information (Funding of the CoE)

Total NRF funding for 2010 (entire year) – CoE only	:	R 7 339 882
CoE-specific Funding from Host institution in 2010 – WITS	:	R 220 000
– SU	:	R 678 134
Funding from other sources for the CoE in 2010	:	R 40 522 296
Total funding	:	**R 48 760 312**

Funding for 2010 for Wits node: (Total: R 9 214 465)

- CoE funding from NRF: **R1 918 123**
- Funding from Wits and NHLS (best estimate): **R2 742 722**, made up as follows:
  - Wits R 902 722 (1 Jan 2010 – 31 Dec 2010)[1]
  - NHLS R 1 840 000 (1 Jan 2010 – 31 Dec 2010)[2]
- Funding from other sources (best estimate): **R4 553 620**, made up as follows:
  - MRC Unit (MMRU) R 964 434 (1 Apr 2010 – 31 Mar 2011)
  - MRC Capital Equipment grant R 530 000 (1 April 2010 – 31 March 2011)
  - MRC Career Dev. Award (B. Kana) R 230 000 (1 Jan 2010 – 31 Dec 2010)
  - MRC Staff salary R 309 492 (1 Jan 2010 – 31 Dec 2010)
  - HHMI (V. Mizrahi) R 702 026 (1 Sep 2009 – 31 Aug 2010)
  - BMGF (sub-contract from SBRI) R 935 557 (Sept 2009-Aug 2010)[3]
  - Swiss/SA Joint Research Prog R 882 111

Funding for 2010 for SU node: (Total: R39 545 847)

- CoE funding from NRF : **R5 421 759**
- Other Funding from SU: (best estimate): **R2 750 000,** incl. some salaries, student bursaries, excl.space, basic infrastructure, secretary, cleaners. SU contributed **R2 468 696** to acquire an Orbitrap instrument.
- Funding from other sources (best estimate): **R28 905 392**, made up as follows:
  - MRC Centre R6 100 000 (1 Jan 2010 - 31 Dec 2010, incl. salaries)
  - PAWC R1 700 000 (salaries only)
  - IAEA R2 520 000
  - EDCTP R2 700 000
  - BMGF R5 300 000
  - Welcome Trust R1 200 000
  - Harry Crossley Foundation R 100 000
  - Optimus Foundation R 700 000
  - WOTRO R 350 000
  - NRF-RCN (Norway) R 440 000
  - SATBAT R 55 000
  - EDR R1 700 000
  - LifeLab R 278 000
  - Other NRF funding R 965 000
  - NRF Orbitrap funding R4 797 392

[1] Comprising R 220 000 commitment from the Central Research Office of Wits in fulfillment of the 10% institutional commitment to the CBTBR; R 427 722 from the Faculty Research Committee towards staff salary costs; R 145 000 for a Mellon Postgraduate Mentoring Award (to D. Ndwandwe, V. Mizrahi & D. Warner) and R 110 000 (Friedel Sellschop Award to B. Kana)
[2] Staff salaries
[3] Calc based on an average exchange rate of R7.00/$. SBRI, Seattle Biomedical Research Institute; BMGF, Bill & Melinda Gates FDN

2. Summary of progress against 5 KPAs

(i) Research

The research productivity of the CBTBR remained particularly high in 2010 as evidenced by the fact that 2 book chapters, 46 articles in peer-reviewed journals, 3 non-peer-reviewed articles were published, and 89 conference presentations were made, including 6 plenary/ keynote lectures, and numerous invited talks. Of the research articles published, 42 were in journals with an impact factor (IF) >2.
Progress against targets SLA 4 targets: The outputs under this KPA greatly exceeded the SLA target (≥10 publications of which ≥5 are in journals with an IF ≥2).

(ii) Education and Training

A total of 4 postdoctoral fellows, 2 PhD students, 6 MSc students and 7 Honours students from the CBTBR graduated or completed their training in 2010. All postgraduate students completed their maximum allowable time agreed upon in the SLA. A number of new postdoctoral, PhD and MSc students were enrolled in both nodes of the CBTBR. Other students were also afforded the opportunity to work in international labs (details provided below).
Progress against SLA 4 targets: The total of 67 postgraduate students associated with the CBTBR in 2010 is more than double the SLA target of ≥25. The student breakdown according gender (64% female) and percentage of postdoctoral fellows (19% of total student complement) equalled or exceeded the SLA targets of ≥50% and ≥10%, respectively. The percentage of black students (51%) exceeded the SLA target of ≥50%. The percentage of Honours students was 12% in 2010. No honours students were rejected unless they did not meet the entrance requirements set by the university.

(iii) Knowledge Brokerage

Members of the CBTBR remained heavily involved in disseminating scientific information at the operational, scientific community and stakeholder levels. Apart from enjoying country-wide and sometimes international publicity in various media platforms, the CBTBR was involved in outreach activities in almost every month of the year with a strong focus on targeting school teachers and learners, and on science communication in general. The co-directors and other senior members of the CBTBR were heavily involved in organising and participating in two international workshops on TB hed in Pretoria in March 2010 – the NIAID *US-Southern Africa Joint Research Forum on Tuberculosis*, and the Joint Institute of Medicine (IOM) and Academy of Science of South Africa (ASSAf) workshop on the *Emerging Threat of Drug-Resistant Tuberculosis in Southern Africa: Global and Local Challenges and Solutions*These meetings brought together world-leading experts in the field and the latter provided the opportunity for CBTBR researchers to contribute to formulating policy for the management of drug-resistant TB (http://www.assaf.org.za/wp-content/uploads/2011/03/final-TB-in-South-AfricaUNEMBARGOED.PDF). A number of meetings/workshops were held with provincial health authorities, MSF and NHLS, particularly regarding drug resistantTB. Presentations on drug resistance testing to NHLS were made in Durban, Bloemfontein and Cape Town. Meetings with SANParks were held, to advise them with regard to TB in wildlife.

(iv) Networking

Considerable effort was devoted to maintaining existing local and international collaborative networks and to developing new linkages. The CBTBR received many contacts and requests for collaboration confirming that the Centre and its Team Members are highly sought-after research partners. Researchers in the CBTBR were invited to join the MM4TB and HIT-TB drug discovery consortia led by Prof. Stewart Cole (EPFL, Switzerland; MM4TB) and Dr. Clifton E. Barry III (NIAID; HIT-TB) which were approved for funding under the EU FP7 and Bill & Melinda Gates Foundation TB Drug Accelerator programs, respectively. These projects will commence in the first quarter of 2011 and the South African components will be led by Prof. Mizrahi and Dr. Warner at UCT. The SU node continues to be a major contributor to a BMGF Grand Challenge grant (Prof. Walzl via Prof. Kaufmann in Berlin). Furthermore, Prof Walzl has obtained new BGMF grants:1) Biomarker Discovery with a consortium including Drs. Clif Barry (NIH), David Alland and others, 2) Hazel Dockrell (LSHTM) on surrogate

markers. In addition he is PI on a new EDCTP grant, African/European consortium with 7 African and 5 European sites, a further EDCTP consortium (TESA) for clinical trials cap dev and a new DFG (Germany) grant to study helminth effects in TB. The SU node (Prof. van Helden and Prof. Warren) is part of an EU consortium "TB Adapt" which was awarded an EU grant from 2007. The SU node has also been granted funds from the Optimus Foundation for development and testing of a new quantitative drug testing system. The SU node is interacting intensively and extensively with the pharmaceutical industry, GATB and FIND, to evaluate new candidate antibiotics and diagnostics in 2010. The SU node is also involved in the final stages of the ZAMSTAR project, which will involve a very large (n=40000) prevalence survey in 8 local communities. A number of persons from SU node have been asked to assist with advising WHO (Prof. van Helden, Prof. Walzl and Prof. Diacon) and other similar bodies. Prof. Mizrahi served on Scientific Advisory Committees of the TB Alliance (New York) and to the Scientific Advisory Committee of the Kwa-Zulu Natal Research Institute for TB and HIV (K-RITH) and worked closely with the management of the TB Centre of Competence of the DST on developing a strategy for supporting TB drug discovery research in SA.

(v) Service rendering

Activities in this area included the provision of technical/ scientific services to the Western Cape Provincial Health Department, the gold mines, Tygerberg Hospital and various TB clinics, the provision of advice and assistance to individuals, research groups and institutions, locally (including NHLS) and abroad, committee membership and scientific review work at the institutional, regional, national and international levels. For example, we have tested (and continue to do so for local scientists) candidate drug compounds for UKZN, UWC and UCT. Members of the CBTBR again played key advisory and participatory roles in the national and regional responses to the extensively drug-resistant (XDR) TB crisis. Assistance to SANParks regarding TB in wild animals has been given, and to the AACL for companion animals. The SU node also assisted the MRC (Delft) and SAAVI with infection problems in their animals. In addition, we have expanded our activities and work closely with MSF and the City of Cape Town in Khayelitsha, and the Eastern Cape DoH, regarding drug resistant TB in particular. We continue to provide a close service to clinics in George and a new site in Fisantekraal, and have expnded our activities to service the whole Western Cape with specialised services.. In particular, we are pleased to report that we have continued to cultivate improved contact with health authorities in many regions, such as the Western Cape and Eastern Cape DoH.

3. Gender Impact

From the "Science by Women" perspective, it is important to note that 64% of post graduate students in the CBTBR in 2010 were female. The very high representation by women at the lower levels of this research enterprise is consistent with the broader demographic picture for the Health Sciences in SA.

4. Other key developments

Establishment of a third node of the CBTBR at UCT

In May 2010, Prof. Mizrahi, co-director of the CBTBR, accepted the position as Director of the Institute of Infectious Disease and Molecular Medicine (IIDMM) at the University of Cape Town. She relocated to UCT at the end of December 2010 together with wits/NHLS Team member, Dr. Digby Warner. Following an approach that was made in April by the co-directors of the CBTBR, with the strong support of the co-chairs of the Board of the CBTBR, Profs. Belinda Bozzoli and Arnold van Zyl, the NRF approved the allocation of R 1.2m of additional funding to support the establishment of a third node of the CBTBR at UCT, to be led by Prof. Mizrahi. Over the ensuing months, a succession plan for leadership of the Wits node was developed leading to the appointment of Wits node Team Member, Dr. Bavesh Kana, as head of the Wits node of the CBTBR, effective 1 January 2011. A Memorandum of Understanding outlining the changes in structure and management of the CBTBR resulting from the creation of a new node at UCT was produced by the CBTBR and signed by the DVCs (Research) of SU, Wits and UCT in March 2011. Under the new dispensation, Prof. Van Helden will serve as the sole director of the CBTBR, with Prof. Mizrahi and Dr. Kana serving as heads of the UCT and Wits nodes, respectively. As in the past, the CoE grant from the NRF will be awarded to SU, which will serve as the principal host institution responsible for distribution of the grants to the UCT and Wits nodes.

The support of the NRF, Wits, SU, UCT and other stakeholders affected by this development, most notably, the MRC and NHLS, and the collegial manner in which the negotiations proceeded, is most gratefully acknowledged. The expansion of the CBTBR to UCT is likely to be strategically important in the long term. It has the potential to significantly increase the reach and scope of the CBTBR by involving other groups from UCT which has a very strong TB research focus within the IIDMM. Moreover, the SU-Wits-UCT alliance is expected have the strength and capacity to interact as an equal partner in biomedical TB research initiatives with K-RITH.

Training

Further details are in the report provided and nothing specific is highlighted here.

Staffing and Staff Development

(i) In 2010, as before, three Team Members, Drs. Kana (Wits), Gey van Pittius, and Streicher (SU) were employed on contract, with the employment contracts of Drs. Streicher and Gey van Pittius being linked to the existence of the CBTBR and Dr. Kana to the MRC recognition cycle of the MMRU, which was renewed in 2009 for a further five-year term (to March 2015). Unless permanent positions can be found for these individuals, there is a chance that they may take up permanent positions elsewhere, outside of the field of TB research, as occurred in other cases (Drs. C. Pheiffer, N. Brown and B. Bapela). Furthermore, Dr. Williams has maintained close ties with the Wits node and continues to provide valuable intellectual input into the supervision of an MSc student who is also co-supervised by staff at the newly established UCT note. Hence the secondment of Dr. Williams to the SU node has provided the idea backdrop to cultivate a collaboration between all three nodes of the CBTBR and to foster closer working relations between members at the distinct nodes.

5. General Comments

The CBTBR has continued to make excellent progress over the past year. The Board met at SU in March 2010 and held a virtual meeting, run by e-mail exchange, in September/October 2010, which culminated in the approval of an amended budget with an overall 2% increase for the CBTBR for 2011.

PROGRESS REPORT

1. Scientific Research

THEME I: TARGET VALIDATION AND CHARACTERIZATION THROUGH BASIC RESEARCH IN MYCOBACTERIAL METABOLISM

Mechanisms of DNA metabolism in mycobacteria. Work in this area focused on: (i) elucidating the role of specialized DNA polymerases in the genetic adaptation of mycobacteria under conditions of stress; (ii) development of methods for measuring dNTP pools in mycobacteria; and (iii) the role of oxidative damage base excision repair (BER) systems in growth, survival and mutagenesis in mycobacteria. A research highlight for 2010 was the publication in *Proceedings of the National Academy of Sciences of the USA* of a paper describing the identification and functional characterization of two additional and essential components, ImuA' and ImuB, which together with DnaE2, constitute a split *imuA'-imuB*/*dnaE2* mutagenesis cassette and identification of the DNA polymerase responsible for error-prone translesion synthesis (TLS). This paper forms part of an ongoing collaboration with Prof. Česlovas Venclovas (Institute of Biotechnology, Lithuania). A related study on the role of the *dinB1* and *dinB2* genes which encode putative Y-family DNA polymerases, was also published this year in the *Journal of Bacteriology*. These two papers represent the culmination of many years of work in an area of TB research for which the Wits node is particularly renowned. Adding to the MMRU's publication outputs in this area was an authoritative review article by Dr. Digby Warner on the role of DNA repair in M. tuberculosis pathogenesis that was published in *Drug Discovery Today: Disease Mechanisms* (see below) and an invited commentary article by Dr. Warner and Prof. Mizrahi published in *Molecular Microbiology*.

The role of resuscitation-promoting factors (Rpfs) in growth and resuscitation of *M. tuberculosis*. A paper describing the effect of Rpf deficiency on the susceptibility of *M. tuberculosis* to a large panel of antitubercular drugs was published in the *Journal of Antimicrobial Chemotherapy*. Dr. Kana and Prof. Mizrahi also published two review articles on the biology of Rpfs in mycobacteria, one in FEMS Immunology and Medical Microbiology and the other in *Drug Discovery Today: Disease Mechanisms*. The latter appeared in the same issue of the journal as Dr. Warner's article. Interestingly, this special issue of *Drug Discovery Today: Disease Mechanisms* was edited by Dr. Helena I. Boshoff, a former graduate of the MMRU (PhD, Wits University, 2000). Ongoing work that forms part of Ms. Lusanda Mapela's Masters' project is aimed at developing tools in *M. smegmatis* for further analyzing the function of Rpfs in mycobacterial cell wall metabolism.

The biosynthesis, transport and function of vitamin B_{12} in mycobacteria and the role of B_{12}-dependent enzymes in mycobacterial growth and persistence. Excellent progress has been made on this project which is supported by a three-year research grant from the Swiss/South Africa Joint Research Programme (SSAJRP) awarded jointly to Prof. Mizrahi and Prof. John McKinney (École Polytechnique Fédérale de Lausanne, Switzerland), which funds research activities in both the Swiss and South African laboratories. Doctoral student Atica Moosa made excellent progress on validating two key findings made previously. Her finding that associates the function of a member of the PPE family of proteins with the assimilation of Co2+ into the vitamin B_{12} biosynthetic pathway in *M. tuberculosis* is particularly novel and exciting. She has been granted a Keystone Symposia Global Health Travel Award to present this work at the Keystone Symposium on Mycobacteria, to be held in Vancouver in January 2011. She also won the prize for Best Poster in the Infectious Disease category of the Faculty of Health Sciences Research Day for her presentation on this work. She will complete her PhD in early 2011 and will be joining the MMRU as a postdoctoral fellow at UCT. Postdoctoral fellow, Dr. Krishnamoorthy Gopinath, has made impressive progress on using forward genetic screens to identify genes involved in the transport of vitamin B_{12} in *M. tuberculosis*, and in constructing *prpDC* and/or *mutAB* mutants of a PDIM-producing strain of *M. tuberculosis* H37Rv which will be used to assess the combined roles of methycitrate cycle and the B_{12}-dependent *mutAB*-encoded methylmalonyl pathway for propionate metabolism in growth and persistence of *M. tuberculosis* vivo. Approval has been granted by the DST, CSIR, Wits and UCT to transfer the SSAJRP grant to UCT to enable this project to be completed.

Elucidation of the physiological role of toxin-antitoxin modules of the VapBC family in growth, persistence and drug susceptibility of *M. tuberculosis*. This project has been completed. Ms. Ahmadou Ahidjo is in the process of completing her PhD thesis which will be submitted by December 2010 and a manuscript describing the key findings from this study is in preparation. This study yielded a number of new insights into the function of VapBC-type toxin-antitoxin modules from *M. tuberculosis*. First, the growth inhibitory effects conferred by VapCs was shown to be related to their function as nucleases. Second, the interaction of VapCs with their cognate VapBs was found to be highly specific inasmuch as non-cognate VapB expression was not able to neutralize VapC toxicity. This finding was further corroborated by the observed exacerbation of VapC toxicity in *M. tuberculosis* resulting from by loss of cognate VapB function. Third, and most importantly, the differential toxicity conferred on *M. smegmatis* by ectopic expression of *M. tuberculosis* VapCs was shown to be due, at least in part, to a high level of variability in the level of VapC protein produced using a common, conditional expression system. These findings, which describe the first attempt to monitor VapC protein levels by means of epitope-tagging, have a significant bearing on work from other groups which have used such systems to 'rank' VapCs in terms of their growth inhibitory effects on heterologous hosts.

The biosynthesis and function of molybdopterin cofactor (MoCo) in mycobacteria. A paper describing the results of Dr. Monique Williams' postdoctoral research on this topic has been accepted for publication in the *Journal of Bacteriology*. Dr. Williams has been awarded a Keystone Symposia Global Health Travel Award to attend the Keystone Symposium on Mycobacteria in Vancouver in January 2011, where she will present this work. This project is being continued by Ms. Nicole Narrandes, who is following up on promising leads generated by Dr. Williams. She has developed tools to assess the extent of molybdopterin synthase subunit interplay in *M. tuberculosis*, and to address the question of whether MoaX, the novel, single-polypeptide MPT synthase identified by Dr. Williams, is post-translationally modified in *M. smegmatis*. In an interesting new development, she has shown that a deletion mutant of *M. smegmatis* in *narB*, which encodes a soluble, MoCo-dependent nitrate reductase, retains the ability to assimilate nitrate, implicating another NR – presumably, the respiratory NarGHI molybdoenzyme, in this activity. This work is forming the basis of Ms. Narrandes' Masters project.

Tuberculosis drug discovery research. Outstanding progress was made by Dr. Garth Abrahams on the development of target-based whole-cell screening as a tool for accelerating TB drug discovery. This work has been carried out under the auspices of the Integrated Methods for Tuberculosis Drug Discovery (IMTB) project which is led by Dr. David Sherman and funded by a grant from the Bill & Melinda Gates Foundation to the Seattle Biomedical Research Institute (SBRI) and involves collaborating groups in the USA and UK. Work over the past year focused on developing and optimizing methods and assays for using conditional knockdown mutants in prioritized targets in high-throughput, whole-cell screens. Dr. Abrahams spent three months working with Dr. Helena Boshoff in the laboratory of Dr. Clifton E. Barry at the NIAID (NIH), completing this study. The IMTB project will come to an end in February 2011. A manuscript describing Dr. Abrahams results is in preparation. His work has formed the basis of two new international grants that will support further TB drug discovery work in the MMRU after relocation of the unit to UCT. A new postdoctoral fellow, Dr. Joanna Evans, who joined the MMRU in July 2010, will be responsible for this work. A second project in the drug discovery area was completed in 2010. This work formed the basis of the Masters project of Ms. Krupa Naran, and involved a preliminary characterization the anti-microbial action of a *Pseudomonas*-derived activity. She found that the activity, which is restricted to mycobacteria and related actinomycetes, shows potent growth-inhibitory effects against *M. smegmatis* and *M. tuberculosis*, raking alongside standard anti-tuberculars such as the aminoglycoside, kanamycin. Ms. Naran's dissertation has been examined and she will receive her MSc degree in December 2010. As part of a proof-of-capability exercise, the MMRU was requested to determined the MICs against *M. tuberculosis* H37Rv of compounds identified as hits from a quantitative high-throughput whole-cell screen (qHTS) of a 35,000-compound library that had been was performed at the NIH by Drs. Barry and Boshoff under the auspices of the SA TB Research and Innovation Initiative (SATRII).[4] The MICs showed a high level of concordance with the inhibitory activities identified by qHTS thus validating the methodology and confirming the ability of the Wits/NHLS laboratory (Wits node of the CBTBR) to provide the necessary microbiological support required to drive a hit-to-lead program for TB drug discovery that is likely to take place in SATRII

The effects of *rpoB* mutations associated with rifampicin resistance on mycobacterial physiology. Ms. Anastasia Koch has made very impressive progress on this project which is aimed at investigating the effects of *rpoB* mutations associated with rifampicin resistance on mycobacterial physiology. She has established and applied methods for transferring point mutations into the *M. smegmatis* genome allowing naïve, *rpoB* mutants to be obtained. Assessment of one such mutant revealed a high level of rifampicin resistance which was fully reversed to wild type levels of susceptibility by restoration of the wild type sequence. This is the first demonstration that the S531L mutation is both necessary and sufficient to confer high-level rifampicin resistance in a mycobacterial species and raises questions about the role – if any – of compensatory mutations in resistance and/or fitness. Ongoing work is aimed at exploring the effects of *rpoB* mutations on fitness when *M. smegmatis* is grown on different carbon sources. Ms. Koch presented her work at two international conferences and was awarded prizes for both presentations. The first prize was for the best oral presentation in the Basic Sciences Track of the 2nd SA TB Conference (Durban, July 2010) and the second was for the best poster presentation at the FEBS/EMBO Lecture Course on Host-Microbe Interactions, held in Spetses, Greece (September 2010). Ms. Koch's experimental work is almost complete and she will submit her MSc dissertation by February 2011.

Joint projects. Dr. Monique Williams, who was appointed by the MRC on a three-year contract as a Senior Scientist in the MMRU (Wits node of the CBTBR), was seconded on a full-time basis to the SU node of the CBTBRin 2010. The purpose of this secondment was to strengthen ties between the two nodes. In addition to completing a study on the molecular mechanisms of molybdopterin biosynthesis in *M. tuberculosis*, which was initiated during her postdoctoral fellowship in the Wits node (2008-2009) and resulted in a publication in *J. Bacteriol*., Dr. Williams became heavily involved in various projects in the SU node through the expertise in mycobacterial genetics and physiology which she has been willing and able to contribute. She has participated actively in discussions on projects studying drug resistance and efflux, and played a key role in designing of experiments for transcriptome analysis as part of the MSc project of Melanie Grobbelaar. She also assisted an MSc student, James Mazorodze, with designing a strategy for generating a deletion mutant in *M. smegmatis,* and is currently training a PhD student, Magareta De Vos, in the procedures involved in making allelic

[4] SATRII presents a major opportunity to support new and exciting drug discovery efforts both in the Wits node of the CBTBR (Johannesburg) as well as the MMRU (Cape Town) from 2011 onwards. At the time of writing of this report, the DST was in the final stages of negotiation with the Technology Innovation Agency (TIA) regarding the structure, research program and funding of SATRII.

exchange mutants in *M. tuberculosis.* She has assisted Philippa Black with the design and construction of a reporter plasmid for use in her MSc project, and Mae Newton-Foot (PhD student) with RNA isolation from *M. smegmatis*. The first year of Dr. Williams' secondment has therefore turned out to be very successful, boding well for the future.

THEME II: BRIDGING THE GAP BETWEEN BASIC AND CLINICAL RESEARCH

Molecular Epidemiology and Evolution, Mycobacterial Genetics and Diagnostics

Many studies were completed during 2009-2010, which can be seen from the publications listed. These are not discussed in detail, but dealt with as concepts, as outlined below. The molecular epidemiological studies have formed the foundation for the formulation of scientific questions which have enabled scientists at the Centre to challenge existing dogmas and, most importantly, to develop new hypotheses, sometimes annually.

1) Molecular Epidemiology Databases: As part of an ongoing molecular epidemiological investigation into the disease dynamics of TB in a high incidence urban setting, a molecular epidemiological database has been constructed to record clinical, epidemiological and molecular data from patients diagnosed with TB. This molecular epidemiology database now comprises strain data of more than 8400 clinical *M. tuberculosis* isolates from approximately 4000 patients spanning the period 1993 to 2008. These isolates have been classified into 1118 strains representing 35 major clades. This represents the largest molecular epidemiological database in the Southern Hemisphere and one of the largest in the world. This is probably the only longitudinal data set which is capturing epidemiological phenomena at the peak of the epidemic and which is measuring the gradual influence of co-infection with HIV on the epidemic.

A second molecular epidemiological study focusing on a high HIV prevalence community has been conducted in collaboration with the University of Cape Town and the University of McGill, Montreal, Canada. This data set includes all patients diagnosed with tuberculosis over a period of two years in Gugulethu and includes over 1700 clinical isolates. This data set represents one of the most intensive studies conducted in an urban community where HIV co-infection is extremely high. A consortium of scientists has been formed to analyze this data set with the view to understanding the factors perpetuating the TB epidemic in this setting. The formulation of the above databases provides a unique opportunity to compare the disease dynamics of TB in two vastly different contexts. For the first time it will be possible to compare the behaviour of the same strains in different settings which may provide novel insights into pathogenesis.

In 2005, a third molecular epidemiological study was initiated to investigate how molecular biology could enhance the diagnosis of tuberculosis in patients attending a primary healthcare clinic. This study has three additional aims: a) Retrospective analysis of molecular epidemiological data to provide an understanding of the disease dynamics in this setting. b) To measure the level of nosocomial transmission occurring within the primary healthcare clinic. c) To determine the period of diagnostic delay. This is an ongoing study, in partnership with the local Department of Health, which focuses on patients attending a single primary healthcare clinic in the town of George.

In 2006, a fourth molecular epidemiological study was initiated to investigate the XDR-TB epidemic in the Western Cape. This study will aim to determine the mechanisms underlying this epidemic to establish whether XDR-TB develops through acquisition or transmission. Included in this study is a study of all cases of pre-XDR-TB (strains with only a single maker for XDR-TB) with the view to determine whether such strains evolve over time to become the future XDR-TB strains.

In 2008, a fifth molecular epidemiological study was initiated to collect and genotypically characterize all drug resistant isolates cultured from patients resident in the Western Cape Province. Currently these drug resistant isolates are being preserved by storage on glass balls at -80°C and only subsets of the isolates are being characterized due to limited funds.

In November 2008 a sixth molecular epidemiological study was initiated to collect and genotype drug resistant isolates identified by the National Health Laboratory Service in the Eastern Cape region. Approximately 2000 MDR isolates have been collected and genotyped by spoligotyping. In turn, target genes have been sequenced with the view to describe the mechanisms underlying drug resistance in this province. In consultation with the DoH of the region it has been emphasized that these isolates should be further characterized to determine the extent of transmission and the role of streptomycin on the emergence of pre-XDR and XDR-TB.

Additional molecular epidemiological studies are currently being done in the Centre. These include:

1. Assisting MSF with understanding TB dynamics in Khayelitsha, Cape Town.
2. Studies focusing on the disease dynamics of drug resistant tuberculosis in:
   a) Boland, Overberg, Karoo and Southern Cape region.
   b) The gold mines in Orkney.
   c) The correctional services in Gauteng

We are currently exploring ways to attempt to match our genotype data with those of others worldwide. Our spoligotyping data is compatible and we share this with the international spoligo database maintained in Guadeloupe. Currently our IS*6110* typing results are not compatible with many others, as we use a more accurate typing standard. However, in conjunction with RIVM in the Netherlands, where the largest database currently exists, we are exploring ways to share even this data internationally.

We have also initiated a DNA sample bank (currently containing around 400 samples) and a linked database focusing on non-tuberculous mycobacterial (NTM) species of the genus *Mycobacterium*. Non-tuberculous mycobacteria are increasingly recognized as playing a role in disease pathogenesis and are becoming a threat due to the spread of the HIV epidemic. In this database we are trying to obtain an overview of species distribution, spread and variation and are developing novel techniques of identification (see below). Samples from this samples bank come from a variety of sources including animal and human.

2) Whole Genome sequencing of XDR-TB: The Beijing genotype of *M. tuberculosis* is a virulent strain that is disseminating worldwide and has a strong association with drug resistance. In the Western Cape of South Africa, epidemiological studies have identified the R220 cluster of the Beijing genotype as a major contributor to a recent outbreak of drug-resistant tuberculosis. Although the outbreak is considered to be due to clonal transmission, the relationship among drug resistant isolates has not yet been established. To better understand the evolution of drug resistance among these strains, 14 drug-resistant clinical isolates of the Beijing genotype were sequenced by whole-genome sequencing, including eight from R220 and six from a more ancestral Beijing cluster, R86, for comparison. While each cluster shares a distinct resistance mutation for isoniazid, mapping of other drug-resistance mutations onto a phylogenetic tree constructed from single nucleotide polymorphisms shows that resistance mutations to many drugs have arisen multiple times independently within each cluster of isolates. Thus, drug resistance among these isolates appears to be acquired, not clonally derived. This observation suggests that, although the Beijing genotype as a whole might have selective advantages enabling its rapid dissemination, the XDR isolates are relatively less fit and do not propagate well. Although it has been hypothesized that the increased frequency of drug resistance in some Beijing lineages might be caused by a mutator phenotype, no significant shift in synonymous substitution patterns is observed in the genomes. While MDR-TB is spreading by transmission in the Western Cape, our data suggests that further drug resistance (i.e. XDR-TB) at this stage is acquired.

3) Proteogenomics: Precise annotation of genes or open reading frames (ORF) is still a difficult task which results in divergence even for data generated from the same genomic sequence. This has an impact on further proteomic studies, and also compromises the characterization of clinical isolates with many specific genetic variations that may not be represented in the selected database. We recently developed a software called MSMSpdbb (Multi-Strain Mass Spectrometry Prokaryotic DataBase Builder) that can merge protein databases from several sources and be applied on any prokaryotic organism, in a proteomic-friendly approach. We generated a database for the *M. tuberculosis* complex (using 3 strains of Mycobacterium bovis and 5 of M. tuberculosis), and analyzed data collected from two laboratory strains and two clinical isolates of M. tuberculosis. We identified 2561 proteins, of which 24 were present in M. tuberculosis H37Rv samples, but not annotated in the M. tuberculosis H37Rv genome. We were also able to identify 280 non-synonymous single amino acid polymorphisms (SAP) and confirm 367 translational start sites. As a proof of concept we applied the database to whole-genome DNA sequencing data of one of the clinical isolates which allowed the validation of 116 predicted SAPs and the annotation of 131 N-terminal start sites. Moreover we identified regions not present in the original M. tuberculosis H37Rv sequence, indicating strain divergence or errors in the reference sequence. In conclusion, we demonstrated the potential of using a merged database to better characterize laboratory or clinical bacterial strains.

4) Mixed *M. tuberculosis* infections: The occurrence of mixed infections of *M. tuberculosis* is no longer disputed. However, their frequency, and the impact they may have on our understanding of tuberculosis (TB) pathogenesis and epidemiology, remains undetermined. Most previous studies of frequency applied genotyping techniques to cultured *M. tuberculosis* isolates found mixed infections to be rare. PCR-based

techniques may be more sensitive for detecting multiple M. tuberculosis strains and can be applied to sputum. To date, one study in South Africa has used a PCR approach and suggested that mixed infection could be common. We investigated mixed infections in northern Malawi using two lineage-specific PCR assays targeting the Latin American-Mediterranean (LAM) and Beijing lineages. Compared with spoligotyping, the specificity and sensitivity of both assays was 100%. From 160 culture-positive sputa, mixed LAM and non-LAM strains were detected in 4 sputa belonging to 2 (2.8%) patients. Both patients were HIV positive, with no history of TB. Cultured isolates from both patients showed only LAM by PCR and spoligotyping. In a set of 377 cultured isolates, 4 were mixed LAM and non-LAM. Only one showed evidence of more than one M. tuberculosis strain using IS6110-based restriction fragment length polymorphism (IS6110-RFLP) and spoligotyping analyses. Corresponding sputa for the 4 isolates were unavailable. Mixed Beijing and non-Beijing strains were not detected in this study. Mixed infections appear to be rare in that setting (unlike the Western Cape) and are unlikely to affect findings based on DNA fingerprinting data. Molecular methods, which avoid the selective nature of culture and target distinct strains, are well suited to detection of mixed infections.

5) Genotype vs. Phenotype: To investigate the association between mycobacterial genotype and disease phenotype in children we describe hospitalised children diagnosed with culture-confirmed tuberculosis (TB) in South Africa, a high TB burden setting. Disease phenotype was classified as intrathoracic or extrathoracic based on mycobacterial culture site. Mycobacterial genotyping was completed using spoligotyping. Our analysis included 421 isolates from 392 children (median age 2 years, range 0.1-12). Intrathoracic disease was present in 294 (75%) children and extrathoracic disease in 98 (25%). The Beijing genotype was the most prevalent (32.9%), followed by the Latin American Mediterranean (LAM, 28.8%), and S genotypes (6.4%). Age was significantly associated with genotype. Children with the Beijing (OR = 2.36, 95%CI 1.21- 4.60) and S genotypes (OR = 3.47, 95%CI 1.26-9.56) were more likely to have extrathoracic disease compared to children infected with the LAM genotype, in analyses adjusted for age and drug resistance. TB genotype and disease phenotype in children were associated. Beijing and S genotypes were more frequently cultured from extrathoracic cultures, indicating potential improved ability to disseminate. Strain-related phenotypes could explain different disease spectra in geographic settings where certain strains are successful. Studies of mycobacterial human interaction should consider host immune responses, clinical and epidemiological factors.

6) Virulence of *M. tuberculosis*: Phylogenetic analysis has shown that Beijing genotype strains can be grouped into at least 7 different sublineages. We aimed to test the hypothesis that the virulence of Beijing genotype strains differed among members of the different sublineages and that the level of virulence correlated with their ability to spread and cause disease. BALB/c mice were infected with Beijing strains representative of the different lineages and of different epidemiological characteristics (transmitted vs. non-transmitted). Survival times, lung pathology, bacterial load and immunology kinetics were evaluated at defined intervals post-infection. Transmissibility was determined by co-housing infected and uninfected mice in close contact for 1-2 months. The results show that mice infected with the highly transmitted Beijing strains began showing mortality 3 weeks post-infection and all had died by 5 weeks, suggesting high virulence phenotypes. In contrast, >80% of mice infected with the non-transmitted strains survived 4 months post-infection, suggesting low virulence phenotypes. Our co-housing transmission model confirmed these virulence phenotypes. Extensive tissue damage and the induction of lower levels of IFNγ and iNOS expression, as well as high but ephemeral TNFα expression were associated with the high virulence phenotype. In contrast, minimal tissue damage and progressive expression of IFNγ and TNFα were associated with the low virulence phenotype. Both virulence phenotypes induced similar levels of IL-4 expression during the early stages of infection after which the high virulence strain induced significantly higher levels of IL-4 expression. In conclusion, this study demonstrates that Beijing genotype strains display a spectrum of virulence phenotypes in mice which mimic their epidemiological characteristics. Both transmissible and non-transmissible strains may exist in the same sublineage.

7) Estimating the specificity of laboratory-diagnosis of tuberculosis: Cross-contamination is not uncommon in mycobacteriology laboratories of high-income countries, as documented by bacterial genotyping. The extent of this problem in low-income countries is largely unknown, where this method is impractical. This study aimed to estimate the rate of cross-contamination in a high-volume tuberculosis (TB) laboratory in South Africa. To achieve this aim simulated sputum specimens labelled with false names were sent from a TB clinic, interspersed with patient samples, and processed for culture and microscopy. Results were interpreted in the context of the observed proportion of samples with positive microscopy and culture results. Using microscopy, 6/190 (3.2%) simulated specimens were positive (estimated specificity = 96.8%).

Considering the 881 positive microscopy results in 6093 clinical samples, we extrapolate that 19.3% (95%CI 7.0-42.8) of positive smears were false-positives. On culture, 2/190 (1.1%) of the simulated specimens were positive for Mycobacterium tuberculosis (estimated specificity = 98.9%). Considering the 1862 positive cultures from 6093 clinical samples, we estimate that 2.4% (95%CI 0.3-8.8) of positive cultures were false-positives. We concluded that simulated specimens offer a simple means of estimating the proportion of false-positive results, providing information on all sources of potential error from the clinic, through the laboratory and to reporting of results.

8) Early Outcomes of XDR-TB: Data from Kwazulu Natal, South Africa, suggest that almost all patients with extensively drug-resistant (XDR) tuberculosis are HIV-positive, with a fatal outcome. Since there is little data for the treatment-related outcomes of XDR tuberculosis in settings with a high HIV prevalence, we investigated the associations of these diseases in such settings to formulate recommendations for control programmes. In a retrospective cohort study, we analysed the case records of patients (>16 years old) with XDR tuberculosis (culture-proven at diagnosis) between August, 2002, and February, 2008, at four designated provincial treatment facilities in South Africa. We used Cox proportional hazards regression models to assess risk factors associated with the outcomes-mortality and culture conversion. 195 of 227 patients were analysed. 21 died before initiation of any treatment, and 174 patients (82 with HIV infection) were treated. 62 (36%) of these patients died during follow-up. The number of deaths was not significantly different in patients with or without HIV infection: 34 (41%) of 82 versus 28 (30%) of 92 (p=0.13). Treatment with moxifloxacin (hazard ratio 0.11, 95% CI 0.01-0.82; p=0.03), previous culture-proven multidrug-resistant tuberculosis (5.21, 1.93-14.1; p=0.001), and number of drugs used in a regimen (0.59, 0.45-0.78, p<0.0001) were independent predictors of death. Fewer deaths occurred in patients with HIV infection given highly active antiretroviral therapy than in those who were not (0.38, 0.18-0.80; p=0.01). 33 (19%) of 174 patients showed culture conversion, of which 23 (70%) converted within 6 months of initiation of treatment. We concluded that a substantial proportion of patients with XDR tuberculosis who are not infected with HIV, have poor management outcomes. Nevertheless, survival in patients with HIV infection is better than previously reported. The priorities for the country are still prevention of XDR tuberculosis, and early detection and management of multidrug-resistant and XDR tuberculosis through strengthened programmes and laboratory capacity.

9) *Mycobacterium africanum:* While *M. africanum* is an important cause of TB in several sites in West Africa, its distribution in other African countries is not well documented. In this study, conducted in Cape Town, 1175 isolates yielded 110 unique RFLP patterns; one of each of these 110 strains was tested for genomic deletions characteristic of *M. africanum* and other atypical members of the *M. tuberculosis* complex. As none of these strains was marked by the deletion of RD9, we conclude that *M. africanum* and other atypical members of the *M. tuberculosis* complex are decidedly uncommon in this part of Africa.

10) *Mycobacterium tuberculosis* proteomics*:* Although the genome of the *M. tuberculosis* H37Rv laboratory strain has been available for over 10 years, it is only recently that genomic information from clinical isolates has been used to generate the hypothesis of virulence differences between different strains. In addition, the relationship between strains displaying differing virulence in an epidemiological setting and their behavior in animal models has received little attention. The potential causes for variation in virulence between strains, as determined by differential protein expression, have similarly been a neglected area of investigation. In this study, we used a label-free quantitative proteomics approach to estimate differences in protein abundance between two closely related Beijing genotypes that have been shown to be hyper- and hypovirulent on the basis of both epidemiological and mouse model studies. We were able to identify a total of 1668 proteins from both samples, and protein abundance calculations revealed that 48 proteins were over-represented in the hypovirulent isolate, whereas 53 were over-represented in the hypervirulent. Functional classification of these results shows that molecules of cell wall organization and DNA transcription regulatory proteins may have a critical influence in defining the level of virulence. The reduction in the presence of ESAT-6, other Esx-like proteins, and FbpD (MPT51) in the hypervirulent strain indicates that changes in the repertoire of highly immunogenic proteins can be a defensive process undertaken by the virulent cell. In addition, most of the previously well characterized gene targets related to virulence were found to be similarly expressed in our model. Our data support the use of proteomics as a complementary tool for genomic comparisons to understand the biology of M. tuberculosis virulence.

11) XDR-TB and treatment adherence: We investigated the emergence and evolution of drug-resistant tuberculosis (TB) in an HIV co-infected population at a South African gold mine with a well-functioning TB

control program. Of 128 patients with drug-resistant TB diagnosed during January 2003-November 2005, a total of 77 had multidrug-resistant (MDR) TB, 26 had pre-extensively drug-resistant TB (XDR TB), and 5 had XDR TB. Genotyping suggested ongoing transmission of drug-resistant TB, and contact tracing among case-patients in the largest cluster demonstrated multiple possible points of contact. Phylogenetic analysis demonstrated stepwise evolution of drug resistance, despite stringent treatment adherence. These findings suggested that existing TB control measures were inadequate to control the spread of drug-resistant TB in this HIV co-infected population. Diagnosis delay and inappropriate therapy facilitated disease transmission and drug-resistance. These data call for improved infection control measures, implementation of rapid diagnostics, enhanced active screening strategies, and pharmacokinetic studies to determine optimal dosages and treatment regimens.

12) Whole Genome Sequencing: As part of a collaboration with Dr. K. Kremer (RIVM, Netherlands) two South African Beijing isolates have been selected for whole genome sequencing. These include the sublineage 7 hyper strain as well as a representative of the most ancient atypical Beijing lineage (sublineage 1). Sequencing of these strains is complete and has been used to reconstruct the evolutionary history of the Beijing lineage. A further 50 strains representative of the different lineages have been sent to The Netherlands for SNP mapping and phylogenetic positioning.
Two Beijing genotype strains representative of a hyper and hypo-virulent phenotype have been submitted for whole genome sequencing. Other isolates representing XDR-TB strains which emerged during treatment are being sequenced at Broad Institute in the USA in collaboration with Prof M. Murray.
A total of 40 M(X)DR-TB strains have been submitted for sequencing in collaboration with Prof J. Sacchetini in Texas, USA. The sequencing results of these strains have delivered very valuable insight into the microevolution of the organism and the development of drug resistance and we are currently doing a complete analysis of the data for publication and follow-up experiments.

13) Genotypic Drug-Susceptibility Testing: The rising number of MDR-TB cases worldwide prompted the World Health Organisation (WHO) to release an emergency update on the guidelines for the management of drug-resistant TB. These guidelines recommended that treatment of MDR-TB should include at least 4 effective drugs and that standardised treatment regimens should be based on resistance patterns for each country/region. Most importantly, treatment regimens should not be dependent on results of drug susceptibility testing (DST) for ethambutol (EMB) or pyrazinamide (PZA). In response, the South African Department of Health has prepared a draft drug-resistant TB treatment policy in which PZA remains one of the 4 effective drugs, while EMB should be replaced with terizidone or cycloserine, if resistant to EMB (disregarding inaccurate DST). Analyses of recent drug-resistance patterns in South Africa indicate a high frequency of undetected EMB and PZA resistance and their association with MDR-TB. Accordingly, we recommend that the WHO guidelines be followed more closely in which 4 other effective drugs are used to treat MDR-TB. EMB and PZA can be included provided they are not counted as one of the four effective drugs. However these guidelines do not address the root cause of the amplification of resistance in undiagnosed MDR-TB patients in South Africa. This can only be achieved by the implementation of rapid DST methods in all TB cases prior to initiation of therapy. Ultimately this would curb the amplification of resistance and the evolution of XDR -TB.

14) Mycobacterial Pharmacogenetics: To assess a potential association between the evolution of XDR strains of *M. tuberculosis* and mutations in the *inhA* promoter or the *katG* gene the frequency distribution of isoniazid resistance conferring mutations in a population sample of drug-resistant isolates of *M. tuberculosis* was analysed. This showed that in the Western Cape and Eastern Cape Provinces, respectively, the percentage of isolates exhibiting *inhA* promoter mutations increased significantly from 48.4% and 62.4% in MDR isolates to 85.5% and 91.9% in XDR isolates. Data from the Western Cape revealed that significantly more XDR isolates showed mutations in the *inhA* promoter than in *katG* (85.5% vs. 60.9%; $p<0.01$), whilst the respective proportions were equal for INH resistant non-MDR isolates (~30%). We concluded that *InhA* promoter mutations are strongly associated with XDR tuberculosis in South Africa. We suggest that this is due to the dual resistance to ethionamide and (low-dose) isoniazid conferred by *inhA* promoter mutations. The use of molecular probe assays such as the GenoType® MTBDR*plus* assay, which allow for the detection of *inhA* promoter mutations, could enable adjustment of the treatment regimens depending on the pharmacogenetic properties of the mutations detected.

15) Fitness of a Drug-Resistant Strain: Temporal analysis of drug-resistant tuberculosis (TB) cases in the Western Cape, South Africa, showed a 1.5-fold increase over a 2-year period, suggesting a doubling time of

8.2 years. This increase was strongly associated with multidrug resistance and the Beijing genotype. Forty-two per cent of the overall increase was due to the Beijing genotype strain R220, suggesting that this strain had evolved unique properties that allowed for both acquisition and transmission of drug resistance. To curb the drug-resistant TB epidemic in this setting, it will be essential to implement rapid diagnostics and efficient infection control measures, improve contact screening and ensure treatment adherence.

16) Capreomycin Resistance: The availability of antituberculosis drugs to treat extensively drug-resistant tuberculosis is limited. The aminoglycosides amikacin and kanamycin, and the cyclic polypeptide capreomycin are therefore important injectable drugs in the treatment of MDR TB. Capreomycin is recommended as a substitute for amikacin or kanamycin if resistance to either of them is suspected. However, cross-resistance between amikacin/kanamycin and capreomycin in *M. tuberculosis* was observed in clinical isolates and laboratory-generated mutants that contain single nucleotide polymorphisms. In this study, the genetic mechanisms that confer phenotypic levels of resistance to amikacin and capreomycin in 50 clinical isolates of *M. tuberculosis* were investigated. The isolates were cultured from patients resident in the Eastern Cape Province of South Africa and then subjected to DNA sequencing of the *rrs*- (1400-1500 region) and the *tlyA*- (entire region) genes. The phenotypic resistance of each isolate was quantified in MGIT 960 and compared to the sequenced data. All isolates with a nucleotide substitution at position 1401 (A→G) in the *rrs* gene showed high-level resistance (>20 µg/ml) to amikacin. A 100% correlation was also found between the A1401G mutation and phenotypic resistance to capreomycin, but at lower MICs of 10 to 15 µg/ml. No other mutations in either the *rrs* or *tlyA* genes were detected. Complete (100%) cross-resistance between amicacin and capreomycin in *M. tuberculosis* isolates with an A-to-G change at position 1401 of the *rrs* gene was therefore observed in this study. Our findings have important implications to ensure the appropriate treatment of patients with drug resistant TB.

17) Reinfection induces reactivation: This is a collaborative study with Prof R. Hernandez-Pando (Mexico) based on the hypothesis that a high infection pressure (high incidence setting) may lead to reactivation of a latent infection. This has been noted in the study setting and thus the aim of the study was to test this hypothesis in mice. Strains representative of the observed case were sent to Mexico where a chronic infection model was developed. The results of this study will be available in due course.

19) DNA methylation in *M. tuberculosis*: In collaboration with Prof Alan Christoffels of UWC, 2 *M. tuberculosis* isolates of epidemiological interest (closely related hyper- and hypo-virulent strains) underwent whole genome sequencing of their bisulphite modified DNAs. Whole genome sequencing has been completed by CoFactor Genomics and the sequence reads have been sent to UWC for analysis. We envisage that the methylome will be described in 2011.

20) Identification and description of rare and novel *M. tuberculosis* complex species: *M. tuberculosis* complex species are characterized by 99.9% similarity at the nucleotide level. However, several host-adapted ecotypes of these organisms have been identified. The recently-described oryx bacillus is an extremely rare slow-growing member of the antelope clade of the *M. tuberculosis* complex and is closely related to the dassie bacillus, *M. africanum* and *M. microti*. The antelope clade is a group of strains apparently host-adapted to antelopes as most described infections with this organism were associated with deer and antelope, most specifically the Arabian oryx (*Oryx leucoryx*). In this study, the oryx bacillus was isolated from a free-ranging adult female African buffalo (*Syncerus caffer*), in good physical condition, which tested strongly positive on three consecutive comparative intradermal tuberculin tests. Upon necropsy, a single pulmonary granuloma and an active retropharyngeal lymph node was found. Surprisingly, comprehensive molecular assays, including LSP and SNP analyses confirmed that the causative organism was not *M. bovis*, but oryx bacillus. Spoligotyping, MIRU-VNTR typing and molecular drug resistance determination were performed to confirm the identity and investigate the genomic characteristics of this under-studied organism. Oryx bacillus has never been reported in Southern Africa and has never been found to infect an African buffalo. The buffalo herd originally consisted of some animals imported from a zoo in Portugal prior to 1990, which might be the historical source of the infection, given the apparent absence of the organism in this region. This illustrates the need for vigilance in preventing the translocation of mycobacterial pathogens during the movement of species.

In another seminal study, we described the emergence of a novel *M. tuberculosis* complex (MtbC) pathogen, named *Mycobacterium mungi*. This pathogen was isolated from banded mongoose (*Mungos mungo*) populations, which forage and den amongst waste and human structures in Chobe District, Botswana. *M.*

mungi appears to be environmentally transmitted to mongooses living in these human altered environments. Presently, we do not know the source of infection, although as with all other members of the *M. tuberculosis* complex, aerosols are the most likely route of transmission. Using basic isolation, culture and a range of PCR and PCR-sequencing typing techniques, including deletion region (RD) and single nucleotide polymorphism (SNP), spoligotyping and MIRU-VNTR analysis, we have established that this emerging pathogen is closely related to the dassie bacillus and *M. africanum* within the MtbC. The name *Mycobacterium mungi* sp. nov. is proposed for this novel member of the *M. tuberculosis* complex (N.L. gen. n. *mungi*, of *Mungos*, isolated from banded mongoose *Mungos mungo*).The identification of a novel TB agent presents new concerns and potential threats to human and wildlife health, particularly in light of the immense sub-Saharan HIV/AIDS epidemic and close habitation proximity between the animals and humans. The study also illustrates the molecular complexity of strain identification in the MtbC and the need to incorporate evolving molecular identification techniques in TB surveillance as we seek to control and manage this group of highly successful pathogens.

21) Non-tuberculous mycobacteria and other related genera: Mycobacterial disease can be caused by different species of the genus *Mycobacterium*. A number of reports, both published and unpublished, of rarely reported species of Mycobacteria have surfaced in South Africa in the last few years. Some unusual hosts have also been involved, causing concern in some quarters. These include reports on *Mycobacterium goodii* in a spotted hyaena, *M. xenopi* in a ruffed lemur, *M. intracellulare* in wild-caught chacma baboons, the "dassie bacillus" in free-ranging dassies, the "oryx bacillus" from an African buffalo, *M. bovis* from a black rhino, and *M. tuberculosis* in meerkats, a domestic dog and in baboons. Our studies try to put these in context and show how improved surveillance and technologies have allowed us to more easily and specifically speciate Mycobacteria than before. Most of the unusual Mycobacterial species have most likely been present in our region for many years and have probably caused disease episodes before, but have been misdiagnosed. Each case, with respect to the animal species involved, the environment in which the host is found and the mycobacterial species, must be evaluated carefully and operational decisions made accordingly.

22) Evaluation of new diagnostic tools: There is a push for better diagnostic tools for tuberculosis to be made available in resource limited settings. The use of liquid culture which is known to be more sensitive and faster for detection of TB from clinical samples is being encouraged. In response to this, studies have been funded to evaluate new laboratory techniques. Laboratory mycobacterial culture isolates have to be further identified as *M. tuberculosis* or non tuberculous mycobacteria (NTM). Phenotypic, biochemical and molecular techniques can be used for this purpose. Molecular techniques are less practical for use in resource limited settings because they are expensive and technologically complex needing specialised equipment, good quality control practises and specially trained personnel. Phenotypic and biochemical tests are slow to yield results as they sometimes require setting up of subcultures which takes weeks to grow and also require experienced staff to interpret the results. The Capilia TB assay is an easy to use assay, which does not require specialised equipment and is quick to yield results. This test has been shown to be highly sensitive and specific for identification of *Mtb* from culture isolates.

We evaluated the Capilia TB assay when used in a resource limited setting. Samples used for this evaluation were collected as part of a community based TB and HIV prevalence survey. Samples were processed for culture according to standard laboratory procedures. Samples were inoculated on MGIT and LJ media. Once growth was detected and confirmed to have acid-fast bacilli (AFB) present by Ziehl-Neelsen stain (ZN), an aliquot of the culture was archived in 7H10 medium with 20% glycerol at -20^{o}C. A subculture was set up for Niacin strip test. The Capilia TB assay and the Genotype Mycobacterium CM and MTBC (GTM-CM & GTM-MTBC) were done on subcultures of archived samples confirmed to have AFB present. An aliquot of 100uL from each MGIT was placed in the sample well of the Capilia assay strip and read after 15 minutes according to the manufacturer's recommendations. To isolate DNA, a 100uL aliquot of culture isolate from the MGIT was heat killed at 95% for 20 minutes. Before use, the sample was centrifuged and finally 5µL of the supernatant was used for PCR. The GTM-CM and GTM-MTBC was done according to the manufacturer's standards. To perform the Niacin strip test, MGIT subcultures of the primary MGIT cultures were set up and the test was performed on three to four weeks old isolates. The full economic costs and cost effectiveness were estimated as part of a separate study in Zambia. Costs were established primarily by expenditure reviews and time spent on processing cultures. The incremental costs of Niacin strip test and the Capilia TB assay per test as well as per correct test result were compared. Compared to the Genotype Mycobacterium CM and MTBC assays, the sensitivity and specificity of the Capilia TB assay was 98.5% and 99.5% respectively. On the other hand, the

Niacin strip test had a sensitivity of 88.1% and a specificity of 89.2%. Irrespective of the culture technique used, cultures were less costly when results were confirmed by the Capilia than by Niacin tests. The overall costs per culture varied between USD28 and 32 depending on the culture method used, translating into approximately USD 155 and 274 per positive culture. The incremental cost of Niacin strip test showed costs of USD 7.26 and 7.35 for LJ and MGIT respectively. The incremental cost of the Capilia TB assay was calculated to be USD 1.84 and 1.46 per test for LJ and MGIT cultures, respectively.

As the role of culture becomes increasingly important for TB control in resource limited settings, so does the need for a rapid, simple and inexpensive identification test for the mycobacterial culture isolates. The Capilia TB assay may be such a test. The need for a rapid and easy to use test to identify mycobacterial isolates is becoming more important especially as the roll out of culture for primary diagnosis of TB in resource-limited settings is being considered. The MGIT culture system is a sensitive method for isolation of mycobacteria and shortens the time to detection of growth of mycobacteria compared to traditional solid media. These systems have the potential to reduce the delay in diagnosis of TB, as well as identification of drug resistance cases of TB. However, without a rapid, accurate test for identification of culture isolates, the purpose of introducing these systems in resource limited settings will be defeated. The Capilia TB assay is a quick and easy to use test to differentiate between *Mtb* complex and NTM culture isolates. Our evaluation confirms what has been shown elsewhere, with the sensitivity and specificity all approaching 100%. On the other hand, the Niacin strip test showed low sensitivity and specificity in our hands. This test is not recommended to be used as a stand-alone test because some strains of NTM species are known to give false negative results since they do not accumulate Niacin. False negative results may also be obtained in cases of mixed cultures. These factors all point to the need to use the Niacin test in conjunction with other tests, but in reality, this may be the only test available in many resource limited settings. Using microscopic morphology may improve the sensitivity of mycobacterial culture isolates identification tests, but this requires well trained and experienced personnel to interpret microscopic features and labour costs will be high. However, evaluated under the same conditions, the Capilia TB assay performs better than the Niacin test and the results of this test are comparable to evaluations done in more sophisticated laboratories. The Capilia TB assay also proved to be less costly than the Niacin strip test when added to either solid (LJ) or liquid (MGIT) culture systems with a difference of at least USD5.42 per test. This difference is to a great extent due to staff time and the amount of consumables needed to subculture before a Niacin strip test can be performed. In our study, the Capilia assay was false negative for only three *Mtb*. complex culture isolates. One of these false negative isolates had a CG-insertion in the coding region of the mpb64 gene resulting in a frame shift mutation and consequently a truncated protein. The reason for the false negativity of the other two isolates are unclear, however it is either also due to absence of expression of Mpb64, or due to a false positive result on the Genotype assay. Two NTM isolates were false positive for the Capilia TB assay. Although it was not detected in the Genotype assay, it is possible that these isolates had low levels of *M. tuberculosis* present in the isolate which was outgrown by the NTM during culture, but still enough to produce a Mpb64 reaction. The use of liquid culture systems together with a quick and easy to interpret culture isolate identification test has the potential to contribute towards reducing diagnostic delay, and most importantly quicker identification of drug resistant TB cases. The roll-out of these techniques should be done with investment in laboratory infrastructure and human resource training with safety measures taken into consideration for handling mycobacterial culture suspensions.

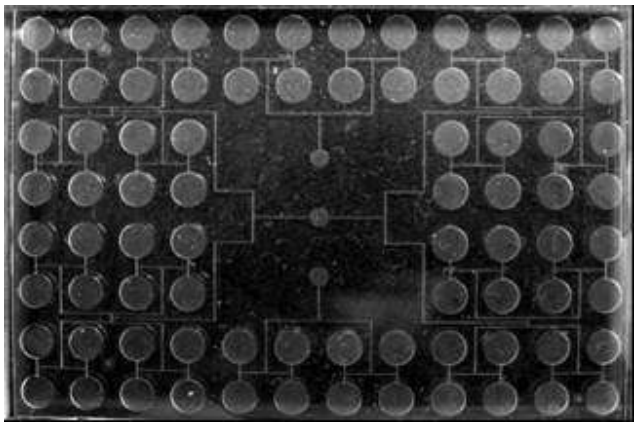
Evaluation of MDRTBsl line probe assay: The project is done in collaboration with Prof K Dheda (UCT) as part of a FIND initiative to improve the genetic diagnosis of drug resistance in *M. tuberculosis*. The project involves a comparative analysis of the Hain Life Sciences MDRTBplus and MDRTBsl line probe assays using crude DNA (250 specimens) and sputum specimens (250 specimens). Preliminary data confirms that the MDRTBplus can be used to genotype crude DNA specimens. However, amplification of the rrs gene proved inconsistent using the MDRTBsl kit. This can be overcome by purification of the DNA.

Evaluation of the AID line probe assay: This project is done in collaboration with AID (Germany) and the NHLS and involves the evaluation of a newly developed line probe assay for genotypic drug resistance testing. Over 2500 sputum specimens have been collected, of which 250 were evaluated using the current assay. Limitations in the specificity of hybridization were noted and have been reported. This product evaluation will continue for 2011 and will make use on new instrumentation and controlled hybridization chambers.

Nanotechnology: 2010 Marked our first venture into the world of “Nanotech”, in collaboration with University of Berkeley, USA. As part of the collaboration, we hosted 2 PhD students from the University of Berkeley, one

a chemical engineer and the other an electrical engineer. These students had no background in TB, and we had none in Nanotech, making it an excellent value-adding experience. Over a 2 month period, the device shown above was designed and manufactured in SA with the help of the Engineering Department at Stellenbosch University. In addition to this device, a complex electrical "reader" prototype was designed and manufactured. The device shown above can take a 2µl sample (placed in small central hole). This sample then diffuses evenly and rapidly to all 80 wells for individual reactions. Such a device can be adapted for diagnosis of XTR TB. The students have since returned to Berkeley and are furthering development of these devices there. It is hoped that they will return to develop this technology further.

"Nanotech" device. Actual size 50 X 35mm



Proteomics – Acquisition of the Orbitrap Velos, a first in Africa

For the past 20 years, biological research has focused primarily on genomic analysis. While this has allowed for comparative analysis studies and provides information on genome variation, it does not provide an understanding of the functional consequences of these variations. It is therefore imperative that we invest in new cutting edge technologies that will allow an accurate analysis of the expression products of a genome. Such technologies will provide the opportunity to gain insights into regulatory pathways, protein modification and biological systems networks. The proteome is the entire complement of proteins (the main components of the physiological metabolic pathways of cells) produced by an organism. This includes the modifications made to a particular set of proteins. It is much more complex than genomics, largely because an organism's genome is more or less constant, whereas the proteome varies from cell to cell and from time to time. mRNA analysis (i.e. by using microarrays) can be used for investigating gene expression, but this does not always correlated with protein content and is prone to experimental variation and other problems. In contrast, proteomics enables the detection and quantitation of most proteins present in a cell. We have successfully obtained funding to acquire an Orbitrap Velos mass-spectrometer, the first one of its kind in Africa. The Orbitrap is the first significant advance in mass-spectrometry in 25 years. It is the only technology that allows for rapid and accurate quantitative analysis of whole cell or sub-cellular proteomes. The Orbitrap Velos represents the pinnacle of ion trap technology. With unparalleled sensitivity and fast cycle times, it delivers more information from less. sample in less time. This instrument has been installed in the SU node of the CBTBR and should be fully operational in beginning of 2011. This technology, combined with our unique population resources and disease profiles unique to Africa, will again enable our researchers to become internationally competitive in their own right, which should lead to reduction of bio-piracy, and will allow our students to move with ease amongst those who have qualified internationally. The instrument has been delivered and should become operational during 2011.

23) Bioinformatics

The ability to identify genetic mutations that result in altered virulence has enormous potential importance for our understanding of *M. tuberculosis* biology and tuberculosis pathology. However, it is the transcriptional and, ultimately, proteomic results of these mutations that will provide us with a more comprehensive understanding, as well as allowing for the identification of potential targets for drug and vaccine research. For over 12 years the SU node of the CBTBR has been conducting an intensive molecular epidemiology-based study aimed at

determining the epidemiological characteristics of tuberculosis in a high incidence area in suburban Cape Town. Within this study site we have identified 40 distinct *M. tuberculosis* strain families that represent all of the major phylogenetic lineages. Our research has also documented numerous phenotypic differences between these strains, particularly with respect to those that differ in virulence as determined by the relative frequencies of strains and how these frequencies change over time. Sequencing of the genomes of selected strains has begun with the aim of generating whole genome sequences for 100 isolates representing these 40 strain families and performing comparative genomics in order to determine their genetic differences. Sequenced isolates of particular epidemiological interest (e.g. hyper-virulent versus hypo-virulent strains) will be selected for further transcriptome and whole proteome analysis. Genetic differences will thus be able to be correlated with transcriptomic and proteomic differences allowing unprecedented insights into the biological basis for *M. tuberculosis* virulence. In collaboration with the group of Prof Alan Christoffels (SANBI) we have embarked on bioinformatics capacity development through the analyses of whole genome sequence data generated within the SU node of the CBTBR. This work is already beginning to produce results that have the potential to vastly increase our understanding of *M. tuberculosis* biology generally, and the genetic alterations and their subsequent influence on protein expression that influence virulence in particular. Our research team at the SU node of the CBTBR already has considerable expertise in classical molecular biology and, more latterly, in proteomics. However, this study aims to address the consequences of the advent of new technologies capable of generating very large and complex datasets which threatens to exceed our analytical capabilities using currently available hardware and expertise. A major intended outcome of this project, therefore, is the acquisition of advanced bioinformatic skills, pertinent to the management and analysis of this type of data in order to retain relevance in the rapidly changing scientific environment. A consequent outcome of the development of such capacity, would be the ability to train future students in these skills, thus equipping them to play a relevant role in South African scientific and industrial research.

Molecular Immunology: Immune responses in tuberculosis, HIV and worm infections

1) Co-infection with *Nippostrongylus brasiliensis* and BCG or *M*. tuberculosis

A co-infection model was established in mice in close collaboration with Dr. Bill Horsnell, Prof. Frank Brombacher and Dr. Muazzam Jacobs from the IIDMM at UCT. Two PhD students from our centre conducted the experiments in the BSL3 animal unit at the IIDMM and have found that *N. brasiliensis* infection, followed 5 days later by BCG infection leads to decreased BCG CFU's in the lungs of co-infected mice than in mice with BCG infection alone. This effect is not seen with Nippostrongylus MTB co-infection. However, if concurrent infection does affect BCG clearance, this may have implications for BCG vaccination efficacy, where infants with other infections may be less protected against MTB disease if the vaccine take is impaired. We are currently investigating the mechanisms for this effect and hypothesise that activation of the innate immune system leads to enhanced killing of the non-pathogenic BCG. Ex vivo experiments with lung macrophages from co-infected mice is showing increased uptake of mycobacteria. We are also investigating different time intervals between the infections and the effect of worm infection on clearance of *M. tuberculosis*. The effect of concurrent Nippostrongylus/BCG infection on subsequent MTB challenge is currently being investigated. The work is expected to lead to a PhD submission during 2011.

2) Biomarkers of protective immunity and surrogate markers of TB disease in Africa- Gates Grand Challenge project 6-74.

The immunology group is part of a consortium led by Prof Stefan Kaufmann from the Max Planck Institute for Infection Biology in Berlin, Germany and that is funded by the Bill and Melinda Gates Foundation (BMGF). The aim of this project is to find biomarkers for protective immune responses against TB by longitudinal follow-up of household contacts of TB patients and by comparing responses in progressors to active disease with those in non-progressors. Prof Walzl is acting as coordinator of one of 6 work packages of the international project and is also member of the steering committee of the consortium. Dr Gillian Black has developed the standard operating procedures for one of the core assays for the project (the diluted 6-day whole blood assay). The group has recruited more than 1200 household contacts of TB patients as well as 180 HIV infected people for this study for two year follow-up.

Harmonization of field and laboratory activities at SUN with the other field sites in Africa was achieved, which was essential prior to commencement of studies. The study designs (clinical protocols), clinical case definitions, clinical procedures, laboratory assays (whole blood assay, ELISPOT assay) and laboratory

standard operating procedures issues were developed and are compatible with the other GC6-74 partners. The consortium has received additional funding from recruitment of another 1000 household contacts and the study is ongoing until December 2013. Our group has completed recruitment of its additional 300 household contacts in December 2010. No assays are currently being conducted on the consortium samples as we need to secure all valuable samples from possible "progressors to active disease". The consortium is expecting to find between 80 and 100 household contacts who progress to active disease and these samples will be of great importance to discover biomarkers for protective immunity.

Database design
We have designed and created a database for capturing data from this project, scheduling and monitoring subject follow-ups and managing the freezer storage of thousands of biological samples. This database uses a Microsoft Access interface and stores the data in a MySQL back-end.
We have also played a major role in the design of the unified database for the international collaborative consortium which will be used to collate all the data from the various field sites for later analysis.

Specific studies:
Diagnostic pilot study: a direct comparison between different tests for latent TB infection was conducted as such comparisons have been lacking in high TB prevalence areas. Tuberculin skin test (TST, the gold standard), Quantiferon TB Gold® (QTB), T Spot TB® and in-house ELISPOT were compared in 60 household contacts of TB patients. We found that none of these tests correlated with exposure gradient and that these tests correlated poorly with each other. We subsequently decided to base our definition of latent TB infection on TST result and not on any of the new tests that rely on INF-γ responses to ESAT-6 and CF-10. Two publications, have resulted from this work.

Contribution to host gene expression profiling: We have recruited more than 120 participants with well defined TB infection and disease status. PBMCs were separated into subsets with MACS beads prior to RNA extraction for micro array analysis. The microarray work has been completed by our collaborators in Berlin and three publications in international journals have resulted from this work Pathway-focused analyses identified a prevalent subset of candidate genes involved in the JAK/STAT signaling pathway including suppressor of cytokine signaling (SOCS) molecules in the subset of protection-associated genes. Differential expression was verified by quantitative PCR analysis for the cytokine-inducible SH2-containing protein (CIS), SOCS3, Janus kinase 3 (JAK3), interleukin-2 receptor alpha chain (IL2RA), and the proto-oncogene serine/threonine-protein kinase (PIM-1). Classification analyses revealed efficient capacity of this gene set to discriminate between T cells from TB patients and people with latent MTB infection, and importantly, optimal discrimination between latent infectiuon and no infection. Further characterization by quantitative PCR revealed highly variable candidate gene expression in CD4(+) and CD8(+) T cells from TB patients and only minor differences between CD4(+) and CD8(+) T cell subpopulations. These results point to a role of cytokine receptor signaling regulation in T cells in susceptibility to TB. Comparing active and latent TB, Fc gamma receptor 1B (FCGRIB) was identified as the most differentially expressed gene, and, in combination with four other markers, produced a high degree of accuracy in discriminating TB patients and latently infected donors. Elevated expression of innate immune-related genes in active TB and higher expression of particular gene clusters involved in apoptosis and natural killer cell activity in latently infected donors are likely to be the major distinctive factors determining failure or success in controlling MTB infection. The gene expression profiles defined in this study provide valuable clues for better understanding of progression from latent infection to active disease and pave the way for defining predictive correlates of protection in TB.

Screening new TB antigens: 86 antigens (85 obtained from Leiden University and one from MPIIB) were screened in 61 participants for their ability to induce IFN-γ production in the whole blood assay. There was a high frequency of responses against control antigens, including ESAT/CFP fusion protein, ESAT-6 and TB10.4. Latency antigens, resuscitation promoting factors and reactivation proteins were all represented in the top group of antigens that elicit IFN-γ responses in a high proportion of HHC's (positive responses in between 40 and 60% of HHC's). The data was combined with the results obtained from the TBRU in Uganda and from the MRC in The Gambia and a combined list of antigens was selected for prospective testing. This panel includes high and low ranking antigens at each site and also includes two antigens provided by SSI. A manuscript has been published on this work.

Recruitment of HIV-uninfected household contacts of TB patient and TB patients: To date, 153 TB index cases and more than 1200 household contacts have been enrolled for PBMC isolation and cryopreservation. Follow up at month 6 and 18 is ongoing. Retrospective analysis of T cell responses will be performed on samples from participants with interesting outcomes. The recruitment, sample processing and data entry mechanisms have been found to function well. To date 22 participants developed incident TB.
Recruitment of HIV-infected participants for WP4 (Gates funded grant):
Recruitment for WP4 started on February 2006. 180 HIV infected participants, including 39 with active TB have been recruited and followed up for two years. Retrospective analysis of T cell responses will be performed on interesting outcomes.

3) Identification of biomarkers that are able to predict tuberculosis treatment response
This work was initially funded by GlaxoSmithKline and continuing work is now funded by EDCTP as well as a BMGF funded TB Drug Accelerator grant to G. Walzl. Host or bacterial surrogate markers for successful TB treatment outcome in pulmonary TB patients are urgently needed to aid clinical trials of new TB drugs. HIV-negative new smear positive pulmonary tuberculosis patients were prospectively studied during treatment with a six month combination therapy of isoniazid, rifampicin, ethambutol and pyrazinamide, daily for two months, followed by daily doses of isoniazid and rifampicin for the next four months, as prescribed by the National Tuberculosis Program. Sputum and other samples were collected at the following time points during treatment: at diagnosis and after day 1, day 3, week 1, week 2, week 4, month 2, month 3, month 6, month 9, month 12, month 18, month 24 and month 30 of treatment. Of the 313 patients enrolled, only 274 completed this study. Patients were excluded from this study if they presented with MDR TB at any time during treatment, were HIV positive, were infected with NTM, had any disease or medication known to affect the immune system, had previous TB or had a lung condition, similar to TB, or were lost to follow up. We have found that simple models, which involve algorithms of microbial load and incorporate the host serum marker granzyme B and peripheral blood white cell counts can predict slow responders to treatment even before treatment is started. Such models will have important implications for TB drug trials and for TB control measures. The predictive model now has to be evaluated prospectively and we are currently recruiting TB patients who are part of a nutritional supplement study in order to test our model.
The TB Drug Accelerator grant of US$ 1 M was awarded in September 2008 and funds the investigation of samples from 14 relapse patients and 30 matched cured patients without relapse. We are focusing on transcriptomic analysis of ex vivo RNA and RNA from live *M. tuberculosis* stimulated whole blood culture samples (to be conducted by our collaborators at the London School for Hygiene and Tropical Medicine in London, UK) and on multiplex cytokine array analysis of sera and whole blood culture supernatants. We have shipped the appropriate samples to the UK and RNA extraction and hybridization is in process. We have analysed sera samples for the levels of 61 cytokines and are in the final stages of analysis of the whole blood culture samples. Statistical analysis is ongoing. An additional amount of just under US$ 500 000 was awarded in 2009 to continue the work.
We have identified the response patterns of 61 host markers from pretreatment to the end of treatment at 5 time points. There is a complex interplay between different types of host markers, including pro-inflammtory markers, innate, adaptive immune system molecules, regulatory markers, and different T helper cell phenotype molecules. The host response to TB treatment has never before been evaluated to this extent and has shed new light on the complexity of this response. It shows that the host immune response is reprogrammed even within a single week of treatment and may offer very sensitive treatment response indicators. There were also differences between relapsing and cured patients that suggest that extent of disease at baseline and residual inflammation at the end of treatment are associated with non-sterilizing cure.

Additionally, we have received serum and whole blood culture supernatants from 13 additional relapse patients and 30 cured patients with non cavitatory TB, who were part of a treatment shortening clinical trial conducted by the TBRU in Uganda and Brazil. Patients without cavitation at baseline and who culture converted at month two of treatment were reandomized into either a conventional 6 month or experimental 4 month treatment duration arm. The shortened treatment group had a higher rate of relapse and the study was terminated early. We will use these samples as a validation cohort for our most promising markers and have decided to use the same set of markers as on our 25 cured patients with moderate disease. This brings our total number of relapse patients to 26 and will significantly increase the power of our study. We have found significant differences in expression levels of the markers in the two studies, highlighting the importance of multisite studies. It is currently unknown if genetic differences, bacterial strain differences or extent of disease phenotypes are responsible for these differences. Analysis is ongoing.

Sputum samples from this sample collection have been made available to Dr Isobel Honeyborne from Dr Timothy McHugh's group (Royal Free Hospital, London) to investigate mycobacterial RNA as possible treatment response markers. Dr Honeyborne has isolated Mtb mRNA from sputum samples at Dx, W1, W2, W4 and W26 in our laboratory in mid April 2009 and returned in October/November 2009 to isolate mRNA from sputum samples at Day 1, Day 3, Week 8 and Week 12 of treatment. Bacterial ribosomal RNA appears to be a sensitive treatment response marker. Additionally we have sent 300 sputum samples of cured patients at various timepoints for microarray analysis to Dr Honeyborne.

4**) Diagnosis of latent TB infection in adults and children**

We are performing the laboratory component of several studies that focus on diagnostic aspects of latent TB infection with the use of the new interferon gamma release assays (IGRA's), namely the Quantiferon TB (QFT) and T Spot TB assays.

a) The ZAMSTAR study (part of the BMGF funded CREATE consortium): A total of 24 communities in Zambia and Cape Town, South Africa, each with 25,000 to 50,000 people, now take part in the ZAMSTAR study. Prof Nulda Beyers from the Desmond Tutu TB Centre is the South African principal investigator for the study. This community-randomized trial is testing two interventions to reduce TB prevalence: improved TB case finding through increased access to TB diagnostics for those with symptoms, and household interventions centred on families with a TB case, offering HIV testing and treatment, TB screening and TB preventive therapy to all contacts of TB patients. The immunology group is performing a substudy on 1 896 participants where QFT tests are being performed at three time points to assess the latent TB infection rate. To date, the first time point has been completed and we are starting the second time point assays.

b) Paediatric IGRA studies: Dr Anneke Hesseling from the Desmond Tutu TB Centre is the SA PI together with Dr. Anna Mandalakas from the Case Western Reserve University on a NIH sponsored study to investigate the performance if the two IGRA tests in children in a high incidence setting. The immunology group is performing the IGRA tests for this study and will do serial tests on approximately 800 infants. Additionally, the Foundation for Innovative Diagnostics in TB (FIND) and collaborators in Norway and Denmark, Dr Harleen Grewal and Dr, Mark Doherty, are part of a study to look at the agreement between TST and the IGRA's, assess the performance of these tests against a standard TB exposure gradient, measure the impact of HIV infection on these tests and to identify modifying factors of these tests in infants.

5) The evaluation of *M. tuberculosis* specific host cytokine signatures in whole blood culture supernatants as diagnostic biomarkers for active TB infection

An EDCTP-funded grant was awarded to the Immunology group to establish a consortium of 7 African institutions in 6 African countries plus 5 institutions in 4 European countries to find host markers for active TB. Gerhard Walzl is the coordinator of this project, which also includes capacity development and networking activities. Interferon gamma release assays (IGRAs), including the QuantiFERON TB Gold In Tube (QFT) accurately indicate *Mycobacterium tuberculosis* infection. These whole blood assays however, do not discriminate between latent *Mtb* infection (LTBI) and active tuberculosis (TB) disease. We have found that several single host markers in whole blood culture assay (WBA) supernatants stimulated with ESAT/TB10.4/Tb7.7 (including epidermal growth factor (EGF), interleukin (IL)-1α and macrophage inflammatory protein (MIP) 1β) have promising discriminating ability on their own to differentiate between latent and active TB. Moreover, combination of these three markers increase this ability substantially. The project will entail the recruitment of 800 HIV negative and 400 HIV positive adults with suspected TB and we will perform WBA tests to assess the ability of combinations of levels of EGF, IL-1α and MIP-1β as measured initially by Luminex technology in WBA supernatants and to be subsequently evaluated by multiplex lateral flow tests to reliably differentiate between active and latent TB (main clinical trial). We will also investigate additional host markers in WBA supernatants which may further improve the ability to diagnose active TB infection as well as evaluate the ability of novel *Mtb* infection phase-associated antigens to elicit host marker responses in overnight whole blood assays. Development of a field-friendly, diagnostic test will subsequently be performed that will include *Mtb* antigen coated tubes (either the currently used QFT test tubes or similar tubes coated with newly identified *Mtb* antigens) combined with lateral flow strips to measure host biomarkers. This test is based on the highly sensitive, field-friendly and stable upconverting phosphor (UCP) technology, and allows simultaneous detection of several host biomarker analytes in culture supernatants.

This approach has promising potential for further development into a rapid (<24 hours) diagnostic test for active TB that relies on *Mtb* specific host biomarker profiles, and does not require any sophisticated laboratory infrastructure. Although such a test would require overnight culture, this can be performed in settings with only

very basic laboratory capability, as only a basic incubator (without CO2 enrichment), a centrifuge, pipettes and a strip reader are required.
The clinical trial will establish a biological sample repositories (including serum, plasma, RNA, WBA supernatants, urine, sputum, peripheral blood mononuclear cells, breath condensate) that will be linked to all relevant clinical information through a study-specific central database to ensure continuous investigation in the search for diagnostic biomarkers (e.g. subsequent 'omics' approaches to identify alterations induced at the gene transcription level that will reveal protein candidates as potential targets for intervention. Parallel metabolomic analyses will reveal information about the most efficacious treatment protocols). The project will also facilitate capacity development to perform clinical trials for prediction of TB disease development in established and less established field sites and their parent African institutions and will foster improved South-South as well as South-North networking between consortium partners.
In 2010 the project had its first consortium annual meeting and harmonized protocols and clinical and laboratory standard operating procedures were created. Ethical approval was obtained by all partners from their local or national ethics review boards. The recruitment started in December for the Stellenbosch component of the study at a newly established research field site, Fisantekraal, where we are working in close collaboration with an NGO, Wonlife.

6) TB Vaccine studies
We have conducted our first Phase I TB vaccine immunogenicity studies with the German vaccine developer Vakzine Projekt Management (VPM). The recombinant BCG vaccine, VPM 1002, was tested in 20 normal adult volunteers. To date, no safety concerns were found. The analysis of the immunogenicity data is ongoing. We are planning a phase II clinical trial, where recruitment of neonates will be conducted by Prof. Mark Cotton's KidCru group and where immunogenicity testing will be performed by our laboratory.
In view of our growing involvement in TB vaccine trials we have initiated the process of accrediting our lab with SANAS for clinical trials. We have appointed a quality assurance officer and plan to apply for accreditation assessment early in 2011

7) The Impact of Steroid Hormones on Protective Immunity to Tuberculosis
This study investigates the effect of steroid hormones including synthetic progestins on anti-mycobacterial immune responses to TB.
Most latently infected individuals contain Mtb infection through a balance of regulatory and effector immune responses. This balance can be influenced by steroid hormones such as glucocorticoids (GCs). Administration of GCs increases the risk of developing active TB in humans and causes reactivation of TB in animal models. Similarly to glucocorticoids the three monthly injectable contraceptive medroxyprogesterone acetate (MPA) has been shown to bind with high affinity to the glucocorticoid receptor (GR) and has been shown by ourselves and others to have partial GR agonist activity. Currently MPA is the most commonly used contraceptive in South Africa and other developing countries. An alternative two monthly injectable contraceptive norethisterone enanthate (NET), which does not have glucocorticoid activity is equally available at no cost at South African health care clinics. Despite the pharmacological differences between these two synthetic progestins, MPA is favoured by many women, as repeat injections are only required four times a year instead of six times a year with NET. In fact in our study setting around Tygerberg Hospital in Cape Town 60.9% of women on contraceptives use MPA, whereas only 17.4% use the two monthly injectible NET. Due to the selective glucocorticoid activity of MPA it is possible that the doses administered for endocrine therapy could have significant immune modulatory effects and impact on susceptibility to as well as clinical manifestation and outcome of infectious diseases.
The role of this hormonal contraceptive in TB infection and disease has never been investigated before. This is surprising as MPA is mainly used in low socioeconomic areas with high TB burden and is the recommended contraceptive for active TB patients as estradiol containing contraceptives are rendered ineffective due to upregulation of P450 cytochromes when used in combination with the anti-TB drug rifampicin.
As described in the previous report, we investigated the effect of MPA on mycobacterial antigen specific expression of cytokines compared the the glucocorticoid cortisol and to progesterone, which MPA is supposed to mimic. We showed that *in vitro* in human PBMCs stimulated with BCG for 3 and 6 days a range of cytokines is differentially expressed in the presence of cortisol, progesterone and MPA. BCG induced expression of IL-1α, IL-1ra, IL-17, IL-17, TNF-α, MIP-1α, IL-5, IL-1β, IL-6 was significantly inhibited by MPA and cortisol, but not by progesterone, indicating that MPA acts very differently from its endogenous analogue progesterone, mimics cortisol and could therefore elicit distinct glucocorticoid side effects. Other cytokines such as INF-γ, IL-12p40, sCD40L, GM-CSF and IL-13 were also significantly inhibited by cortisol and MPA and to lesser extent

also by progesterone. IP-10 expression was upregulated by cortisol and MPA and G-CSF was upregulated by all three steroids. We performed these experiments both at low as well as at high hormone doses and could show that the differential expression of many of these cytokines occurred at high as well as low doses of steroids. To assess whether the inhibition of antigen specific cytokine expression by MPA occurs also in women using MPA as contraceptive we collected PBMCs from 10 MPA users as well as 10 controls and stimulated them with BCG for 3 and 6 days respectively. Despite the small number of samples, we found that production of IL-12p40, IL-1α, IL-10, IL-13 and G-CSF was significantly lower in MPA users than in controls. Deficiences in IL-12p40 have been shown to predispose to mycobacterial disease and in mouse knock out experiments IL-1α has been shown to be important for the early immune response to Mtb, therefore reduced production of these cytokines could impact on susceptibility to TB in MPA users. Furthermore IL-10 and IL-13 are required for prevention of excessive immunopathology in active TB patients and a lack of these cytokines could exacerbate lung inflammation. Therefore our findings suggest that MPA use could not only affect susceptibility to TB, but also TB disease severity. Interestingly we could show *in vitro* that the contraceptive NET does not cause inhibition of IL-12p40 and IL-1α and might therefore be a safer choice for women exposed to Mtb. One PhD student and one BSc honours student (MSc in 2011) have been involved in this study during 2010. Together with Dr Petrus Steyn from the Family planning unit at Tygerberg hospital we plan to extend this study with recruitment of a larger number of study participants including MPA users, NET users and women not using hormonal contraceptives. This study will also elucidate the underlying mechanisms of steroid mediated changes in signal transduction and transcription in PBMCs carried out in collaboration with Dr Jackie Cliff and Prof Hazel Dockrell at the London School of Hygiene and Tropical Medicine.
Increased susceptibility to TB due to MPA use would be of major clinical significance for women living in South Africa and other Sub-Saharan countries and would lead to changes in current family planning policies.
In collaboration with Dr Muazzam Jacobs at the UCT, a PhD student is currently assessing the effect of MPA on mycobacterial burden and pathology in two different mouse strains (BALB/c and C57BL/6) with different susceptibility to TB infection. Additionally we will perform ELISA Arrays and Luminex analysis for differential cytokine expression on the serum and lung homogenates of all animals. A new MSc student will investigate whether MPA causes reactivation of latent TB in this mouse model similarly to classical glucocorticoids.

Host Genetics
The majority of individuals who are infected with *M.tuberculosis*, do not progress to active disease. This is due to the individual host response to infection by TB, and the purpose of this research into the genetics of susceptibility to TB is to find which genes and polymorphisms are involved, and to what degree they impact on the risk of an infected individual progressing to active disease. Socio-economic circumstances play an important role in the development of TB, but as this occurs in only 10% of immunocompetent people, there are clearly many potentially manipulable factors operating, and elucidating these genetic mechanisms could enable us to drop this percentage. The current TB epidemic in the Western Cape is not abating despite our internationally accepted drug regimen. This epidemic is not driven by HIV although exacerbated by it. It may be due to an underlying genetic susceptibility to TB in the affected populations, and this issue needs to be addressed in the field if it is the case. Identification of the relevant genes has involved a number of approaches: nonparametric linkage analysis (genome scanning using sib-pair studies), direct candidate gene association studies, and recently, genome-wide approaches such as admixture mapping. We have been involved in a number of these different approaches as the field of human genetics is changing rapidly.

Research projects
1) Genome Wide and Candidate Gene studies: A nonparametric linkage analysis (affected sib-pair study) was done as a follow-up to an earlier scan published in PNAS in 2000. In this second genome wide scan on an independent set of over 140 families, completed with the same collaborators, we identified linkage to TB in a region on chromosome 20q. A multistage strategy was employed (Cooke *et al* 2008) to identify a novel locus for tuberculosis susceptibility in African populations. Forty SNPs within the genomic region found in the South African Coloured (SAC) population and Malawians were used to screen a large independent Gambian population, and two genes, melanocortin 3 receptor (*MC3R*) and cathepsin Z (*CTSZ*), showed evidence of disease association. Polymorphisms in these genes were further genotyped in populations from Guinea-Bissau and the Republic of Conakry. A polymorphism in the 3'UTR in *CTSZ* showed significant disease association (P=0.005). To validate these findings, we conducted an independent, unrelated case-control study and found that the same SNP implicated in *CTSZ* by Cooke *et al* showed evidence of disease association in the SAC population ($p < 0.0001$) and a SNP located 373bp upstream of the *MC3R* gene was also significantly associated with TB (p=0.0004, adjusted for age and gender). This SNP is predicted to create an alternative

transcription factor binding site (Adams *et al*, 2011, accepted). This study provided convincing evidence to motivate investigation into the mechanisms of action of the respective pathways of these previously unsuspected molecules in TB progression; cathepsins in the lysosomal proteolytic system and MC3R in inflammation and energy homeostasis.

We also did case-control studies of the following genes, some of which were novel polymorphisms investigated by us and found to be either not associated with TB, such as a number of genes modulating interferon-gamma (Moller et al, 2010b), or associated, such as *TNFRSF1B* (Moller et al, 2010a). *TNF receptor 2:* the pleiotropic cytokine tumour necrosis factor-α (TNF-α) is essential to control tuberculosis infection and various TNF family members and their respective receptors may contribute to tuberculosis risk. We investigated four functionally relevant polymorphisms in the TNF receptor 2-encoding gene, TNF receptor superfamily member 1B, for association with tuberculosis susceptibility. Genotyping of four polymorphisms was done in independent populations from South Africa (cases=429, controls=482) and Ghana (cases=640, controls=1158). Single-point and haplotype analysis in South Africans revealed an association in the 3' untranslated region of the gene. The T allele of rs3397 alone and/or the 3´ untranslated region haplotype GTT may confer protection against tuberculosis. The GTT genotype had previously been shown to increase the decay of TNF receptor 2 mRNA, and it is possible that mRNA destabilization represents a molecular key mechanism for disease susceptibility. Interestingly, the association signal appeared to be restricted to women. The genetic finding was validated in female participants from Ghana, and the combined p value in the haplotype analysis was p=0.00011. The lack of association found with the genes modulating interferon-γ was unexpected, but serves to emphasize the complexity of the immune response to TB. These studies added substantially to the existing work on the above genes and polymorphisms, and introduced new candidate genes to the field.

2) Genetic Epidemiology of TB: As part of the establishment of a clinical trial site in the Western Cape for testing new TB vaccine candidates, we were (Prof E Hoal) asked by local colleagues and Prof Erwin Schurr of McGill University, Montreal, Canada, to be the local Principal Investigator of a human genetic epidemiology study funded by the Sequella Global TB Foundation (now Aeras). Our aim was to study about 400 children and young adults in 128 families in of a genome-wide linkage search. This family-based study to look at genetic factors impacting on the immune response to mycobacterial antigens was designed and implemented, and has resulted in four publications in the last year.

While many studies have compared in-vitro tuberculosis diagnostic tests with the venerable tuberculin skin test (TST), there is little understanding of the quantitative relationship between critical measures of anti-mycobacterial immunity used to detect tuberculosis infection. We therefore decided to determine the degree of redundancy between quantitative read-outs of in-vivo and in-vitro assays of anti-mycobacterial immunity. We measured in-vivo TST responses and a whole blood assay was used to determine the in-vitro antigen-specific IFNγ cytokine release. The frequency of CD8+ cells was tested using intracellular CD4+ and IFNγ antigen-specific IFNγ cytokine staining. We found that in-vivo TST responses segregated into two well separated groups with either no measurable response (TST induration < 5mm; n=164) or a normally distributed group with TST indurations ≥5mm with peak at 15mm (n=260). In-vitro assays provided a less pronounced separation of responders and non-responders. Correlation analysis of responses among persons with TST ≥5mm demonstrated that extent of TST response was poorly correlated with IFNγ release (commonly accepted as the method of choice) and frequency of IFNγ+CD4+/CD8+ cells across three stimulating antigens (BCG, PPD, ESAT-6). We could therefore conclude that in-vivo and in-vitro assays are non-redundant, complementary measures of anti-mycobacterial immunity. We showed that the two assays measure very different features of the immune response, and cannot act as predictors of each other (Gallant et al 2010a). Both TST and in-vitro assays provided valuable information about anti-mycobacterial immunity and by interference latent tuberculosis. There was no correlation with gender and the immune response to mycobacterial antigens, but a strong positive correlation with age (Gallant et al 2010b).

By analysing the immunological correlates with the genetic data, we could determine the degree to which human anti-mycobacterial immunity (often used to infer presence of TB infection) is inherited. We found high heritability (> 50%) for in-vitro secretion of TNFα and IFNγ, and the frequency of antigen-specific IFNγ+CD4+ and IFNγ+CD8+ cells in the response of whole blood to mycobacterial challenge (Cobat et al 2010). In principal component analysis, the first three components explain 75% of the overall variance. This is consistent with the effect of pleiotropic regulatory genes of human anti-mycobacterial immunity. These results directly demonstrate the pivotal role of host genetics in quantitative measures of anti-mycobacterial immunity and suggest that immune-diagnosis of TB infection may be confounded by host genetics.

A genome scan was performed with the quantitative immunological reaction to injected PPD (Mantoux) as the measured trait. We analysed the results in two ways: by dividing the children into responders vs non-responders, and by quantitative analysis of the extent of the Mantoux reaction. Most of the subjects had been exposed to TB, but about 20% did not show delayed type hypersensitivity (DTH) in the skin test, appearing to be naturally resistant to infection by *M. tuberculosis*. This strong resistance mapped to a 6-Mbp chromosome region on 11p14 called *TST1,* suggests that we could one day manipulate cellular mechanisms to prevent TB. The second locus, in the 2.9-Mbp 5p15 region called *TST2*, segregated with differing extents of T-cell-mediated DTH. These genetic factors might contribute to whether an infected individual keeps the bacterium dormant or develops the disease. The measurement of TST did not correlate with interferon-gamma release, indicating that other pathways could be more important. In addition, our collaborators fine mapped the 5p15 region and identified *SLC6A3* as a candidate gene. This gene is a solute carrier family member, which could influence granuloma responses to mycobacteria, and loss of the protein reduces DTH response to ovalbumin. Further fine mapping of the much larger TST1 region is ongoing. This work was the first report of a genetic resistance factor for TB infection. One major locus determines innate resistance to *M. tuberculosis* infection in endemic areas and a second controls the extent of that response via regulators of T-cell dependent DTH. These findings are both ground-breaking in the field.

3) Admixture mapping: This is a novel approach for disease gene discovery and falls between linkage and association studies. It requires a population which has arisen from two or more genetically different parent populations where the frequency of the disease, and therefore underlying risk variants, is different in the founding populations. The aim is to localise the parts of the genome inherited from a specific ancestral population in the patients in order to identify the locus responsible for the phenotype. The tools are genetic markers that occur with different frequencies in different population groups. When risk alleles vary across populations, genetically mixed individuals with the disease under investigation are likely to have a higher probability of having inherited the loci near the disease loci from the population at higher risk of the disease. Admixture mapping has a higher statistical power to detect genes of modest effect than linkage, if these risk alleles are differentially distributed between ancestral populations. The methodology has recently been successfully used in studying hypertension and prostate cancer in a two-way admixed population, i.e. African-Americans. In the random marker approach, random markers throughout the genome are typed in affected individuals. This approach is successfully implemented by using microarrays. We plan to apply this methodology to the investigation of tuberculosis in the local population. We have carried out a whole genome SNP analysis using the GeneChip Human Mapping 500K Array Sets from Affymetrix on 1000 individuals, consisting of 100 controls and 900 TB cases (HIV-). Given the complexity of the data and the analyses we need, this work is being done in collaboration with the computational biologists and bioinformaticists. Preliminary quality control and analysis of data was done in our Centre, up to the limits of our computational capacity. We are also collaborating with Dr Alkes Price (Harvard School of Public Health, USA) due to the novelty of this work.
In preparation for using the Affymetrix 500k chip data for admixture mapping, we needed to investigate the ancestral population contributions to the SAC population. The result was the dissection of the ancestral populations of nearly 1000 individuals living in Cape Town, constituting a definitive study of the SAC in the Western Cape, the home of the majority of this population group (de Wit et al 2010a). In early 2008, few people in SA had worked with genome-wide data, and the data cleaning alone took months. This work has used the publicly available databases of Hapmap and the hgdp population database at Stanford. We have found that there are at least four major ancestral populations contributing to the Coloured group: namely the indigenous Khoisan, African black, European immigrants and people brought in from India and related areas in the east. The structure of the SAC population was not quite what was expected in terms of the high Khoisan input, but was supported by a small study of 39 individuals by Sarah Tishkoff. Previously we had collaborated with Lluis Quintana-Murci at the Institut Pasteur in Paris to study a candidate gene. As he is an international expert on mtDNA in population and evolutionary genetics, we collaborated on the further investigation of the SAC population to determine the relative contributions from the maternal and paternal sides of the various ancestors, and again found exceptionally high Khoisan contribution from the maternal side, providing much historical insight (Quintana-Murci et al 2010).

4) Gene-gene interactions: Another approach was to determine the effect of gene-gene interactions on determining the susceptibility to TB of an individual, as opposed to the more usual scenario of expecting a measurable effect from a single gene. We genotyped over 800 cases and controls for 11 polymorphisms in 9

genes, all of which had shown evidence of association with TB. With eight instances of statistically significant gene-gene interactions, the importance of epistasis was clearly identifiable in this study (de Wit 2010b).

Targets and new drug development.

Pathogenic *M. tuberculosis* is unique in its ability to export glutamine synthetase (GS) and this export mechanism is essential to the survival of the bacillus. GS activity is regulated by adenylylation and the exported adenylylated form makes GS a potential drug target since only the deadenylylated version of GS is present in humans. In collaboration with the CSIR, the research project was directed towards two goals 1) To directly inhibit the GS adenylylated form as a specific drug target (CSIR) and 2) To indirectly target the GS of *M. tuberculosis* by unravelling the unique mechanism of export (Stellenbosch). To determine the level of genetic conservation of the *gln*A1 gene, the *gln*A1 gene as well as the genetic regions immediately upstream and downstream of this gene (that might contain regulatory elements of this gene) was sequenced. The sequencing data showed that the *gln*A1 gene remained genetically conserved in all *M. tuberculosis* strains sequenced, as we detected no mutations or polymorphisms in the *gln*A1 gene from any of the *M. tuberculosis* strains sequenced. The potential highly conserved nature of GS makes it ideal for possible drug targeting. Compounds for the direct and specific inhibition of *M. tuberculosis* GS were developed by the CSIR and we have tested them for biological activity in our BL3 laboratory using the BACTEC system. Four candidate molecules were identified with bactericidal activity, which show no toxicity to eukaryotic cell cultures. The results show strong potential for anti-tuberculosis drugs designed through rational design that would give activity as well as specificity.

1) Elucidation of the Nitrogen metabolic pathway in the *Mycobacteria:* Nitrogen metabolism is an essential metabolic pathway in all living organisms. The regulatory cascade governing this pathway is important, especially in prokaryotes, as they are reliant solely on their immediate environment for nitrogen which may be present in constantly varying concentrations. Nitrogen metabolism has been extensively studied in enteric bacteria such as *E. coli,* as well as in two Actinomycetales, *Corynebacterium glutamicum* and *Streptomyces coelicolor,* due to their industrial importance; however, very little is known about the metabolic pathway in other Gram positive Actinomycetales. This subgroup of prokaryotes is of particular interest as it contains many important pathogens such as *M. tuberculosis, M. leprae* and *Corynebacterium diptheriae.*
Our study has been focused on nitrogen metabolism in the non-pathogenic, rapidly growing *Mycobacterium smegmatis* in order to better understand how nitrogen metabolism may be regulated in disease-causing mycobacteria such as *M. tuberculosis.* Since very limited data is available on this topic in the mycobacteria, we used the closely related *C. glutamicum* and *S. coelicolor* as models for the mechanisms present in *M. smegmatis.* The study involved the identification and analysis of the expression of 10 candidate genes, putatively implicated in nitrogen metabolism in *M. smegmatis.* The genes encoded for major components of the metabolic pathway such as glutamine synthetase, glutamate dehydrogenase, signalling and regulatory proteins and transcriptional repressors. We analysed the relative expression levels of these genes under conditions of nitrogen-limitation and excess using reverse-transcriptase and real-time PCR. Our results indicate that the expression profiles of the candidate genes are affected by both the nature of the nitrogen source as well as its relative concentration in the media. In addition, it was observed that the expression of these genes is affected by the type of culture medium used (minimal medium *vs*. enriched medium) which reflects the interplay between both carbon and nitrogen metabolic pathways. Upon comparison with the data available on *C. glutamicum*, it was observed that there are distinct differences in the levels of expression of these genes under similar conditions. These results indicate that there may be different regulatory mechanisms present in *M. smegmatis*.
Gene deletions of potential transcriptional regulators governing the expression of nitrogen-related genes were undertaken. *M. smegmatis* is unique in that its genome encodes transcriptional regulators found in both *C. glutamicum* and *S. coelicolor.* Transcriptional studies of a select number of genes was undertaken in order to compare the difference (in any) in gene expression between the knockout mutants and wild type *M. smegmatis* under conditions of varying nitrogen availability. It was found that the deletion of the regulator similar to that in *S. coelicolor* resulted in abnormal bacterial colony morphology, growth rate and disrupted regulation of gene transcription. In contrast, the deletion of the regulator similar to that in C. glutamicum did not result in the marked changes in growth in *M. smegmatis;* however there was some modest effect on the transcriptional regulation of a few genes.

In addition to studies done at a transcriptional level, an investigation into the function of the effector enzymes of nitrogen metabolism was undertaken in both *M. smegmatis* and *M. bovis BCG*. The activity of glutamine synthetase as well as three, as yet largely unstudied, glutamate dehydrogenase enzymes was determined under various conditions of nitrogen availability in *M. smegmatis*. It was found that these glutamate dehydrogenase enzymes play a greater physiological role with regards to nitrogen assimilation than previously thought. This was an important finding as the pathogenic *M. tuberculosis* has a homologous glutamate dehydrogenase (NAD-specific glutamate dehydrogenase) that may function in a similar way to *M. smegmatis.* Upon analysis of the activity of the only GDH present in the *M. bovis BCG*, it was found that it functions in a very different from as in *M. smegmatis.* It was found that the enzyme plays a largely assimilatory role and is not regulated as in *M. smegmatis.* It is believed that *M. tuberculosis* has only a single route of nitrogen assimilation which is via the ATP-dependant Glutamine synthetase/glutamate synthase pathway. We have shown that an additional nitrogen assimilatory route is possible via NAD-GDH. This may have significant implications for the survival of *M. tuberculosis* in various environments (e.g. during latency) and is an aspect which requires further investigation.
The knowledge gained from this study may provide insight into how nitrogen metabolism may be regulated in *M. tuberculosis*. Due to the essential nature of this pathway and the central importance of protein interactions in regulating the pathway; elucidation of the mechanisms of control may lead to the discovery of novel drug targets that may help to control the TB epidemic.

2) Elucidation of mode of action of a furanone based antituberculosis compound
F1082 is a novel synthetic furanone based on a plant-derived natural product. Furanones are “privileged chemical scaffolds”, found in a number of biologically important natural products and displaying a broad range of pharmacological properties. An original and efficient synthetic route to F1082 has been developed by partners in the Department of Chemistry, University of Cape Town. We have shown that F1082 has potent activity against *M. tuberculosis* at an MIC of 8 µg/ml. It is highly selective for mycobacteria, as it did not inhibit growth of Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, and *Pseudomonas aeruginosa* ATCC 9027). F1082 thus meets one of the Global Alliance for TB Drug Development criteria for an ideal new TB drug. We therefore wish to study F1082 further, in order to attempt to develop it into a drug candidate. To benchmark the system we used a standard drug (INH). As expected, INH showed a bactericidal effect reducing the bacterial count with more than 5 log units CFU/ml in 24 hours. Once stationary phase had been reached (after 5 days) we observed no further effect of the INH, confirming published data that INH is less effective on slow growing organisms The effect of F1082 is different. This compound reduces bacterial count by two logs in 24 hours. The effect does not appear to be concentration dependent within the first 24 hours, but as the culture entered stationary phase, F1082 further showed an effect which was concentration dependent. Although there was less reduction of bacterial load with F0182 compared to INH, F1082 clearly inhibited the growth of bacteria, showing a bacteriostatic effect. Since the compound inhibited survival of bacteria in stationary phase, it may have more than one active form or more than one target site. In order to determine the mode of action for new or novel compounds, one can identify changes in gene expression. We used the lowest concentration at the earliest time point to determine the gene signature profile of F1082.
At 8 µg/ml (at 4 and 24 hrs) 26 genes were differentially regulated. Of these genes, 7 were of interest (*mbt*B, *mbt*C, *mbt*D, *mbt*E, *mbt*F, *mbt*H and *bfr*B) as they all fall in the same cluster and are involved in iron acquisition. The mbt genes were all up regulated and bfr gene was down regulated in the presence of F1082.The *mbt* genes are mycobactins which are used by the mycobacteria to scavenge for iron extracellularly when there is not enough intracellular iron. Our observations on the bfr gene lead us to suggest that F1082 interferes with iron acquisition in *M. tuberculosis.*
We observed that almost all the genes that were differentially regulated at 8 µg/ml were a subset of genes altered by a high concentration of F1082 (64 µg/ml). This clearly showed that as the concentration increases, more genes were affected but the genes that were affected by low concentration consistently were affected even by high concentration This suggests that the gene signature profile of the compound/mode of action is still the same. For the microarray data analysis two paradigms were used to analyse the data: 1) what are the genes that were differentially regulated between the control and the treated and 2) which genes are coordinately regulated. When we used the latter paradigm, genes that were coordinately regulated by high concentration were all involved in information pathways. These genes are all involved in protein synthesis and this suggests that F1082 inhibits the growth of *M. tuberculosis* by interference with protein synthesis. Our microarray data has been shown to be reproducible and reliable. We conclude that F1082 is bacteriostatic and

its activity appears to be concentration dependent. Its mode of action may involve iron regulation and/or protein synthesis. New analogues of F1082 are being synthesized and we will test these to assess whether we can improve on the activity of this compound.

3) Evaluating other new anti-tuberculosis drug candidates.

Novel thiolactone-isatin hybrids as potential antimalarial and antitubercular agents
The synthesis of a novel series of thiolactone-isatin hybrids led to the discovery of tetracyclic by-products which displayed superior antiplasmodial activity. The tetracycle thus formed the basis of a more focused SAR study. Identified from this series is compound with an IC_{50} of 6.92 μM against the chloroquine-resistant (W2) strain of *P. falciparum*. Useful antimalarial SARs delineated include the need for substitution at C-5 of the isatin scaffold and the enhancement of activity by increasing the linker length. In contrast to their antimalarial activity, other hybrids were devoid of antitubercular activity whereas the advanced intermediates displayed growth inhibitory activity against the H_{37}Rv strain of *M. tuberculosis* as revealed by the BACTEC, MABA and the LORA assays

Derivatives of SQ109
Presently, SQ109 (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) which is a second generation agent developed from the first line drug ethambutol is one of the most promising anti-TB drug candidates at the clinical trials stage. SQ109 displays high potency against H37Rv reference strain and MDR strains of *Mycobacterium tuberculosis*. Although SQ109 is specific for mycobacteria with activity against *M. tuberculosis, M. bovis, M. marinum*, it however, shows little activity against *M. avium* and *M. smegmatis*. As part of an ongoing project to develop highly potent anti-tuberculosis therapeutics, six SQ109 derivatives were synthesized and screened *in vitro* for their anti-tuberculosis activity against the ATCC strain H37Rv and the extensively drug-resistant clinical strain XDR173. One of the compounds showed potent activity against both strains of *Mycobacterium tuberculosis* within a MIC range of 0.5 to 0.25 μM. Another derivative and SQ109 were potent within a MIC range of 1 to 0.5 μM, whilst a third derivative displayed an activity within the MIC range of 0.5 – 2 μM against both *Mycobacterium tuberculosis* strains. This development is done in collaboration with Dr Thavi Govender at UKZN. This work has been published.

Pentacyclo-undecane derived cyclic tetra-amines as potent anti-tuberculosis agents
As part of an ongoing effort to develop highly potent anti-tuberculosis agents, fourteen Pentacyclo-undecane (PCU) tetra-amine compounds were synthesized and screened for their in vitro anti-mycobacterial activity against two TB strains, H37Rv and XDR 194 [an extensively drug-resistant strain of tuberculosis]. Using the broth macrodilution method, nitrofuranylamide based compounds showed almost similar activities against the H37Rv strain of Mycobacterium tuberculosis when compared with the control drug, ethambutol. N-Geranyl piperazine PCU and trans–trans farnesyl piperazine PCU were 3.2 and 3.7 times more potent than commercially available ethambutol. Both isoprenyl PCU tetra-amine derivatives and N-decyl piperazine PCU (9a) were highly active against the XDR 194 strain of tuberculosis with MICs in the range of 0.63–3.02 mM. Cytotoxicities (IC50) of isoprenyl based compounds were tested on a mammalian cell line [MDBK (Madin Darby bovine kidney epithelium)] with values of 30, 24 and 25 mM respectively. Testing new derivatives is ongoing. Development is in collaboration with Dr Thavi Govender of UKZN.

4) Testing drugs designed against mycothiol pathway enzymes
Mycothiol (MSH) is unique to mycobacteria as a major low molecular weight cellular thiol responsible for protection of bacteria against oxidative stress. The design of drugs against enzymes of the mycothiol pathway is based on the premise that mycothiol is unique to mycobacteria and important for its survival.
A number of commercially available substrate analogs were acquired for testing in an extracellular assay. These included the glycosyl transferase inhibitors, nikkomycin and tunicamycin as targets toward the glycosyl tranferase MshA. Tunicamycin inhibits the transfer of Glc-NAc-1-P from UDP-GlcNAc to polyprenyl monophosphates in a variety of organisms including Gram positive bacteria. The most active mimetic *in vitro*, tunicamycin is a well known, non-specific natural product inhibitor of glycosyl transferase. At 10 μg/mL in the *in vitro* assay, tunicamycin exhibited high inhibition (96%) against Mtb in the BACTEC assay. The other glycosyl transferase inhibitor nikkomycin exhibited no activity in the extracellular assay.
M. tuberculosis -infected macrophages subjected to tunicamycin treatment were observed by electron microscopy in order to compare the possible ultrastructural alterations of bacilli in presence of added drugs.

We compared the presence or absence of, intracytoplasmic lipid inclusions (ILI) (normally replicating bacilli display no ILI whereas non-replicating persistent bacilli do), degraded bacilli that correspond to killed bacilli, and whether phagosomes contained a single or several bacilli because arrest of phagosome maturation occurs only in the case of loner phagosomes. For this study, BMDM were infected with *Mtb* H37Rv. At day 5 pi, different concentrations of tunicamycin were added to the infected cells. After 0, 1 or 3 days of treatment, cells were fixed and processed for electron microscopy in order to analyse the fate of bacilli in presence of drugs. In presence of tunicamycin: after a 24-hr treatment, with tunicamycin added at 5µg/ml, more than 95% of the bacilli were morphologically intact. The drug was, however, cytotoxic for macrophages that became permeabilized or underwent lysis. This occurred also with lower concentrations of 1µg/ml.
During 2010, a high throughput enzyme (mshA) screening assay was developed. In 2011, all designed compounds will first be subjected to this assay before testing for antitubercular activity.

5) Investigation of the synergistic effect of Sulfamethoxazole and Trimethoprim in combination with first-line TB drugs

We wished to evaluate the activity of Sulfamethoxazole and Trimethoprim, as individual drugs as well as in combination with each other and first-line TB drugs against Mycobacterium tuberculosis. Trimethoprim did not show any inhibitory effect on H37Rv. However, Sulfamethoxazole showed 80% growth inhibition at 4.75µg/ml. At concentrations lower than 4.75µg/ml Sulfamethoxazole lost most of its' activity. The Sulfamethoxazole-Trimethoprim combination did not show any synergistic effect. We also tested the combination of Sulfamethoxazole and first-line TB drugs; isoniazid (INH), rifampin (RIF) and ethambutol (EMB) in a range of concentrations below the MIC on H37Rv. The combination of SMX-RIF showed a strong synergistic effect, SMX-INH showed no interaction and SMX-EMB showed an additive effect. Although SMX seemed to be effective against H37Rv, the activity was lost against INH mono-resistant strains, a phenomenon that raises questions. We are using qPCR to assess the changes in gene expression in response to SMX, in particular *folP1*, which is a target of SMX. Insights gained from these approaches may give a better understanding of action of this drug and its potential as a candidate drug in the cocktails used against *Mycobacterium tuberculosis*.

Antimycobacterial Clinical Trials

We have noted a constant increase in demand for sites able to perform clinical trials at a quality adequate for registration of novel antituberculosis drugs and regimens. Requirements of Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) must be met. All documentation needs to be complete and ready for inspection by national and international regulatory authorities. Only few centres globally can currently fulfil those requirements. 2010 has seen more drug trials conducted than ever before in our centre. At the level of EBA studies (2 weeks study duration, Phase IIA), we have tested the introimidazole derivative PA-824 in various dosages. For the first time we are evaluating combinations in EBA studies (TMC207, PZA, PA-824, and Moxifloxacin) and have recently started the evaluation of SQ-109. We participated in a phase IIB study (8-week duration) of Rifapentine versus Rifampicin added to HZE in the first 2 months of treatment in adults with pulmonary TB. A large Phase III trial (full treatment duration REMoxTB) was initiated at the centre in 2007 to investigate if tuberculosis treatment duration can be shortened from 6 to 4 months by incorporating Moxifloxacin into the regimen. More than 10,000 samples have been processed for this ongoing study so far. MDR and XDR tuberculosis trials have seen the completion of recruitment of two trials with TMC207 for which follow-up is ongoing.
Investigator driven projects are focusing on the evaluation of endpoints for EBA studies that could make such activities more accessible to centres without access to highly developed technology.
We have during this year collaborated with the Global Alliance for TB Drug Development (USA, NGO with various stakeholders), the Tuberculosis Trials Consortium (CDC, USA), the PanACEA Consortium (EDCTP, European Union), pharmaceutical companies (Tibotec, J&J), and FIND (Switzerland, NGO with various stakeholders). We have processed clinical trial samples from sites based at UCT, Port Elizabeth, Klerksdorp, George and Johannesburg.
For next year we expect activities to stabilize at the current level. There are several EBA studies in planning and Phase IIB work is likely to be re-initiated by the TBTC on a revised protocol. Otsuka (OPC-67683) and Tibotec (TMC207) have both announced new studies with their compounds in MDR and XDR tuberculosis in Phase II and Phase III. REMoxTB is continuing. Capacity of the laboratory is the current bottleneck for further expansion of activities, and its lack of accreditation a point that needs urgent attention as more and more prospective funders are insisting on independent third party verification of standards. Accreditation is an extremely costly and labour intensive exercise. We see this as a weak point in the centre at present.

Veterinary Tuberculosis

1) Diagnostics

Following the successful adaptation of a human TB test, the QuantiFERON-TB Gold assay, for use in non-human primates, we have continued to investigate its adaptation for use in other species. In particular we are investigating its use for the diagnosis of bovine tuberculosis (BTB) in African buffaloes, lions, and rhinoceroses within the context of the extensive *Mycobacterium bovis* epidemics in the Kruger National Park (KNP) and Hluhluwe iMfolozi Park (HiP).

The African buffalo is the most important maintenance host of *M. bovis* amongst South African wildlife and represents a potential reservoir of infection for other wildlife species, domestic animals, and ultimately humans. As part of an annual buffalo BTB testing program performed in the HiP we showed that an adaptation of the QuantiFERON test was able to detect *M. bovis*-infected animals with at least a similar sensitivity and specificity to the commonly used tuberculin skin test (TST). Indeed, results from this study suggested that the QuantiFERON-TB test showed a greater sensitivity that the TST and this observation will be tested in future studies. Use of the modified QuantiFERON assay could potentially make buffalo BTB testing more sensitive and efficient than is currently the case.

Lions with BTB are known to become severely ill, however it is unknown what effect the disease is having on the lion population of the KNP. There is currently a large lion ecology project under way in the KNP which seeks to address this question; however no practical BTB test for lions exists. A project which was initiated in 2010 aims to develop such a test as a modification of the QuantiFERON-TB assay.

Many rhinoceroses are annually translocated from the KNP and the HiP. While rhinoceroses are not regarded as being particularly susceptible to BTB there are a number of cases of rhinoceroses developing clinical BTB and movement of infected individuals could therefore act to spread the disease. In an effort to prevent such spread, a diagnostic test for BTB in rhinoceroses is vital. As such, we have also engaged in a long-term project to validate a rhinoceros BTB test and to survey 200 rhinoceroses in the KNP in order to quantify the risk of such *M. bovis* transmission.

A modification of a highly practical human tuberculosis (TB) interferon-γ release assay diagnostic test, the QuantiFERON-TB Gold (In-Tube Method) (QFG-IT), was evaluated in one of these studies for diagnosing natural mycobacterial infection in rhesus macaques (*Macaca mulatta*). All animals in a captive colony were tested using the QFG-IT and tuberculin skin test (TST). Animals testing positive to these tests were euthanised and necropsied. Selected tissues were processed for histopathology and mycobacterial culture, and positive cultures were speciated by *Mycobacterium tuberculosis* complex polymerase chain reaction (PCR) and 16S rRNA gene PCR-sequencing techniques. *M.tuberculosis* was cultured from a TST-positive/QFG-IT-positive animal which showed gross pulmonary pathology typical of TB. Additionally, *Mycobacterium kansasii* was cultured from a TST-negative/QFG-IT-positive animal which had no pathological or histopathological signs of mycobacterial infection. The detection of *M.kansasii* infection in a QFG-IT-positive animal which showed no evidence of disease indicates that this test might be a highly sensitive tool for the diagnosis of mycobacterial infection in rhesus macaques. However, these findings highlight the limitations of the QFG-IT to specifically detect infection by the pathogens *M.tuberculosis* and *M.bovis*.

In another study, routine meat inspection of antelope carcasses from a South African game reserve revealed a high prevalence of tuberculosis-like lesions. This study aimed to identify the causative agent of this disease outbreak and to describe the pathological features associated with the disease. In total, 139 animals were randomly harvested from the game reserve and subjected to necropsy inspection. Of these animals, 46 (33%) showed gross visible lesions. Histopathological examination revealed the presence of encapsulated necrogranulomas in organs and/or lymph nodes of 22 of 27 animals tested. Tissue samples from lesions were processed for both non-selective bacterial culture and mycobacterial culture following decontamination. In non-selective cultures of lesions from 25 of 31 animals tested, *Corynebacterium pseudotuberculosis* was detected. Isolation of *C. pseudotuberculosis* was closely associated with the presence of necrogranulomas. In mycobacterial cultures of lesions from 9 of 41 animals tested, different species of non-tuberculous mycobacteria (NTMs) were detected. In 5 instances, depending on the culture procedure that was applied, either *C. pseudotuberculosis* or NTMs were isolated from the same tissue sample. Our results suggested that the disease outbreak observed was caused by infections with *C. pseudotuberculosis*. In sub-Saharan Africa, the role of pathogens other than bacteria of the *Mycobacterium tuberculosis* complex may be underestimated in causing tuberculosis-like lesions. In cases where potentially pathogenic NTMs can be isolated from mycobacterial cultures of tuberculosis-like lesions, the non-use of additional non-selective culture techniques could lead to misinterpretations of diagnostic test results and unnecessary veterinary management outcomes.

2) Whole genome and disease susceptibility work

Two pools of African buffalo DNA were prepared and run on the ABI SOLiD next-generation sequencer, at the University of Liverpool genome centre. Both pools were single comparative intradermal tuberculin test positive, with pool A containing four bovine tuberculosis (BTB) culture positive animals, and pool B containing five BTB culture negative animals. The output data was converted into useable file formats, collated, mapped to the reference genome of *Bos taurus*, (domestic cattle) and annotated using various bioinformatics techniques. This enabled the identification of single nucleotide polymorphisms (SNPs) found in the buffalo genome relative to that of the cow. It also enabled the identification of SNPs that exist in one pool of the buffalo DNA (i.e. infected or non-infected) but not in the other pool. Annotation and further analysis will allow the selection of particular SNPs, genes or pathways of interest for further investigation in our entire African buffalo sample set.

Data Management

As a result of the extensive nature of our centre's data requirements, we have chosen to manage our own information technology systems. We currently have in excess of 110 computers in the centre, including 5 Linux-based servers which supply the infrastructure requirements of staff and students involved in our many projects. All these systems are developed and maintained in-house by Dr G van der Spuy. Amongst the various services provided by the server platforms are two key components.

The first is the provision of a data backup system which daily backs up all data stored on all the personal computers as well as the data servers in the centre. This data is archived at various intervals for one year and currently comprises 0.5 terra-bytes of compressed data. The entire backup is also mirrored on a second server outside the centre.

Secondly, many of our projects generate large quantities of often complex data. In order to manage this data and facilitate its integration and analysis, we have implemented a number of open source-based databases with web-based interfaces. Several of these systems are also responsible for managing entire projects, besides the data collection process. We currently have eight data management systems running or in development, three of which directly service international collaborative projects and collect data from a number of study sites in Africa.

2. Education and Training

Breakdown of postgraduate students and postdoctoral fellows in the CBTBR in 2010

Student category	Number/percentage	Target based on SLA4 (for Performing Phase, 2009-2010)
Total number of students	67	≥ 25
% Postdoctoral fellows	19%	≥10%
% PhD students	33%	N/A
% MSc students	36%	N/A
% BSc (Hons) students	12%	N/A
% Women students	64%	≥ 50%
% Black students	51%	≥ 50%

Degrees conferred and postdoctoral fellowships completed

The CBTBR graduated 4 postdoctoral fellows, 2 PhD, 6 MSc, and 7 Honours students in 2010.

Dissertations and theses

MSc dissertation:

1. Krupa Naran – 'Characterization of the anitmycobacterial effect of a Pseudomonas-derived activity""
2. Lindsey Adams - "Investigating the roles of CTSZ, MC3R and MC4R in host susceptibility to tuberculosis".
3. Claudio Laisse - "Characterization of tuberculous lesions in naturally infected African buffalo (*Syncerus caffer)"*
4. James Mazorodze -"Mycobacterium tuberculosis, a major threat to South African health: intracellular survival after treatment with novel drugs designed against the mycothiol pathway".
5. Michelle Smit - "Investigation of the ESX-4 secretion system interactome of *Mycobacterium tuberculosis".*
6. Muneeb Salie – Investigating candidate genes identified by genome-wide studies of granulomatous diseases in susceptibility to tuberculosis: ANXA11 and the CADM family"

PhD theses:

1. Kim GP Hoek – "Investigation into Genotypic Diagnostics for *Mycobacterium tuberculosis"*

Research interns (MRC sponsored)

1. Ms NC Ngombane (Research Intern) Registered for 2nd year of MSc degree in 2010
2. Ms P Seepe (Research Intern) Registered for 2nd year of MSc degree in 2010

Recruitment of new postgraduate students

A number of new students have joined the team already or will do so during the course of 2010. Applications from other students are under consideration, pending availability of supervisory capacity, laboratory and office space and/or funding, including bursary support (see above). At the SU node, we enrolled 2 Postdoctoral fellows, 7 PhD, 10 MSc and 8 Honours students into the CBTBR in 2010. Given the changes occurring in the Wits node with the transfer of Prof. Mizrahi and Dr. Warner to UCT, the emphasis in 2010 was on supporting existing students and ensuring that projects were brought to completion rather than on the recruitment of new students. Therefore, only one MSc student and one Honours student were recruited to the Wits node in 2010.

Honours and awards to students

- Ms. Anastasia Koch won the Discovery Clinical Excellence Award for Track 1 – Basic Sciences for the presentation that she gave at the 2nd SA TB Conference, Durban, 2-5 June 2010
- Ms. Krupa Naran and Ms. Anastasia Koch were awarded bursaries from the FEBS Youth Travel Fund to attend the EMBO-FEBS Lecture Course on Host-Microbes Interactions, held in Spetses, Greece, in September, 2010. Ms. Anastasia Koch won the prestigious Poster Prize at this Lecture Course. Interestingly, this prize was won by her supervisor, Dr. Warner, when he attended the same course when it was last held in 2006. What makes this award of Ms. Koch's even more noteworthy is the fact this course was targeted at doctoral and postdoctoral students, and Ms. Koch is only a Masters student.
- Ms. Bintou Ahidjo Ahmadou won the Best Young Researcher prize in the Infectious Diseases category, Faculty of Health Sciences Research Day and Postgraduate Expo, Wits University
- Ms. Atica Moosa won the Poster Prize in the Infectious Diseases category, Faculty of Health Sciences Research Day and Postgraduate Expo, Wits University
- Dr. Cliff Magwira was awarded a Global Infectious Diseases Research Training Program fellowship for short-term training in the laboratory of Dr. Petros Karakousis at Johns Hopkins University.
- Ms. Duduzile Ndwandwe was awarded a Columbia University-southern African Fogarty AITRP pre-doctoral training grant for short-term training in TB Basic Sciences in the laboratories of Prof. Eric Rubin and Dr. Sarah Fortune at the Harvard School of Public Health.
- Ms. Bintou Ahmadou Ahidjo's attendance at the NITD Symposium on Tuberculosis 2010 in Yaounde, Cameroon was sponsored in full by a travel award from the Novartis Institute of Tropical Diseases (NITD), Singapore.
- Dr. Monique Williams, Ms. Atica Moosa, Ms. Léanie Kleynhans, Ms. Natalie Bruiners and Mrs Margareta de Vos were awarded Keystone Symposia Global Health Travel Awards to attend the Keystone Symposium on Mycobacteria to be held in Vancouver in January 2011.
- Dr RJ Keyser (postdoctoral researcher) was awarded an Oppenheimer Memorial Trust grant to spend 2 months (February-March) overseas in the laboratory of Dr S Lesage and Prof A Brice in Paris, France.
- Masters student James Hove Mazorodze received a Fogarty Fellowship for a research training visit for 3 months (July-September 2010) to the Lab of Prof William R. Jacobs at the HHMI in New York, USA.
- Masters student Muneeb Salie received a Fogarty Fellowship for a research training visit for 3 months (October-December 2010) at the Public Health Research Institute (PHRI) in New Jersey, USA.
- Doctoral student Sureta Fortuin received a Fogarty Fellowship for a research training visit for 6 months (January-June 2011) at the Public Health Research Institute (PHRI) in New Jersey, USA.
- Doctoral student Andile Ngwane received a Fogarty Fellowship for a research training visit for 3 months (September-December 2010) at the Public Health Research Institute (PHRI) in New Jersey, USA.
- Doctoral student Sven Parsons received a Fogarty Fellowship for a research training visit for 6 months (May-October 2010) at the Public Health Research Institute (PHRI) in New Jersey, USA
- Postdoctoral student Gail Louw received a Fogarty Fellowship for a research training visit for 3 months (September-December 2010) at Harvard University, USA.
- Masters student James Hove Mazorodze visited the Lab of Dr Chantal de Chastellier at INSERM in Marseille, France during the month of October 2010.

- Immunology research group study clinician Daleen Kriel was awarded a Novartis bursary to complete a 2 year MSc in Clinical Epidemiology and Biostatistics at SUN and her research project and study involvement will be TB research.
- Honours student Mr Kabengele Keith Siame won the Poster Presentation Prize in the Basic Sciences category, Faculty of Health Sciences Academic Year Day 2010 Stellenbosch University.

Hosting of international exchange students

The SU node hosted Mr Sebastian Knackstedt in the first quarter of 2010 on a three month internship in order to learn new techniques and gain insight into the everyday working habits of the CBTBR. In the 3rd quarter of 2010 the CBTBR was visited by Michel Labohm an electrical engineer (PhD candidate, Netherlands) and Rick Henrikson a chemical engineer (Ph.D. candidate at the UC Berkeley/UC San Francisco USA) for 2 months to work on a nanotech device for diagnosis of XTR-TB. Hopefully this technology will be developed further.

Molecular Epidemiology Course

Prof. Rob Warren ran Molecular Epidemiology courses for African/Asian/South America fellows. In 2010 trainees from Tanzania (Mr A Busiry, Ms B Mehaki) and S. Herron (CCTR) and for postgraduate students at the Honours level from the faculty of Health Sciences. This was a comprehensive course where all participants had hands-on experience for the extraction of DNA from *Mycobacteria tuberculosis*, restriction enzyme digests, southern blotting, probe labelling and hybridisation. The course equipped all participants with the necessary skill to enable them to perform world class DNA fingerprinting. The participants from Africa and Asia also received a comprehensive manual and components such as standards, probes and internal markers to ensure that they could immediately begin DNA fingerprinting in their respective countries.

Tibotec Training courses

Ms Amour Venter conducted training for Tibotec Inc. on the Microbiology Manual and trial procedures of study protocol TMC207-C209 at one more clinical site. The training involved laboratory procedures for processing of tuberculosis specimens which are multi-drug resistant. The laboratory personnel as well as site monitors were trained on the trial procedures. Training was done during May 2010 at KEMRI, Centre for Respiratory Disease Research, Nairobi, Kenya.

Amour Venter also conducted initiation training for the laboratory personnel involved with clinical drug trials on the following protocols:

- Global Alliance Protocol TMC207-CL001; 08 April 2010
- REMoxTB updated; 08 April 2010
- Quality Control Procedures; 08 April 2010
- Clinical Drug Trials Overview; 09 September 2010
- TBTC Study 29; 09 September 2010
- Global Alliance Protocol NC-001-(J-M-Pa-Z); 09 September 2010
- PanACEA Protocol LMU-IMPH-SQ109-01; 08 December 2010

Training courses attended by staff and students

- Suereta Fortuin, Marieta Burger, Louise Vos, Keith Siame, Nastassja Steyn, Natalie Bruiners, Mae Newton-Foot, Zhuo Fang, Michelle Smit attended a Bioinformatics Course offered by Gene Diagnostics.
- Various members of staff and students attended the MERCK Laboratory Safety and Chemical Grades course in April 2010.
- Masters student Melanie Grobbelaar and Doctoral student Margaretha de Vos attended a short course for Next-Generation sequencing and Transcriptomics at SANBI (June 2010), University of the Western Cape.
- Doctoral student Margaretha de Vos attended the short course “Analysis of Next Generation Sequencing data and functional annotation” held at the University of Cape Town in December 2010.
- Prof. Nico Gey van Pittius, Dr. Novel Chegou, Ms. Léanie Kleynhans and Ms. Nelita du Plessis attended the ‘Introduction to Biostatistics Course’ hosted and presented by the Biostatistics Unit of the South African Medical Research Council, 30 August – 1 September 2010.
- Various members of staff and students attended the SafeNet (AFRICA) – Fire Fighting and First Aid Training courses in October 2010.
- Dr. Novel Chegou and Dr Daleen Kriel participated in the ‘Training and Educational Workshop in Immunology and Cell Biology for PhD students and young postdocs in the AE-TBC project’ at the Max-Planck-Institute for Infection Biology (MPIIB) in Berlin, Germany, 11-12 November 2010.

- Liesel Muller and Kim Stanley attended the SANAS (South African National Accreditation System) Requirements for ISO accreditation - New Applicants Workshop in December 2010.
- Ms Liesel Muller attended the Accreditation training workshop in 2010.
- Ms Laurianne Loebenberg (PhD), Ms Karien Viljoen, Ms Belinda Kriel, Ms Angela Menezes, Ms Nelita du Plessis (PhD) and Ms Leanie Kleynhans attended the Basic and advanced flow cytometry workshop in 2010.
- Dr Daleen Kriel attended the Chest Radiology Review System Intensive Training Course at UCT (Prof. Dheda's TESA site).
- Dr Novel Chegou attended the Research Presentation skills at Stellenbosch University in 2010.
- Dr Daleen Kriel, Dr Andre Loxton, Ms Suereta Fortuin (MSc), Ms Laurianne Loebenberg (PhD) attended the Statistica and Grant writing workshop (cofounded by UCT and SUN from TESA budget) in 2010.
- Ms Belinada Kriel, Ms Angela Menezes, Ms B Thompson, Ms Hannique Human, Dr Novel Chegou, Dr Andre Loxton, Ms Nelita du Plessis and Ms Kim Stanley attended the PBMC workshop in 2010.
- Doctoral student Nikki le Roex visited the lab of Dr Harry Noyes at the University of Liverpool, UK during the month of October 2010, to participate in Next Generation Sequencing.
- Dr Natalie Roetz attended a Wellcome Trust Advanced Course on Genomic Epidemiology in Africa held in Kilifi, Kenya, from 28th November to 3rd December 2010.

Staff members studying for higher degrees (all registered at SU)

- Cedric Werely (PGWC) is a 5th year PhD student, working on Arylamine N-acetyltransferase genes in Tuberculosis to study the influence of host genetics on disease susceptibility.

Other capacity development activities

- The Wits node's commitment to transformation is reflected in the fact that the majority of postdoctoral fellows and postgraduate students who were trained in the unit in 2010 are from previously disadvantaged backgrounds.
- The Departmental allocation from Prof. Mizrahi's HHMI grant was used to provide BSc Hons bursaries to four students (Ms. Kealeboga Mokolobate, Mr. Dale Liebenberg, Ms. Michelle Robinson and Ms. Raees Khan) and support the running costs of these students as well as four others (Ms. Suzanne Nicholson, Mr. Greg Meyer, Ms, Ryan Tucker, Ms. Gabi Bradshaw, and Ms. A. Papadopoulos). In addition, travel support for conference attendance was provided to one student (Ms. S. Saayman). In addition, funds were used to partly support the travel costs for attendance by 10 department students at the SASBMB conference in Bloemfontein.

Exchange visits and strengthening of collaborative linkages

- Dr. Garth Abrahams (postdoctoral fellow) spent 3 months (May-July) working at the NIAID (NIH) I the laboratory of collaborator, Dr. Clifton E. Barry. Dr. Abrahams worked under the supervision of former MMRU member, Dr. Helena Boshoff, on a project supported by the IMTB grant from the Bill & Melinda Gates Foundation.
- Dr. William R. Bishai, a leading TB researcher from Johns Hopkins University in Baltimore, USA, and newly appointed director of K-RITH, visited the MMRU several times during the year. He met with staff and students and the MMRU and with other Wits researchers with an interest in TB/HIV research
- Long-time collaborator, Prof. Gilla Kaplan (PHRI, New Jersey) also visited the MMRU in August, where she discussed ongoing and future collaboration with Prof. Mizrahi and Dr. Kana.
- Prof. Gilla Kaplan (Public Health Research Institute Center, International Center for Public Health, New Jersey, USA) – October - To meet with various members of the Department and foster collaborations.
- Prof A Steyn, Univ Alabama, USA - Discussions on the mycothiol and ergothioneine pathways.
- Prof B Bishai, USA - Discussions on TB research in South Africa and collaboration with the HHI HIV/TB institute in Natal.
- Dr C de Chastellier, INSERM, France – November - Discussions on EM and Mycothiol pathway.
- Dr RJ Keyser (postdoctoral researcher) spent 2 months (February-March) working in the laboratory of Dr Suzanne Lesage and Prof Alexis Brice at INSERM UMR/S 679, Pitié Salpêtrière Hospital, Paris, France.
- Dr Karen Jacobs,Harvard University, USA - Visited twice to discuss collaborative work on drug resistant TB.
- Prof Eric Bottger visited to discuss existing collaborative work.

International Atomic Energy Agency (IAEA)

The International Atomic Energy Agency (IAEA) awarded eight candidates a two-month fellowship to be trained in theory and practical techniques in molecular diagnostic methods of communicable diseases. The emphasis was on the application of these techniques for the detection of mutations in genes associated with drug resistance in TB. The following candidates were trained in 2010: Ms Betty Mehaki and Mr Ally Busiry from Tanzania.
For the past 10 years we have been involved in development and transfer of molecular technology to various countries in Africa through funding mostly from the IAEA. In this initiative we use tuberculosis as a model disease to transfer molecular technology. Our involvement includes planning and report meetings with IAEA (Prof. T Victor), the running of training workshops in Africa and the hosting of African Fellows for training purposes. Advice is given electronically to numerous participating countries and 24 students were trained between 2004 and 2010. The technologies and experience have helped other countries in Africa to get a better understanding of tuberculosis, and the value of this initiative is shown in collaborative papers.

3. Knowledge Brokerage

The operational environment

Both nodes are actively involved in the sharing of knowledge amongst researchers within the CBTBR through lab meetings held at least weekly. Journal Club meetings, held weekly at both sites, also provide an opportunity to share broader-based scientific issues and ideas within the field of biological sciences. We also attend numerous local and international conferences, often as invited speakers, where we share our work with the international community. Prof. Van Helden, Prof. Warren and Prof. Mizrahi were involved in organising and participating in two international workshops on TB held in Pretoria in March 2010 – the NIAID *US-Southern Africa Joint Research Forum on Tuberculosis*, and the joint Institute of Medicine (IOM) and Academy of Science of South Africa (ASSAf) Workshop on the *Emerging Threat of Drug-Resistant Tuberculosis in Southern Africa: Global and Local Challenges and Solutions*. These meetings brought together world-leading experts in the TB field from the USA and southern Africa. The NIAID workshop was aimed at identifying opportunities for collaboration in South Africa and its neighboring countries, and to explore opportunities for collaboration between southern African countries and the United States. The IOM-ASSAf workshop provided an opportunity for members of the CBTBR to contribute to formulating policy for the management of drug-resistant TB. The summary of the key findings from this workshop is contained in a report released by the IOM and ASSAf on World TB Day, 24 March 2011 (http://www.assaf.org.za/wp-content/uploads/2011/03/final-TB-in-South-AfricaUNEMBARGOED.PDF). We have had numerous meetings and contacts with health authorities, such as W and E Cape Departments of Health, to share with them our findings and the implication of these. These are just some of the bodies we have met with. We have also been invited to assist with adviceto international organisations, such as GATB and WHO.

Knowledge translation to stakeholder groups

CBTBR members were involved in numerous public awareness activities countrywide in 2010:

(i) Public awareness, public engagement, and publicity

- Prof. Warren gave a talk in 2010 on the Molecular Epidemiology of XDR-TB to health care professionals at 2 Military Hospital.
- Prof. Warren gave a talk in 2010 on the Tuberculosis to members of the SANDF at 9 SAI
- Prof. Warren presented two lectures in the MBChB module on Infections and Clinical Immunology in 2010. Title: South African Molecular Epidemiological Data.
- Numerous radio, TV and newspaper interviews locally and abroad. Owing to extreme administrative burden, opportunistic interviews, no accurate records were kept.

(ii) Outreach activities

Prof Corfield has continued her involvement in outreach activities that engage the general public in a greater awareness and appreciation of biomedical science; in the past 12 years, she has received support and encouragement for this work from many different role players and has actively encouraged the participation of others in many events. During 2010, there were several requests to present popular previously developed workshops and exhibits at national, regional and local forums, such as National Science Week, Scifest Africa,

Science Centre school visits, a Netherlands NGO (SEEDS) outreach road show; meetings of lay groups, namely, supporting cross cultural adoptions or persons with certain heart conditions (PACE) and the SA Defense Force's HCT roll-out. The activities variously featured at these events use a workshop "*HIV comes to the Party*", which explains immunology and the virus; a TB exhibit and accompanying workshop ("*The Trouble with TB"*), which emphasises drug resistance and antibiotics; a workshop "*TIK's Tricks*", which highlights the neurophysiology of the drug Tik (methamphetamine); a skin exhibit, "*The Skin you're in*" which looks at the skin in health and disease; a workshop entitled "*DNA Detective; what's in your genes*?", which examines genomics and forensic applications in DNA fingerprinting and "*Enzyme Antics*", which introduces the role of enzymes in digestion and in biotechnology applications. In 2010, in response to public interest in the microbicide Tenofovir, she developed an HIV exhibit which looks at anti-retrovirals and clinical trials.

Highlights in 2010 were activities that empowered others to become involved in furthering public awareness and engagement in biomedicine and in examining the ethical and societal issues raised by new technologies. Two tools that particularly helped accomplish this were the completion of phase two of the Wellcome Trust International Engagement WTIE grant awarded to Prof Corfield (Principal awardee) in partnership with the MTN Sciencentre, Cape Town and the production of a Biotechnology DVD for the Public Understanding of Biotechnology (PUB) initiative of SAASTA (a division of the Department of Science and Technology).

The WTIE project entitled "Catalysing partnerships: the role of science centres as intermediaries between the public and scientists in engagement with biomedical sciences in South Africa", plans to bring science centres and scientists together to make biomedical science issues more assessable to the general public. The first phase, completed in 2009, defined which interventions best engage target audiences (scholars, teachers, the general public). Based on knowledge gained, regional workshops were held at three Science Centres, namely the Cape Town Science Centre, Unizul in KZN and Scibono in Johannesburg. About 30 participants attended each workshop by invitation; those attending represented a mix of scientists from local tertiary and research institutions and from science centres across the region (including science centres in rural areas). Several MRC employees attended these workshops, as well as postgraduate students and staff from MRC Centres/Units in the Western Cape, KZN and Gauteng. As a result of their participation, several new public engagement activities have been/are being developed. The final phase of the project will involve the production of a "how-to-engage" manual and a website.

The second new activity highlighted above was the production of a Basic Biotechnology DVD by Contrast Films which was commissioned by PUB to serve as a resource for high schools to increase awareness and understanding of biotechnology and to explore the ethical issues raised. The DVD featured Prof Corfield presenting four workshops that she developed which look at the science of DNA genetics and forensics, genetically modified organisms, cloning and bioinformatics. The DVD has been distributed to schools and interested persons across South Africa.

During 2010, Prof Corfield was involved in other activities that furthered public awareness of various aspects of science. One of these is the DNA Project, which is a non-profit organisation which seeks to raise awareness of the importance of DNA forensic evidence through many activities. The two facets in which Prof Corfield has been involved are the development of teaching modules for a BSc Hons course in DNA Forensics (currently offered of UOFS) and a workshop, *DNA CSI*, which she has helped develop and has presented to ADT Security staff at their Cape Town training academy. She was also involved in further projects with PUB, including assessing the Basic Biotechnology programme rolled out by the Gateway Science Centre in Umhlanga and she updated several PUB fact sheets (including Biotechnology in Medical Research, Cloning and Stem Cells, DNA Profiling, GMOs).

Several of the events in which she is involved have received media coverage and she has featured on radio interviews about Scifest, the Gauteng WTIE workshop, as well as a "State of the Nation" debate on FM Classic Gauteng "Has the Human Genome Project delivered?" with David Gleeson and Prof Michelle Ramsay. The Star carried a feature on the Murder Mystery event that she developed to look at the issues raised by DNA profiling.

TB, HIV, DNA, TIK, ENZYME and BIOTECHNOLOGY WORKSHOPS and TB and SKIN EXHIBITS

During the course of 2010, the six workshops "*The Trouble with TB*", "*HIV comes to the party*", "The *DNA Detective, what's in your genes?*", "*TIK's Tricks*", "*Enzyme Antics*" and "*Basic Biotechnology*" and exhibits "*The Trouble with TB*" and "*The Skin you're in*" developed by Prof Corfield were presented at a number of venues across the country, not only by her, but also by others who have been trained in the past, or in the year of this report. The workshops in which members of SU node of the CBTBR were involved are detailed below:

20-21 January assessment for PUB of Gateway Umhlanga Basic Biotechnology activities for high school learners

Late January: three day communication skills for BSc Hons students in Dept Medical Biosciences

16-17 March: WTIE workshop at Cape Town Science Centre

24-30 March: Scifest Africa workshops *DNA Detective* and *TIK's Tricks* and the *Trouble with TB* exhibit

17 April: lead discussion for cross-cultural adoption lay group on the role of genetics

19-23 April: series of workshops (DNA, TIK, HIV) for schools in socio-economically depressed areas of the Western Cape funded by Dutch NGO SEEDS, run in partnership with Cape Town Science Centre – trained their facilitators to present in mother-tongue

19-20 May: WTIE workshop at Unizul Science Centre (Richard's Bay)

21 May: DNA genetics workshop to PACE lay group (Prevent Cardiac Arrhythmic Events) – persons and families of persons who have suffered arrhythmic events

25 May: Oral Communication Skills workshop University of Stellenbosch

16 July presentation on Careers in Health Care for bridging course students at University of Stellenbosch

28 July: meeting at SAASTA Pretoria to discuss rollout of Basic Biotechnology workshops at national science centres

2-7 August: National Science Week, workshops and exhibits manned by Prof Corfield, Mrs Benita Mayosi and Mrs Debbie Railloun of Research Translation Office of the MRC and several students from SU node of the CBTBR.

18 August: *HIV comes to the party* presented to SA Defense Force 9th Infantry Battalion Eersterivier as part of national HIV counselling and testing (HCT) roll out

7-8 September: WTIE workshop at Scibono Science Centre, Johannesburg

13 September: University Research Development Office workshop to look at issues of fraud in Research

17 November: Oral Communication Skills workshop University of Stellenbosch

Late November-mid-December: 8 *DNA CSI* workshops presented to ADT security staff

Interactions with National Department of Education (DOE)
There was no direct contact with the DOE in 2010, but Prof Corfield presented basic biotechnology workshops to educators under the PUB programme and the SEEDs workshops and via the Scifest and Nat Sci week to students which represent DOE schools.

4. Networking

Existing networks and linkages

Both nodes of the CBTBR are involved in wide collaborative networks that involve TB researchers and research institutions in a large number of countries. Maintaining existing collaborative networks and developing new linkages is of critical importance to the CBTBR. For this reason, members continued to devote significant time and effort to networking.

New networks and linkages

The Wits node became a participant in a 24-member consortium of research teams from Europe, South Korea, India and South Africa, led by Prof. Stewart Cole (EPFL, Switzerland) that was awarded for funding for 2011-2014 for a TB drug discovery project under the EU FP7 program. This grant has been transferred to UCT and will be run by Prof. Mizrahi and Dr. Warner.

NAME	INSTITUTION	NATURE/ PURPOSE, OUTPUTS AND FUTURE DIRECTION OF COLLABORATION
Prof. John D. McKinney	École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland	Collaboration on the mechanisms of propionate catabolism, co-author on a manuscript published in 2008 and joint awardee (with Prof. Mizrahi) on a four-year grant from Swiss/ SA Joint Research Programme which was transferred to UCT in January 2011.
Dr. Clifton E. Barry III and Dr. Helena Boshoff	Tuberculosis Research Section, Laboratory of Host Defenses, National Institute of Allergy & Infectious Diseases, NIH, Rockville, MD	Collaborating members of the IMTB Consortium funded through SBRI. Hosted Dr. Garth Abrahams during his 2-month working visit to the NIAID in 2010
Prof. Gilla Kaplan	Public Health Research Institute, International Center for Public Health, Newark, NJ	Prof. Kaplan serves the international member on the Board of the CBTBR. She and Prof Mizrahi jointly run the TB Basic Sciences component of the CU-SA Fogarty AITRP. Visited the MMRU in August 2010
Prof. Česlovas Venclovas	Institute of Biotechnology, Vilnius, Lithuania	Ongoing collaboration on the structure and function of a novel mutagenic complex in mycobacteria. Co-author on 2010 publication in PNAS
Prof. Heini Dirr	University of the Witwatersrand	Ongoing collaboration on the expression and structure determination of members of a novel mutagenic complex in mycobacteria
Prof. Eric Rubin	Microbiology and Immunology, Harvard Medical School, USA	Collaborating member of the IMTB Consortium
Prof. David Sherman	Seattle Biomedical Research Institute, USA	PI of the IMTB Consortium
Prof. James Sacchettini & Dr. Tom Ioerger	Biochemistry and Biophysics, Texas A&M University, College Station, TX	Collaborating members of the IMTB Consortium. Also collaborating on whole-genome sequence analysis of strains of *M. tuberculosis*. Co-authored one publication in 2010
Prof. Sir Tom Blundell and Prof. Chris Abell	Cambridge University, UK	Collaborating members of the IMTB Consortium . Collaboration to be strengthened through joint participation in both the MM4TB and HIT-TB consortia
Prof. Chris Sassetti	University of Massachusetts, USA	Collaborating member of the IMTB Consortium
Prof. Tanya Parish	Barts and the London, UK & Infectious Diseases Research Institute (IDRI), Seattle, USA	Collaborating member of the IMTB Consortium
Prof. Stewart Cole	EPFL, Lausanne, Switzerland	PI of the *More Medicines for Tuberculosis* (MM4TB) Consortium, approved for funding under EU FP7
Prof. Vickery Arcus	AgResearch, University of Waikato, New Zealand	Collaboration on the role of VapBC toxin-antitoxin modules in the physiology of *M. tuberculosis*

Prof. Gregory Cook	University of Otago, Dunedin, New Zealand	Collaboration on the role of VapBC toxin-antitoxin modules in the physiology of *M. tuberculosis*
Prof. Petros Karakousis	Johns Hopkins University, Baltimore	Nucleotide pool determination in mycobacteris. Hosted Dr. Cliff Magwira for short-term training fellowship in 2010
Dr. S. Sampson	Imperial College, UK	The evolution and function of the PE and PPE gene families (2001-present) & the ESAT-6 secretion system interactome (2007- present).
Dr. H. Mardassi, Mr. A. Karboul and Mr. A. Namouchi	Institut Pasteur, Tunisia	Characterization of *M. tuberculosis* lineages through the PE/PPE gene family (2002 - present)
Dr. W. Bitter and Mr A. Abdallah	Vrije Universiteit, Amsterdam, Netherlands	The trafficking of the *M. tuberculosis* PE and PPE proteins (2006 – present).
Dr. John Ho	Cornell University, New York, USA	Characterization of *M. tuberculosis* lineages through the PE/PPE gene family (2007 – 2009).
Prof. J. Ho, Dr. A. Gibson and Prof. R. Huard	Cornell University, New York, USA	The dissemination of the major RDRio sub-lineage of the LAM *M. tuberculosis* spoligotype family in Luso-American countries, Portugal and Africa
Dr. H. Mardassi	Institut Pasteur de Tunis, Tunisia	Characterisation of LAM evolutionary history (2007-present).
Prof. A. Steyn	University of Alabama, Birmingham, USA	The ESAT-6 secretion system interactome (2007-present).
Prof. Dr. VPMG Rutten, Dr. I. van Rhijn, Dr. A.P. Koet	Utrecht University	Non-tuberculous mycobacteria in wildlife (WOTRO Integrated program proposal) (2007 - present).
Dr. R. Anthony	KIT The Netherlands	MLPA assay for the detection of ofloxacin resistance
Prof. D. van Soolingen	RIVM The Netherlands	Evolution of the Beijing genotype Lineage
Dr. K. Kremer	RIVM The Netherlands	Whole genome sequencing of Beijing genotype strains
Dr. V. Dartois	Novatis Singapore	MassArray detection of mutations conferring drug resistance
Prof. E. Böttger	University of Zurich	Development and evaluation of novel genetic based diagnostics for drug resistance.
Prof. E. Böttger	University of Zurich	Evolution of ofloxacin resistance
Prof. E. Nardell	AIR facility, Witbank	Transmissibility of drug resistant TB
Prof. Erwin Schurr	McGill University, Montreal, Canada	Genetic epidemiology. Poster outputs; 4 papers published 2009-2010.
Prof. Laurent Abel & Alexandre Alcais	INSERM / Université Paris 5, France	Analysis of genetic epidemiology. Poster outputs; 4 papers published 2009-2010.
Dr. Iris Grossman	GlaxoSmithKline, NC, USA	Genetic susceptibility to TB.
Dr. Alkes Price	Harvard School of Public Health, Boston, USA	New collaboration. Analysis of admixture mapping.
Dr. Ingileif Jonsdottir	deCODE, Iceland	Genetic susceptibility to TB.

Dr. Lluis Quintana-Murci	Institut Pasteur, Paris, France	Genetic susceptibility to TB and population structure. Paper expected 2010.
Prof. Stefan Schreiber and Dr. Almut Nebel	Christian Albrechts University, Kiel, Germany	Investigation of candidate genes in TB. Resulted in 2 co-authored publications in 2007, and 2 co-authored publications in 2009
Prof .Megan Murray	Harvard / Broad institute	Various project including the evolution of XDR-TB strains; other mechanisms of drug resistance (in addition to genomic mutations); mechanisms of resistance to 2nd line drugs; strain fitness; certain strain families may have both increased fitness and increased potential for acquiring drug resistance. All of these projects involve whole-genome sequencing, proteomics, microarray. Prof. Murray is directly involved in project planning, paper writing, funding proposals (NIH and Wellcome trust).
Dr. Judit Nagy	Imperial College London	Proteomics of large clusters (more transmitted) vs. small clusters (less transmitted) in the same strain family after other criteria to select isolates have been taken into consideration. The aim is to identify proteins that are differentially expressed in the same strain family which may give them an advantage to transmit better than others.
Prof. Harald Wiker	Bergen University, Norway	Ongoing collaboration on the *M. tuberculosis* phosphorylome New collaboration on the detection of drug resistance by single run multi-locus sequencing. New collaboration on the *M. tuberculosis* secretome.
Dr. Kristin Kremer	RIVM, The Netherlands	Ongoing collaboration on *M. tuberculosis* adaptation.
Dr. Hernandez Pando Rogelio	National University of Mexico	Test different drug resistant strains (MDR / XDR) in a mouse model for strain fitness/virulence. The isolates are the same as described above and will compliment the data obtained by molecular investigations. To determine whether reinfection induces reactivation.
Dr. Helen Cox	MSF	New collaboration on drug resistance in Khayelitsha, Western Cape. Impact of mixed infection on treatment outcome.
Prof. Tom Alber	Berkeley	New collaboration on the *M. tuberculosis* lipidome.
Prof. Brigitte Gicquel	Pasteur Institute	New collaboration on mutation in *M. tuberculosis* DNA repair genes
Prof. K. Dheda	UCT	Molecular epidemiology of XDR-TB
Prof. R. McNerney	LSTHM	Whole genome sequencing of drug resistant M. tuberculosis strains
Dr. Kim Mallard	LSTHM	Mixed *M. tuberculosis* infection in Malawi
Prof. Kathy Eisenach	Arkansas, USA	Mechanisms of strain fitness in an in vitro THP-1 cell line model. Project is in planning phase.
Prof. Stefan Kaufmann	Max Planck Institute for Infection Biology, Berlin, Germany	Collaborators on BMGF-funded project.
Prof. Henry Boom	Cleveland, Ohio, US	Collaborators on BMGF-funded project.

Prof. Hazel Dockrell	London School for Hygiene and Tropical Medicine, London, UK	Collaborators on BMGF-funded project, Co-applicants on grant application to BMGF.
Dr. Mark Doherty	Statens Serum Institute, Kopenhagen, Denmark	Collaborator on BMGF-funded project, collaborators on NIH-sponsored study
Dr. Martin Ota	MRC, The Gambia	Collaborators on BMGF-funded project.
Prof. Harriet Mayanja	Makarere University, Uganda	Collaborators on BMGF-funded project.
Prof. Willem Hanekom and Dr. Hassan Mahomed	SATVI, UCT	Collaboration on TB vaccine studies
Dr. Carol Holm-Hansen	Norwegian Institute for Public Health	Collaboration on BMGF Grand Challenge Exploration grant, 2010-2011
Dr. Christoph Lange and Dr. Barbara Kalsdorf	Clinical Infectious Diseases, Center for Clinical Studies, Medical Clinic, Research Center Borstel, Germany	Collboration on TB diagnostic study 2011
Dr. Jeff Boyle	R&D, Cellestis, Australia	Collaboration on diagnostic TB study 2010-2011
Dr. Volkmar Schoellhorn	Auto-Immune Diagnostics (AID)	Collaboration on TB diagnostic study 2010-2011
Prof. Ronnie Anderson & Dr. Caroline Cholo	MRC/ UP Unit on Inflammation & Immunity, University of Pretoria	Collaboration on the role of potassium transport in mode of action of anti-tubercular action of riminophenazines.
Prof N. Beyers, Dr A. Hesseling, Dr S. Tonkin, Prof B. Marais	SU	Non-tuberculous Mycobacteria (NTM) - Prevalence and Clinical relevance in HIV-infected and HIV-uninfected children (2006 - present).
Prof. N. Beyers	DTTC, SU	Ongoing collaboration of the molecular epidemiology of *M. tuberculosis* in the W. Cape.
Dr. A. Michel, J. Godfroid, K. Coetzer and N. Kriek	Onderstepoort Veterinary Institute	Non-tuberculous mycobacteria in wildlife (WOTRO Integrated program proposal) (2007 - present).
Dr. Mary Jackson	Colorado State University	Screen anti-TB compounds against RIF-resistant *M. tuberculosis* strains.
Dr. Dorian Bevec	MondoBiotech, Switzerland	Screen peptides for anti-TB activity.
Prof. Kelly Chibale	Dept Chemistry University of Cape Town	Screen antituberculosis lead compounds
Dr. Corli Witthuhn	Food Science, SU	Fermentation Processes to kill *M. Tuberculosis*
Dr. Thavi Govender	Dept. Chemistry, UKZN	Test antituberculosis activity of existing antituberculosis drug derivatives. K. Onajole 2009
Prof. Green	Dept Chemistry, UWC	Screen new compounds and derivatives for antituberculosis activity
Dr. S. Todorov	Univ. Sao Paulo, Brazil	Antituberculosis activity of Bacteriocins Todorov, 2008
Dr C. Kenyon	CSIR, Pretoria	Dormancy regulators of M.tb in human macrophages.
Dr. Haynes	Hong Kong University of Technology	Testing new compounds for antituberculosis activity

Prof Peter Folb	Pharmacology, UCT	Testing derivatives of Diphenyl Oxazole for antituberculosis activity
Ms. Marlein Bosman	NHLS , Green point	Collaborator on all our projects – provides routine samples.
lily telisinghe, Dr Salome Charalambous	Arum Health	TB in the correctional services
Dr. Gerrit Coetzee	NHLS	Drug resistant TB in South Africa
Dr. Sias May	TB Control program in Suidkaap/ Lawwaaikamp	TB Control strategy.
Dr. Danie Theron	Eben donges hosp, Worcester	New project on DOTS program on farms.
Prof C. Reinecke & Dr du Toit Loots	North West University	*M.tuberculosis* metabolome.
Prof. C. Wright	NHLS Tygerberg	The diagnostic utility of FNAB
Dr. Alistair Calver	Gold Mine in Northern province	Ongoing, outbreak of drug resistance in a setting with a good control program.
Prof. Willem Hanekom	IIDMM, UCT	Sharing of technology (multicolour FACS, Luminex machine), sharing of samples, manuscript accepted for publication.
Prof. Frank Brombacher	IIDMM, UCT	Sharing of expertise (murine helminth models).
Dr A. Hesseling	SU	New collaboration to investigate genotype-immunological phenotype correlations in children.
Mrs. Lungi Kwitshana	MRC, Durban	Collaboration in project on worm-HIV co-infection.
Prof. Keertan Dheda	Lung Institute, UCT	Collaboration in diagnostic/biomarker project.
Dr. Anna Mandalakas	Case Western Reserve University, USA	Collaboration of diagnostic studies in paediatric TB.
Dr. Marc Jacobsen	Bernhard Nocht Insitute, Hamburg, Germany	Collaboration on helminth/TB co-infection studies.
Prof. Muazzam Jacobs	IIDMM, UCT	New collaboration to assess the impact of steroid hormones on protective immunity to Mycobacterium tuberculosis in a mouse animal model.

5. Service rendering

As per our Business Plan for the current phase of the CBTBR, the following services were provided in 2010:

The provision of scientific/ technical service, advice and assistance to local Government, regional services, institutions, research groups and individuals

Thesis examination

- Dr. Warner reviewed a PhD upgrade proposal and examined a PhD thesis from SU. Drs. Kana and Gordhan reviewed Masters and PhD proposals from Wits.
- Numerous external examinations were done by members of the SU node. These include examining PhD or MSc theses for WITS, Pretoria, UCT, UWC and other universities and Universities of Technology.

Journal editing and reviews

- Prof. Mizrahi served on the Editorial Advisory Boards of the *Biochemical Journal, Tuberculosis*, and the *International Journal of Biochemistry and Cell Biology* and was appointed to the Editorial Board of *Cellular Microbiology,* the pre-eminent journal in the field of host-pathogen interactions with an impact factor of

5.725. In 2010, she also reviewed manuscripts submitted to the following journals: *Cell, Molecular Microbiology, Journal of Bacteriology, Cell Host and Microbe,* and *PLoS Medicine*.

- Dr. Warner reviewed manuscripts submitted to *Molecular Microbiology, Future Medicinal Chemistry, Immunobiology*, *Journal of Bacteriology*, *Antimicrobial Agents and Chemotherapy* and *Nature Communications*
- Dr. Kana reviewed manuscripts submitted to *FEMS Microbiology, Biochimie, African Journal of Microbiological Research* and *DNA and Cell Biology*
- Dr. Gordhan reviewed a manuscript submitted to *Genetics and Molecular Biology*
- Most if not all senior members of the SU node review numerous manuscripts for international journals. Records are not kept, but journals include *Nature Reviews, Lancet, Lancet Infectious Diseases, PLoS, J Antimicrobial Chemotherapy, J Mol Med, BMC, Tuberculosis, IJTLD, JID, J Biotech, IJMS, Indian Heart Journal, Cardiovasc. J SA, J Biotech, IJMS, Molecular Biology and Evolution, Journal of Infection in Developing Countries, Journal of Bacteriology, Journal of Medical Microbiology, American Journal of Respiratory Critical Care Medicine, Tuberculosis and Journal of Molecular Biology and Biotechnology.*

Expert Panel or Committee Membership

- Prof. Mizrahi served on the Scientific Advisory Committee of the Global Alliance for TB Drug Development (New York).
- Prof. Mizrahi served on both the Board of Directors and the Scientific Advisory Board of K-RITH (Washington and Durban).
- Prof. Mizrahi served on the Management Committee of the IMTB Project, SBRI, USA
- Prof. Mizrahi served on the Council of Scientific Advisors of the International Centre for Genetic Engineering and Biotechnology (Trieste, Italy) and was re-appointed for a further three-year term on this committee.
- Prof. Mizrahi served on the Steering Committee of the Columbia University-southern Africa Fogarty AIDS and TB Research Training Program (CU-SA Fogarty AITRP).
- Prof. Mizrahi served on the Scientific Advisory Committee of the EU-funded New Medicines for TB Program (NM4TB) based at the École Polytechnique Fédérale de Lausanne (EPFL, Switzerland).
- Prof. Mizrahi served on the Planning Committee of the NIH/HHMI/SA MRC US-Southern African Research Forum on TB, held in Pretoria, 1-2 March 2010.
- Dr. Gordhan served on the Postdoctoral Fellowship Review Panel of the NRF.
- Dr. Kana served as a judge for the Discovery Health Journalism Awards.
- Dr. Kana chaired the sessions on Tuberculosis and Genomics at the MRC Research Day (Cape Town, October 2010) and judged presentations in these sessions.
- Prof. Mizrahi served on the Executive Committee of the School of Pathology of Wits University.
- Drs. Gordhan and Kana served on the Postgraduate Committee, Faculty of Health Sciences, Wits University.
- Dr Warner was appointed to, and served on the Steering Committee of iThemba Pharmaceuticals, a non-profit organization based in Modderfontein funded by the Department of Science and Technology. This organization, specializes in Medical Chemistry with a strong research focus on tuberculosis drug discovery.
- Dr. Gordhan served on the Postgraduate Assessor Group Committee, Wits Faculty of Health Sciences.
- Dr. Kana served as a judge for the Infectious Diseases Track at the Faculty of Health Sciences Research Day and Postgraduate Expo.
- Profs van Helden and Walzl served on MSF, GATB, WHO and Stop TB Partnership.
- Prof van Helden served on the GMO Advisory Committee for the Dept. Agriculture.
- Prof van Helden served on the Interim Steering Committee of the DST TB Centre of Competence.
- Profs Walzl and Wiid served on the Ethics Committee for Experimental Animal Research of Stellenbosch University.
- Prof Warren served on the Centre for Infectious Diseases of Stellenbosch University.
- Profs Walzl and Warren served on the Research Committee of Stellenbosch University Faculty of Health Sciences.
- Prof Gey van Pittius served on the Committee for Postgraduate Education and Health Research Ethics Committee of Stellenbosch University Faculty of Health Sciences.

Examples of Research Funding Reviews

- Wits node staff participated actively in reviewing proposals submitted to international and local funding agencies/ institutions. Prof. Mizrahi served as a reviewer of proposals for the Wellcome Trust (UK), Wellcome Trust/ Department of Biotechnology (India), The Royal Society (UK), NRF, and CU-SA Fogarty AITRP. She also carried out a programme review for the National Institute of Medical Research (UK). Dr. Warner reviewed an application submitted to the Wellcome Trust (UK), K-RITH and the NRF. Dr. Gordhan reviewed proposals from the NRF and Dr. Kana reviewed proposals from the NRF, K-RITH, the CU-SA Fogarty AITRP, the Wits URC (NRF National Equipment Programme, National Nanotechnology Equipment Programme, Fellowships Committee (Carnegie Programme)) and the Wits Faculty of Health Sciences (Minor Equipment Review Panel).
- Numerous grant application reviews were done by the staff of the SU node for the MRC, NRF and NHLS, as well as other national and international funding organisations by all the senior members of the SU node of the CBTBR. No accurate records are kept, as these are too numerous. International bodies include the Wellcome Trust, Royal Society, Swiss Science Foundation, WOTRO, WHO, Alexander von Humbolt Foundation, European Young Investigator Award for European Science Foundation, Rolex Awards, etc.

Other services rendered

- Speciation of Non-Tuberculous Mycobacteria for Onderstepoort Veterinary Institute & Kruger National Park
- Identification of *M. tuberculosis* in tissue specimens for the NHLS
- Genotyping of clinical isolates (RFLP or mutation detection) for the NHLS.
- Prof V Corfield was NRF rating panel moderator in 2010
- Specialist Diagnostic service for CV diseases listed earlier
- Specialist diagnostic service for MDR or XDR TB cases
- Specialist diagnostic service for suspect extra-pulmonary TB cases
- Hospital medical specialist clinical services, eg pulmonology and genetics
- Advice to National Dept Agriculture on GMOs and veterinary TB
- **Seegene (Korea):** We have been approached and are currently in a collaborative venture with Seegene, Inc. to design and improve *M. tuberculosis* diagnostics. Seegene is a molecular diagnostic company based in Korea well known for its pioneering R & D activities and novel technologies. Seegene, Inc. has continued to develop innovative technology platforms, ACP™ (Annealing Control Primer), DPO™ (Dual Priming Oligonucleotide), and READ™ (Real Amplicon Detection). With these cutting-edge molecular diagnostic technologies applied to diagnostic kits and various practical approaches, Seegene has enhanced the sensitivity and specificity of PCR (polymerase chain reaction) up to unprecedented level. This advance enables Seegene Inc. to provide multiplex PCR products by which multiple target genes of pathogens can be simultaneously detected, saving time and cost.
- **AID (Germany):** Evaluation of a genetic drug susceptibility test.
- **Hain Life Sciences (Germany):** Evaluation of the MDRTBsl genetic drug susceptibility test.

6. Gender impact of research

"Science for Women" (gender-sensitivity of the research agenda)
The work being undertaken in the CBTBR is aimed at contributing towards global efforts in researching and developing new laboratory-based tools for reducing the societal burden of TB. TB is the greatest single infectious cause of death in young women, and causes more deaths among women than all causes of maternal mortality combined. The particularly high rates of HIV co-infection in women are expected to fuel an increased prevalence of TB in women over time. In addition to the disease burden, TB also imposes a massive, but largely hidden burden of social impact on women. The long-term nature of the research projects being undertaken within the CBTBR makes it impossible to assess their gender impact in the short-term.

"Science by Women" (the participation by women in the research programme)
Five out of the 13 Core Team Members of the CBTBR are women. The CBTBR has also maintained a high percentage of female students (64% of all students and 45% of postdoctoral fellows), which is in line with demographic norms for the Life and Health Sciences at a national level. Both nodes have demonstrated that they are able to provide an environment which is attractive to, and supportive of women researchers at all levels, from Honours students to senior postdoctoral fellows and Core Team Members. These indicators confirm that the CBTBR serves as a centre in which women researchers are nurtured and developed.

HUMAN RESOURCES

1. Core Team Members

Title	Surname	Citizenship	Institution	Gender	Race	% Time spent in CBTBR
Prof.	Mizrahi	Italy	Wits/ NHLS	F	W	100
Dr.	Gordhan	SA	Wits	F	B	100
Dr.	Kana	SA	Wits	M	B	100
Dr.	Warner	SA	Wits	M	W	100
Dr.	Gey van Pittius	SA	US	M	W	100
Prof.	Hoal van Helden	SA	US	F	W	100
Dr.	Ronacher-Mansvelt	SA	SU	F	W	100
Dr.	Streicher	SA	SU	F	W	100
Prof.	Van Helden	SA	MRC	M	W	100
Prof.	Victor	SA	PAWC	M	W	100
Prof.	Walzl	SA	US	M	W	100
Prof.	Warren	SA	MRC	M	W	100
Dr.	Wiid	SA	PAWC	M	W	100

2. Postdoctoral Fellows

Title	Surname	Citizenship	Institution	Gender	Race	% Time spent in CBTBR
Dr.	Abrahams	South African	Wits	Male	Black	100
Dr.	Black	British	SU	Female	White	95[a]
Dr.	Esterhuyse	South African	SU	Female	White	90[b]
Dr.	Evans	South African	Wits	Female	White	50[c]
Dr.	Hayward	South African	SU	Male	White	70[d]
Dr	Krishnamoorthy	India	Wits	Male	Black	100[e]
Dr.	Louw	South African	SU	Female	Black	100
Dr.	Magwira	Malawian	Wits	Male	Black	100
Dr.	McEvoy	Australian	SU	Male	White	100
Dr.	Möller	South African	SU	Female	White	100
Dr.	Muller	Swiss	SU	Male	White	100

a. Left the SU node in December 2010
b. Left the SU node in November 2010, to work in Germany
c. Joined the Wits node in July 2010 and will move to UCT in January 2011
d. Left the SU node in September 2010
e. Will move to UCT in January 2011

3. Students

Title	First Name	Surname	Degree	Institution	Race	Gender	Nationality	Status
Ms	Lizaan	Ehlers	Hons	SU	White	Female	South African	Complete
Mr	Paulin Essone	Ndong	Hons	SU	Black	Male	Gabonese	Complete
Ms	Suzanne	Nicholson	Hons	Wits	White	Female	South African	Incomplete
Ms	Khutso Germina	Phalane	Hons	SU	Black	Female	South African	Complete
Ms	Carine	Sao Emani	Hons	SU	Black	Female	Cameroonian	Complete
Mr	Kabengele Keith	Siame	Hons	SU	Black	Male	Zambian	Complete
Ms	Nastassja Lise	Steyn	Hons	SU	White	Female	South African	Complete
Ms	Leani	Thiart	Hons	SU	White	Female	South African	Complete
Ms	Lindsey	Adams	MSc	SU	White	Female	Canadian	Complete
Ms	Philippa	Black	MSc	SU	White	Female	South African	Incomplete

Mr	Zhuo	Fang	MSc	SU	Black	Male	Chinese	Incomplete
Ms	Suereta	Fortuin	MSc	SU	Black	Female	South African	Incomplete
Ms	Melanie	Grobbelaar	MSc	SU	White	Female	South African	Incomplete
Ms	Andrea	Gutschmidt	MSc	SU	White	Female	German	Incomplete
Ms	Anastasia	Koch	MSc	Wits	White	Female	South African	Incomplete
Mr	Claudio Joao Moura	Laisse	MSc	UEM	Black	Male	Mozambican	Complete
Mr	Lance Andrew	Lucas	MSc	SU	White	Male	South African	Incomplete
Mr	Lubabalo	Macingwana	MSc	SU	Black	Male	South African	Incomplete
Ms	Lusanda	Mapela	MSc	Wits	Black	Female	South African	Incomplete
Mr	Thabiso	Masotla	MTech	CPUT/SU	Black	Male	South African	Incomplete
Mr	James Hove	Mazorodze	MSc	SU	Black	Male	Zimbabwean	Complete
Ms	Angela Maria	Menezes	MSc	SU	White	Female	South African	Incomplete
Ms	Zandile	Mlamla	MSc	SU	Black	Female	South African	Incomplete
Ms	Krupa	Naran	MSc	Wits	Black	Female	South African	Complete
Ms	Nicole	Narrandes	MSc	Wits	Black	Female	South African	Incomplete
Mrs	Nokwanda Crystal	Ngombane	MSc	SU	Black	Female	South African	Incomplete
Mr	Muneeb	Salie	MSc	SU	Black	Male	South African	Complete
Ms	Prudy Mashika	Seepe	MSc	SU	Black	Female	South African	Incomplete
Ms	Michelle	Smit	MSc	SU	White	Female	South African	Complete
Ms	Marisa	Tait	MSc	SU	White	Female	South African	Incomplete
Mr	Albertus	Viljoen	MSc	SU	White	Male	South African	Incomplete
Ms	Chandré Kim	Wagman	MSc	SU	Black	Female	South African	Incomplete
Ms	Bintou	Ahmadou Ahidjo	PhD	Wits	Black	Female	Cameroonian	Complete
Dr	Adane Mihret	Bekele	PhD	SU	Black	Male	Ethiopian	Incomplete
Ms	Maria Magdalena	Botha	PhD	CSIR	White	Male	South African	Incomplete
Ms	Natalie	Bruiners	PhD	SU	Black	Female	South African	Incomplete
Mrs	Violet	Chihota	PhD	SU	Black	Female	South African	Incomplete
Ms	Margaretha	de Vos	PhD	SU	White	Female	South African	Incomplete
Ms	Nelita	Du Plessis	PhD	SU	White	Female	South African	Incomplete
Ms	Kim	Hoek	PhD	SU Tygerberg Hosp	White	Female	South African	Complete
Ms	Catriona Jane	Kirsten	PhD	SU	White	Female	South African	Incomplete
Ms	Leanie	Kleynhans	PhD	SU	White	Female	South African	Incomplete
Mrs	Lungile	Kwitshana	PhD	U Kwazulu Natal	Black	Female	South African	Incomplete
Ms	Laurianne	Loebenberg	PhD	SU	White	Female	South African	Incomplete
Ms	Nikki	Le Roux	PhD	SU	White	Female	South African	Incomplete
Ms	Charmaine	Mlambo	PhD	SU/Wits	Black	Female	South African	Incomplete
Ms	Atica	Moosa	PhD	Wits	Black	Female	South African	Incomplete
Ms	Duduzile	Ndwandwe	PhD	Wits	Black	Female	South African	Incomplete
Ms	Mae	Newton-Foot	PhD	SU	White	Female	South African	Incomplete
Mr	Andile	Ngwane	PhD	SU	Black	Male	South African	Incomplete
Mr	Lyndon	Oldfield	PhD	CSIR	White	Male	South African	Incomplete
Ms	Chrisna	Swart	PhD	SU	White	Female	South African	Incomplete
Mr	Ruben	van der Merwe	PhD	SU	White	Male	South African	Incomplete
Mr	Cedric John	Werely	PhD	SU	Black	Male	South African	Incomplete
Dr	Garth	Abrahams	Postdoctoral	Wits	Black	Male	South African	Complete
Dr	Gillian	Black	Postdoctoral	SU	White	Female	British	Complete
Mr	Novel	Chegou	Postdoctoral	SU	Black	Male	Cameroonian	Incomplete

Dr	Marna	Esterhuyse	Postdoctoral	SU	White	Female	South African	Complete
Mr	Joanna	Evans	Postdoctoral	Wits	White	Female	South African	Incomplete
Dr	Krishnamoorthy	Gopinath	Postdoctoral	Wits	Black	Male	Indian	Incomplete
Dr	Don	Hayward	Postdoctoral	SU	White	Male	South African	Complete
Dr	Gail	Louw	Postdoctoral	SU	Black	Female	South African	Incomplete
Dr	Andre	Loxton	Postdoctoral	SU	Black	Male	South African	Incomplete
Dr	Sven	Parsons	Postdoctoral	SU	White	Male	South African	Incomplete
Dr	Cliff	Magwira	Postdoctoral	Wits	Black	Male	Malawian	Complete
Dr	Marlo	Möller	Postdoctoral	SU	White	Female	South African	Incomplete
Dr	Borna	Muller	Postdoctoral	SU	White	Male	Swiss	Incomplete

4. Administrative and Other Staff

Title	Surname	Position	Based at	Gender	Race
Dr	Baker	Project Manager	SU	M	B
Ms	Peachy	Bookkeeper/ Admin. Assistant	WITS	F	B
Dr	Williams[a]	Senior Scientist	WITS	F	B
Dr	Machowski[b]	Senior Scientist	WITS	F	W

a. On a three-year contract appointment with MRC, seconded to SU node in January 2010
b. Emigrated in July 2010
c. Appointed in June 2010
d. Left in July 2010

OUTPUTS

Books / Chapters in Books (Total: 2)

McEvoy CRE, Warren RM, van Helden PD. 2010. Molecular Methods and Their Application in TB Epidemiology. *In*: Schaaf HS, Zumla AI (ed.) *Tuberculosis. A Comprehensive Clinical Reference*. Elsevier Publications. pp 28 – 37.

Dheda K, Warren RM, Zumla A, Grobusch MP. 2010. Extensively drug-resistant tuberculosis: epidemiology and management challenges. Infect Dis Clin North Am 24:705-725.

Articles in Peer-Reviewed Journals (Total: 46)

Warner DF, Ndwandwe DE, Abrahams GL, Kana BD, Machowski EE, Venclovas C, Mizrahi V. (2010) Essential roles for imuA'- and imuB-encoded accessory factors in DnaE2-dependent mutagenesis in Mycobacterium tuberculosis. Proc Natl Acad Sci USA. 107(29):13093-13098. (IF=10.312)

Kana BD, Mizrahi V, Gordhan BG. (2010) Depletion of resuscitation-promoting factors has limited impact on the drug susceptibility of Mycobacterium tuberculosis. J Antimicrob Agents Chemother. 65(8):1583-1585 (IF=4.294)

Ioerger TR, Feng Y, Ganesula K, Chen X, Dobos KM, Fortune S, Jacobs WR Jr, Mizrahi V, Parish T, Rubin E, Sassetti C, Sacchettini JC. (2010) Variation among genome sequences of H37Rv strains of Mycobacterium tuberculosis from multiple laboratories. J Bacteriol. 192(14):3645-3653. (IF=4.035)

Kana BD, Abrahams GL, Sung N, Warner DF, Gordhan BG, Machowski EE, Tsenova L, Sacchettini JC, Stoker NG, Kaplan G, Mizrahi V. (2010) Role of the DinB homologs Rv1537 and Rv3056 in Mycobacterium tuberculosis. J Bacteriol. 192(8):2220-2227. (IF=4.035)

Capron M, Mizrahi V. (2010) Highlights from the first meeting of the Europe-Africa frontier research conference series infectious diseases: from basic to translational research. FEMS Immunol Med Microbiol. 58(1):1-2. (IF=2.352)

Kana BD, Mizrahi V. (2010) Resuscitation-promoting factors as lytic enzymes for bacterial growth and

signalling. FEMS Immunol Med Microbiol. 58(1):39-50. (IF=2.352)

Aguilar D, Hanekom M, Mata D, Gey van Pittius NC, van Helden PD, Warren RM, Hernandez-Pando R. (2010) Mycobacterium tuberculosis strains with the Beijing genotype demonstrate variability in virulence associated with transmission. Tuberculosis (Edinb) 90:319-25. (IF=2.986)

Alexander KA, Laver PN, Michel AL, Williams M, van Helden PD, Warren RM, Gey van Pittius NC. (2010) Novel Mycobacterium tuberculosis complex pathogen, M. mungi. Emerg Infect Dis 16:1296-9. (IF=6.497)

Calver AD, Falmer AA, Murray M, Strauss OJ, Streicher EM, Hanekom M, Liversage T, Masibi M, van Helden PD, Warren RM, Victor TC. (2010) Emergence of increased resistance and extensively drug-resistant tuberculosis despite treatment adherence, South Africa. Emerg Infect Dis 16:264-271. (IF=6.497)

Cilliers K, Labadarios D, Schaaf HS, Willemse M, Maritz JS, Werely CJ, Hussey G, Donald PR. (2010) Pyridoxal-5-phosphate plasma concentrations in children receiving tuberculosis chemotherapy including isoniazid. Acta Paediatr. 99(5):705-710 (IF=1.995)

Cobat A, Gallant CJ, Simkin L, Black GF, Stanley K, Hughes J, Doherty TM, Hanekom WA, Eley B, Beyers N, Jaïs J-P, van Helden PD, Abel L, Hoal EG, Alcaïs A, Schurr E. (2010) High heritability of antimycobacterial immunity in an area of hyperendemicity for tuberculosis disease. J Infect Dis 201:15-9. (IF=5.698)

De Souza GA, Fortuin S, Aguilar D, Hernandez Pando R, McEvoy CRE, van Helden PD, Koehler CJ, Thiede B, Warren RM, Wiker HG (2010). Using a label-free proteomic method to identify differentially abundant proteins in closely related hypo- and hyper-virulent clinical Mycobacterium tuberculosis Beijing isolates. Mol. Cell Proteomics. 9: 2414-2423. (IF=9.606)

Demers A-M, Boulle A, Warren RM, Verver S, van Helden PD, Behr MA, Coetzee D. (2010) Use of simulated sputum specimens to estimate the specificity of laboratory-diagnosed tuberculosis. Int J Tuberc Lung Dis 14:1016-23. (IF=2.442)

Demers A-M, Mostowy S, Coetzee D, Warren RM, van Helden PD, Behr MA. (2010) Mycobacterium africanum is not a major cause of human tuberculosis in Cape Town, South Africa. Tuberculosis (Edinb) 90:143-4. (IF=2.986)

Dheda K, Shean K, Zumla A, Badri M, Streicher EM, Page-Shipp L, Willcox P, John M-A, Reubenson G, Govindasamy D, Wong M, Padanilam X, Dziwiecki A, van Helden PD, Siwendu S, Jarand J, Menezes CN, Burns A, Victor TC, Warren RM, Grobusch MP, van der Walt M, Kvasnovsky C. (2010) Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study. Lancet 375:1798-807. (IF=29.443)

Dheda K**,** Warren RM**,** Zumla A, Grobusch MP**.** 2010. Extensively drug-resistant tuberculosis: epidemiology and management challenges. Infect Dis Clin North Am 24:705-725. (IF=2.290)

Diacon AH, Dawson R, Hanekom M, Narunsky K, Maritz SJ, Venter A, Donald PR, Van Niekerk C, Whitney K, Rouse DJ, Laurenzi MW, Ginsberg AM, Spigelman MK. (2010) Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive Tuberculosis patients. Antimicrob Agents Chemother. 54(8): 3402-3407. (IF=4.294)

Diacon AH, Maritz JS, Venter A, Van Helden PD, Andries K, McNeeley DF, Donald PR. (2010) Time to detection of the growth of Mycobacterium tuberculosis in MGIT 960 for determining the early bactericidal activity of antituberculosis agents. Eur J Clin Microbiol Infect Dis. 29 (12): 1561-5. (IF=2.560)

Gallant CJ, Cobat A, Hoal EG, Schurr E. (2010) Quantifying latent TB infection: The dilemma continues. Chest. 138: 460-461. (IF=5.442)

Gallant CJ, Cobat A, Simkin L, Black GF, Stanley K, Hughes J, Doherty TM, Hanekom WA, Eley B, Beyers N, Jaïs J-P, van Helden PD, Abel L, Alcaïs A, Hoal EG, Schurr E. (2010) Impact of age and sex on mycobacterial immunity in an area of high tuberculosis incidence. Int J Tuberc Lung Dis 14:952-9. (IF=2.442)

Gallant CJ, Cobat A, Simkin L, Black GF, Stanley K, Hughes J, Doherty TM, Hanekom WA, Eley B, Beyers N, Jaïs J-P, van Helden PD, Abel L, Alcaïs A, Hoal EG, Schurr E. (2010) Tuberculin skin test and in vitro assays provide complementary measures of antimycobacterial immunity in children and adolescents. Chest

137:1071-7. (IF=5.442)

Govender L, Abel B, Hughes EJ, Scriba TJ, Kagina BM, de Kock M, Walzl G, Black G, Rosenkrands I, Hussey GD, Mahomed H, Andersen P, Hanekom WA. (2010) Higher human CD4 T cell response to novel Mycobacterium tuberculosis latency associated antigens Rv2660 and Rv2659 in latent infection compared with tuberculosis disease. Vaccine 29(1): 51-57. (IF=3.508)

Harper CJ, Hayward D, Kidd M, Wiid I, van Helden PD. (2010) Glutamate dehydrogenase and glutamine synthetase are regulated in response to nitrogen availability in Myocbacterium smegmatis. BMC Microbiol 10:138. (IF=3.329)

Hesseling AC, Marais BJ, Kirchner HL, Mandalakas AM, Brittle W, Victor TC, Warren RM, Schaaf HS. (2010) Mycobacterial genotype is associated with disease phenotype in children. Int J Tuberc Lung Dis 14:1252-8. (IF=2.442)

Hesseling AC, Walzl G, Enarson DA, Carroll NM, Duncan K, Lukey PT, Lombard C, Donald PR, Lawrence KA, Gie RP, van Helden PD, Beyers N. (2010) Baseline sputum time to detection predicts month two culture conversion and relapse in non-HIV-infected patients. Int J Tuberc Lung Dis 14:560-70. (IF=2.442)

Human H, Hagen CM, de Jong G, Harris T, Lombard D, Christiansen M, Bardien S. (2010) Investigation of mitochondrial sequence variants associated with aminoglycoside-induced ototoxicity in South African TB patients on aminoglycosides. Biochem Biophys Res Commun 393:751-6. (IF=2.270)

Innes S, Schaaf HS, Hoek KGP, Rabie H, Cotton MF. (2010) Unsuspected fatal drug-resistant tuberculosis in a closely monitored child: a plea for improved source-case tracing and drug susceptibility testing. South Afr J Epidemiol Infect. 25(2): 30-32. (IF not available)

Ioerger TR, Feng Y, Chen X, Dobos KM, Victor TC, Streicher EM, Warren RM, Gey van Pittius NC, Van Helden PD, Sacchettini JC. (2010) The Non-clonality of Drug Resistance in Beijing-genotype Isolates of Mycobacterium tuberculosis from the Western Cape of South Africa. BMC Genomics. 11: 670. (IF=4.179)

Johnson R, Warren RM, van der Spuy GD, Gey van Pittius NC, Theron D, Streicher EM, Bosman M, Coetzee GJ, van Helden PD, Victor TC. (2010) Drug-resistant tuberculosis epidemic in the Western Cape driven by a virulent Beijing genotype strain. Int J Tuberc Lung Dis 14:119-21. (IF=2.442)

Koegelenberg CFN, Bolliger CT, Theron J, Walzl G, Wright CA, Louw M, Diacon AH. (2010) Direct comparison of the diagnostic yield of ultrasound-assisted Abrams and Tru-Cut needle biopsies for pleural tuberculosis. Thorax 65:857-62. (IF=6.906)

Mallard K, McNerney R, Crampin AC, Houben R, Ndlovu R, Munthali L, Warren RM, French N, Glynn JR. (2010) Molecular detection of mixed infections of Mycobacterium tuberculosis in sputum samples from patients in Karonga District, Malawi. J Clin Microbiol. 48 (12): 4512-4518. (IF=4.126)

Michel AL, Müller B, van Helden PD. (2010) Mycobacterium bovis at the animal-human interface: a problem, or not? Vet Microbiol 140:371-381.

Möller M, de Wit E, Hoal EG. (2010) Past, present and future directions in human genetic susceptibility to tuberculosis. FEMS Immunol Med Microbiol 58:3-26. (IF=2.352)

Möller M, Flachsbart F, Till A, Thye T, Horstmann RD, Meyer CG, Osei I, van Helden PD, Hoal EG, Schreiber S, Nebel A, Franke A. (2010) A functional haplotype in the 3'untranslated region of TNFRSF1B is associated with tuberculosis in two African populations. Am J Respir Crit Care Med 181:388-93. (IF=9.881)

Möller M, Hoal EG. (2010) Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. Tuberculosis (Edinb) 90:71-83. (IF=2.986)

Möller M, Nebel A, van Helden PD, Schreiber S, Hoal EG. (2010) Analysis of eight genes modulating interferon gamma and human genetic susceptibility to tuberculosis: a case-control association study. BMC Infect Dis 10:154. (IF=2.715)

Muyoyeta M, de Haas PEW, Mueller DH, van Helden PD, Mwenge L, Schaap A, Kruger C, Gey van Pittius NC, Lawrence K, Beyers N, Godfrey-Faussett P, Ayles H. (2010) Evaluation of the Capilia TB assay for culture confirmation of Mycobacterium tuberculosis infections in Zambia and South Africa. J Clin Microbiol

48:3773-5. (IF=4.126)
Onajole OK, Govender P, van Helden PD, Kruger HG, Maguire GEM, Wiid I, Govender T. (2010) Synthesis and evaluation of SQ109 analogues as potential anti-tuberculosis candidates. Eur J Med Chem 45:2075-9. (IF=3.363)
Parsons SDC, de Villiers C, Gey van Pittius NC, Warren RM, van Helden PD. (2010) Detection of Mycobacterium kansasii infection in a rhesus macaque (Macaca mulatta) using a modified QuantiFERON-TB Gold assay. Vet Immunol Immunopathol 136:330-4. (IF=2.211)
Repsilber D, Kern S, Telaar A, Walzl G, Black GF, Selbig J, Parida SK, Kaufmann SHE, Jacobsen M. (2010) Biomarker discovery in heterogeneous tissue samples -taking the in-silico deconfounding approach. BMC Bioinformatics 11:27. (IF=4.108)
Roberts L, Passmore JS, Williamson C, Little F, Bebell LM, Mlisanac K, Burgers WA, van Loggerenberg F, Walzl G, Djoba Siawaya JF, Abdool Karim Q and Abdool Karim SS. (2010) Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. AIDS 24(6):819–831. (IF=5.334)
Sester M, Giehl C, McNerney R, Kampmann B, Walzl G, Cuchí P, Wingfield C, Lange C, Migliori GB, Kritski AL, Meyerhans A; European Network for global cooperation in the field of AIDS and TB (EUCO-Net). (2010) Challenges and perspectives for improved management of HIV/Mycobacterium tuberculosis co-infection. Eur Respir J. 36(6):1242-1247. (IF=5.076)
Stefan DC, Dippenaar A, Detjen AK, Schaaf HS, Marais BJ, Kriel B, Loebenberg L, Walzl G, Hesseling AC. (2010) Interferon-gamma release assays for the detection of Mycobacterium tuberculosis infection in children with cancer. Int J Tuberc Lung Dis. 14(6): 689-694. (IF=2.442)
Wallis RS, Pai M, Menzies D, Doherty TM, Walzl G, Perkins MD, Zumla A. (2010) Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet 375:1920-37. (IF=29.443)
Wright CA, Bamford C, Prince Y, Vermaak A, Hoek KGP, Marais BJ, Warren RM. (2010) Mycobacterial transport medium for routine culture of fine needle aspiration biopsies. Arch Dis Child 95:48-50. (IF=2.620)
Wright CA, Hoek KGP, Marais BJ, van Helden PD, Warren RM. (2010) Combining fine-needle aspiration biopsy (FNAB) and high-resolution melt analysis to reduce diagnostic delay in Mycobacterial lymphadenitis. Diagn Cytopathol 38:482-8. (IF=1.215)

Non Peer-Reviewed Articles (Total: 3)

Marais BJ, van Helden PD, Donald PR. (2010) Adequacy of 6-month follow-up in chemotherapy trials. Int J Tuberc Lung Dis 14:927; author reply 928. (IF=2.442)
Van Helden PD. (2010) Is bigger better? EMBO Reports 11:408.
Van Helden PD. (2010) Managing Academic Research. EMBO Reports 11:648.

Published Abstracts (Total: 0)

Technical Reports (Total: 0)

Products / Artefacts / Patents (Total: 0)

Conferences/Meetings Attended & Invited Talks/Seminars Presented (Total: 89)

Abrahams GL, Wei H, Ciulli A, Sherman DR, Abell C, Barry CE III, Boshoff HIM, **Mizrahi V.** New tools for tuberculosis drug discovery: Development of novel screening strains of M. tuberculosis and application in target-based approaches. Plenary lecture presented at the Keystone Symposium on Antibiotics and Resistance: Challenges and Solutions, Santa Fe, NM, 14-19 February 2010.
Wei AH, Silvestre HL, Wen S, Abrahams GL, Sherman DR, Barry CE III, Boshoff HIM, Ciulli A, Blundell TL, **Abell C.** Application of fragment-based approaches to the discovery of new agents against Mycobacterium tuberculosis. Plenary lecture presented at the Keystone Symposium on Antibiotics and Resistance: Challenges and Solutions, Santa Fe, New Mexico, 13-19 February, 2010

Mizrahi V. Early-stage drug discovery. Plenary lecture presented at the NIH/HHMI/SA MRC US-Southern Africa Joint Research Forum on Tuberculosis, Pretoria, 1-2 March 2010.
Mizrahi V. Summary of the NIH/HHMI/SA MRC US-Southern Africa Joint Research Forum on Tuberculosis. Talk presented at the Research Forum (2 March 2010), and at the following Institute of Medicine- Academy of Science of South Africa Workshop on the Emerging threat of drug-resistant tuberculosis: Global and local challenges and solutions, Pretoria, 3-4 March 2010.
Abrahams GL, Mizrahi V. Target-based whole-cell screening. Talk presented at the IMTB Annual Meeting, Martha's Vineyard, MA, 5-6 May 2010.
Mizrahi V. Taking advantage of stress: DNA damage, mutagenesis and the evolution of drug resistance in M. tuberculosis. Division U Plenary Lecture, presented at the 110th General Meeting of the American Society for Microbiology, San Diego, CA. 24-28 May 2010.
Cholo MC, Cockeran, R., Mizrahi, V., van Rensburg, E.J., Stoker, N.G., and Anderson, R. Evaluation of the Relative Efficiencies and Preferential Utilization at Various Stages During Growth of the Trk and Kdp Potassium Transporters of Mycobacterium Tuberculosis. Talk presented at the 2nd SA TB Conference, Durban, 2-5 June 2010.
Koch A, Mizrahi V, Warner DF. The fitness cost of drug resistance mutations in mycobacteria. Invited talk presented at the 2nd SA TB Conference, Durban, 2-5 June 2010.
Gordhan BG. The role of DNA glycosylases in mutagenesis and adaptation to stress in mycobacteria. Invited talk presented at the 2nd SA TB Conference, Durban, 2-5 June 2010.
Kana BD. From TB Pathogenesis to Drug Development. Plenary lecture presented at the 2nd SA TB Conference, Durban, 2-5 June 2010
Warner DF. A novel mechanism of damage-induced mutagenesis in Mycobacterium tuberculosis. Invited talk presented to the National TB Reference Laboratory, National Institute for Communicable Diseases, Johannesburg, 19 July 2010.
Warner DF. Taking advantage of stress: DNA damage-induced mutagenesis in Mycobacterium tuberculosis. Invited talk presented to a visiting delegation led by Prof. Ronald Atlas under the People To People Citizen Ambassador Programme, University of the Witwatersrand, 21 July 2010.
Mizrahi V. TB Drug Discovery Challenges from a Mycobacterial Physiology Perspective. Invited talk presented at the Scientific Advisory Committee meeting of the Global Alliance for TB Drug Development, New York, 28 July 2010.
Mizrahi V. Taking Advantage of Stress: DNA damage, mutagenesis and the evolution of drug resistance in M. tuberculosis. Plenary Lecture delivered at the UKZN College of Health Sciences Research Symposium, Durban, 15 September 2010.
Ahidjo BA, Gordhan BG, Mizrahi V. Nuclease-like VapC toxins from mycobacterium tuberculosis differentially inhibit mycobacterial growth and are specifically neutralised by their cognate antitoxins. Invited talk presented at the Faculty of Health Sciences Research Day and Postgraduate Expo, University of the Witwatersrand, 22 September 2010. [Winner, Best Young Researcher Award, Infectious Diseases category]
Koch A, Mizrahi V, Warner DF. The fitness cost of rifampicin resistance in mycobacteria. Invited talk presented at the Faculty of Health Sciences Research Day and Postgraduate Expo, University of the Witwatersrand, 22 September 2010.
Koch A, Mizrahi V, Warner DF. The physiological implications of drug resistance in Mycobacteria. Talk presented at the 3rd Postgraduate Cross-Faculty Symposium, Wits University, 27 October 2010.
Ndwandwe DE, Mizrahi V, Warner DF. A novel mutasome in Mycobacterium tuberculosis. Talk presented at the 3rd Postgraduate Cross-Faculty Symposium, Wits University, 27 October 2010.
Moosa A, Mizrahi V, Warner DF. The metabolism of vitamin B_{12} in Mycobacterium tuberculosis: role of alternate B_{12} cofactors. Talk presented at the 3rd Postgraduate Cross-Faculty Symposium, Wits University, 27 October 2010.
Naran K, Veale R, Mizrahi V, Warner DF. Characterisation of the anti-mycobacterial effect of a

Pseudomonas-derived activity. Talk presented at the 3rd Postgraduate Cross-Faculty Symposium, Wits University, 27 October 2010.
Abrahams GL, Wei H, Ciulli A, Sherman DR. Abell C, Barry CE III, Boshoff HIM, **Mizrahi V.** New tools for tuberculosis drug discovery: Development of novel screening strains of M. tuberculosis and application in target-based approaches. Poster lecture presented at the Keystone Symposium on Antibiotics and Resistance: Challenges and Solutions, Santa Fe, NM, 14-19 February 2010.
Kana BD, Mizrahi V, Gordhan, BG. The role of resuscitation promoting factors in drug susceptibility and stress adaptation of Mycobacterium tuberculosis. Poster presented at the Gordon Research Conference on Bacterial Cell Surfaces, Colby Sawyer College, NH, USA, 27 June – 2 July 2010.
Koch A, Mizrahi V, Warner DF. The fitness cost of rifampicin resistance in mycobacteria. Poster presented at the FEBS-EMBO Lecture Course on Host-Microbe Interactions, Spetses, Greece, 3-10 September 2010. [Winner, Best Poster Prize]
Naran K, Mizrahi V, Warner DF. A Pseudomonas-derived extract that inhibits growth of Mycobacterium tuberculosis Poster presented at the FEBS-EMBO Lecture Course on Host-Microbe Interactions, Spetses, Greece, 3-10 September 2010.
Moosa A, Mizrahi V, Warner DF. A role for a mycobacterium-specific protein family in vitamin B12 biosynthesis in Mycobacterium tuberculosis. Poster presented at the Faculty of Health Sciences Research Day and Postgraduate Expo, University of the Witwatersrand, 22 September 2010. [Winner, Best Poster Prize, Infectious Diseases category]
Naran K, Mizrahi V, Warner DF. A Pseudomonas-derived extract that inhibits growth of Mycobacterium tuberculosis. Poster presented at the Faculty of Health Sciences Research Day and Postgraduate Expo, University of the Witwatersrand, 22 September 2010.
Narandes N, Mizrahi V, Kana BD. Functional characterisation of molybdopterin synthase-encoding genes in mycobacteria. Poster presented at the Faculty of Health Sciences Research Day and Postgraduate Expo, University of the Witwatersrand, 22 September 2010.
Ndwandwe DE, Mizrahi V, Warner DF. A novel mutasome in Mycobacterium tuberculosis. Poster presented at the Faculty of Health Sciences Research Day and Postgraduate Expo, University of the Witwatersrand, 22 September 2010.
Ahmadou Ahidjo B, Kuhnert D, Machowski EE, Gordhan BG, Abrahams GL, Mizrahi V. VapC toxins from Mycobacterium tuberculosis differentially inhibit mycobacterial growth and are specifically neutralised by their cognate antitoxins. Poster presented at the NITD Symposium on Tuberculosis 2010, Yaounde, Cameroon, 11-15 October 2010.
Mapela L, Kana BD. Characterisation of resuscitation promoting factors in M. smegmatis. Poster presented at the MRC Research Day, Cape Town, 14 October 2010.
Narrandes N, Mizrahi V, Kana BD. Functional characterization of molybdopterin synthase-encoding genes in mycobacteria. Poster presented at the MRC Research Day, Cape Town, 14 October 2010.
Lucas LA, Möller M, Nebel A, Van Helden PD, Schreiber S, Hoal EG. The role of Toll like receptors in genetic host susceptibility to tuberculosis. Talk presented at the 10th AstraZeneca Health Sciences Research Day. University of Stellenbosch, South Africa, 25 February 2010.
Muleya V. Structural Characterisation of the Interaction Between RBBP6 and the Multifunctional Protein YB-1. Talk presented at the 10th Astrazeneca Health Sciences Research Day. University of Stellenbosch, 25 February 2010.
Ngombane N, Ottenhoff THM, Kidd M, Walzl G, Black G. The predictive role of cytokine expression patterns of recently exposed tuberculin skin test negative TB contacts in the Western Cape Province. Talk presented at the 10th AstraZeneca Health Sciences Research Day, University of Stellenbosch, South Africa, 25 February 2010.
Salie M, Möller M, Van Helden PD, Schreiber S, Hoal EG, Nebel A, Hofmann S. A 3'UTR polymorphism in ANXA11 is associated with pulmonary tuberculosis in the South African coloured population. Talk presented at the 10th AstraZeneca Health Sciences Research Day. University of Stellenbosch, South Africa, 25

February 2010.
Wagman C, Möller M, Hoal EG. Marco haplotypes and susceptibility to tuberculosis. Talk presented at the 10th AstraZeneca Health Sciences Research Day. University of Stellenbosch, South Africa, 25 February 2010.
Warren RM. Molecular and Genetic epidemiology of TB in South Africa. Invited talk presented at the US-Southern Africa Joint research forum on Tuberculosis. Roode Valley Country Lodge, Pretoria, South Africa, 1-2 March 2010.
Warren RM; Genetic analysis of drug resistant strains in South Africa. Invited stalk at the ASSAF meeting on MDR-TB, Pretoria, 3-4 March 2010.
De Vos M. Rifampicin induces differential protein expression in Mycobacterium tuberculosis. Invited talk at the 2nd TB Conference on forging strategic partnerships to Fight TB and HIV. Durban, South Africa, 2-3 June 2010.
Van Helden PD. Quantitative first- and second-line drug susceptibility testing (DST) on MGIT 960 using BD EpiCenter Software. Invited talk presented at the 2nd TB Conference on Forging strategic partnerships to Fight TB and HIV. Durban, South Africa, 2-3 June 2010.
Black PA, Warren RM, Louw GE, Van Helden PD, Victor TC. Identification of novel mechanisms of drug resistance in Mycobacteria byTransposon Mutagenesis. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
De Vos M, Warren RM, Johnson R, Van Helden PD, Ndimba BK, Victor RC. Rifampicin induces differential protein expression in Mycobacterium Tuberculosis. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Hoal EG. The History in our Genes: the Complex Structure of the South African Coloured Population. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Macingwana L, Baker B, Ngwane A, Van Helden PD, Cotton M, Wiid IJF. Investigation of synergistic effect of Isoniazid, Sulfamethoxazole and Trimethoprim as potential first-line combination drug regimen against Mtb. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Streicher EM, Gey Van Pittius NC, De Kock M, Van Helden PD, Victor TC, Warren RM. Acquisition of resistance to anti-Tuberculosis drugs may compromise the utility of genetic based drug resistance tests. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Tait M, **Sirgel F,** Trollip A, Warren RM, Coetzee G, Victor TC. Cross-resistance compromises the use of Capreomycin in the treatment of XDR-TB. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Hoek KGP, Wright CA, Marais BJ, Van Helden PD, Warren RM. High-resolution melt analysis for the detection of Mycobacterial Lymphadenitis using Whatman Fta® Elute cards. Talk presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Warren RM. Recent findings in antibiotic resistance in TB. Joint IPCAN/IFIC. Invited talk presented at Spier, Stellenbosch, South Africa, 29 August - 1 September 2010.
Warren RM. Molecular Epidemiology and the Genesis of Drug-Resistant TB. Talk presented at the Workshop on Drug-Resistant TB: Current Practise, Controversies, and Clinical Challenges. University of Cape Town, Cape Town, South Africa, 3-5 September 2010.
Diacon AH. Emerging drugs for the management of drug-resistant TB. Talk presented at the Workshop on Drug-Resistant TB: Current Practise, Controversies, and Clinical Challenges. University of Cape Town, Cape Town, South Africa, 3-5 September 2010.
Ronacher K, Kleynhans L, Ehlers L, Walzl G. Medroxyprogesterone-acetate alters cytokine expression in response to mycobacterium bovis BCG in vitro and in PBMCs of contraceptive users. Talk presented at the SATBAT Meeting, Johannesburg, South Africa, 12 October 2010.
Ronacher K, Kleynhans L, Ehlers L, Walzl G. Medroxyprogesterone-acetate alters cytokine expression in

response to mycobacterium bovis BCG in vitro and in PBMCs of contraceptive users. Talk presented at the South African Immunology Society Conference. Lanseria, South Africa, 5-8 Dec 2010.
Walzl G, Chegou NN, Loxton A, Ronacher K. Biomarkers for different forms of tuberculosis. Talk presented at the South African Immunology Society Conference. Lanseria, South Africa, 5-8 December 2010.
De Souza GA, **Fortuin S**, Aguilar D, Hernandez-Pando R, McEvoy CRE, Van Helden PD, Koehler CJ, Thiede B, Warren RM, Wiker HG. Using a label-free proteomic method to identify differentially abundant proteins in closely related hypo and hyper-virulent clinical Mycobacterium tuberculosis Beijing isolates. Talk presented at the Analitika International Conference on Analytical Science, Stellenbosch, South Africa, 5 - 9 December 2010.
Gey van Pittius NC. Tuberculosis in South Africa- problem setting - epidemiology data and current trends. Invited talk presented at Koch-Metschnikow Forum Fourth Scientific Symposium on the occasion of World Tuberculosis Day 2010. Berlin, Germany, March 22-23 2010.
Warren RM, Gey van Pittius NC, Streicher EM, Louw GE, Calver A, Hernando-Pandez R, De Vos M, Tait M, Trollip A, Chiota V, Hanekom M, Coetzee G, Van der Spuy G, Muller B, Victor TC, Van Helden PD. Mycobacterium tuberculosis adaptation: answers or questions. Invited talk presented at the TB Adapt meeting. Rio De Janeiro, Brazil, 25-26 May 2010.
Warren RM, Gey van Pittius NC, Streicher EM, Louw GE, Calver A, Hernando-Pandez R, De Vos M, Tait M, Trollip A, Chiota V, Hanekom M, Coetzee G, Van der Spuy G, Muller B, Victor TC van Helden PD. A compilation of recent findings in molecular epidemiology and diagnostics of tuberculosis in South Africa. Talk presented at the IV National Tuberculosis Meeting/ 1st Brazilian STOP TB Partners' Forum. Rio De Janeiro, Brazil, 26 - 29 May 2010.
Gey van Pittius NC. Tuberculosis in South Africa - from a treatable disease to total drug resistance. Invited talk presented at the Institute of Medicine (IOM) of the USA and the Russian Academy of Medical Sciences (RAMS) Forum on Drug Discovery, Development, and Translation. Moscow, Russia, 26-27 May 2010.
Victor TC, Streicher EM, Tait M, Johnson R, Van der Spuy GD, Gey van Pittius NC, Theron D, Bosman M, Coetzee GJ, Van Helden PD, Chabula-Nxiweni EM, Trollip A, Warren RM. Molecular insights Into the XDR-TB Epidemic in the Western And Eastern Cape Regions of South Africa. Invited talk presented at the ASM conference. San Diego, USA, 23-27 May 2010.
Louw GE, Warren RM, Gey van Pittius NC, McEvoy CRE, Murray M, Van Helden PD, Victor TC. Activated efflux pumps define the level of Rifampicin resistance in M. tuberculosis. Invited talk presented at the ASM Conference, San Diego, USA, 23-27 May 2010.
Victor TC. Molecular insights Into the XDR-TB Epidemic in the Western and Eastern Cape Regions of South Africa. Invited speaker at the Global Forum for infectious diseases. University California, San Diego, USA, 23-27 May 2010.
Van Helden. Susceptibility testing of Mycobacterium tuberculosis: The why and how in the developing world. Invited talk presented at the 50th Interscience conference on antimicrobial agents and chemotherapy. Boston, USA, 12-15 September 2010.
Victor TC. Insights into MDR- and XDR-TB in South Africa. Invited talk presented at a meeting to discuss the application of genetics- and genomics-based tools for diagnosis, drug susceptibility testing and epidemiology. Tunis, Tunisia, 20-22 September 2010.
Diacon AH. TMC-207 versus placebo plus OBT for the treatment of MDR-TB: a prospective clinical trial. Invited talk presented at the 41st Union World Conference on Lung Health. Berlin, Germany, 11-15 November 2010.
Van Helden PD. Microbial genetic determinants of TB infection and disease progression. Invited talk presented at the 41st Union World Conference on Lung Health. Berlin, Germany, 11-15 November 2010.
Walzl G. Biomarkers for use in clinical trials. Invited talk presented at the 41st Union World Conference on Lung Health. Berlin, Germany, 11-15 November 2010.
Stead M. Frequency and clinical outcome of mixed infections in South African TB patients. Invited talk

presented at the 41st Union World Conference on Lung Health. Berlin, Germany, 11-15 November 2010.
De Vos M, Warren RM, Johnson R, Van Helden PD, Ndimba BK, Victor TC. RIF induces diferential protein expression in Mycobacterium tuberculosis. Poster presented at the 10th AstraZeneca Health Sciences Research Day. University of Stellenbosch, South Africa, 25 February 2010.
Ngwane A, Wiid IJF, Van Helden PD. Elucidation of the mechanism of action of a novel furanone based antituberculosis compound (F1082). Poster presented at the 10th AstraZeneca Health Sciences Research Day. University of Stellenbosch, South Africa, 25 February 2010.
Fortuin S. A label-free method identified differentially abundant proteins in related M. tuberculosis Beijing strains, Poster presented at the ESF 4th conference on Functional Genomics and Disease, Dresden, Germany, 14–17 April 2010.
Bruiners N, Gey Van Pittius NC, Warren RM. Investigation Of Complement C1q Polymorphisms In A Cohort Of Tuberculosis Infected Patients. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Dramowski A, Morsheimer MM, Jordaan A, Victor TC, Donald PR, Schaaf HS. Rifampicin Mono-Resistant Mycobacterium Tuberculosis Disease: An Increasing Trend Among Children In Cape Town. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Fang Z, Gey Van Pittius NC, Van Helden PD. Elucidation Of The Substrates Of Mycosin 3, An Essential Protease of M.Tuberculosis. Poster presented at the 54th Academic Year Day, University Of Stellenbosch, South Africa, 11-12 August 2010.
Friedrich SO, Nabeta P, Boehme C, Venter A, Diacon AH. Direct comparison of GeneXpert with smear microscopy, solid and liquid culture for sputum monitoring of tuberculosis (TB) patients. Poster presented at the 54th Annual Academic Day, Stellenbosch University, South Africa, 11-12 August 2010.
Grobbelaar M, Louw GE, Warren RM, Gey Van Pittius NC, Van Helden PD, Victor TC. New hope for treatment of drug resistant Tuberculosis. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Kirsten CJ, Hayward D, Wiid IJF, Van Helden PD**.** Identification of the Transcriptional Regulator of Nitrogen Related Genes In Mycobacterium Smegmatis. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Lucas LA, Möller M, Nebel A, Van Helden PD, Schreiber S, Hoal EG. Genetic Variants of Toll- Like Receptors 2 & 8 And Their Possible Role In Susceptibility To Pulmonary Tuberculosis. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Newton-Foot M, Warren RM, Van Helden PD, Gey Van Pittius NC. The Mycobacterium Tuberculosis Esx-3 Secretion System Interactome. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Phalane KG, Esterhuyse MM, Hoal EG. Evaluation of Differential Dna Methylation Levels In The Interleukin 8 (Il8) Gene Of Tuberculosis Infected Cases And Controls. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Seepe PM, Warren RM, Johnson R, Van Helden PD, Louw GE, Victor TC. Differential Expression Of Genes In Clinical Strains of Mycobacterium Tuberculosis In Response to Isoniazid. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Siame KK, Gey Van Pittius NC, Warren RM. Investigating the Genome Variation and Evolution of Group 1 M. Tuberculosis Strains. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Steyn NL, Smit M, Newton-Foot M, Gey Van Pittius NC. The Construction of Genetic Knock-Outs of the Esx Secretion Systems In Mycobacterium Smegmatis. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Viljoen AJ, Kirsten C, Hayward D, Wiid IJF, Van Helden PD. Regulation of the Nitrogen Assimilatory Enzymes, Glutamine Synthetase And Glutamate Dehydrogenase In Mycobacterium Smegmatis And M.

Bovis BCG. Poster presented at the 54th Academic Year Day, University of Stellenbosch, South Africa, 11-12 August 2010.
Seepe PM, Johnson R, Louw GE, Van Helden PD, Warren RM, Victor TC. Differential expression of genes in clinical strains of Mycobacterium Tuberculosis in response to isoniazid. Poster presented at the MRC Research Day, MRC Conference Centre, Cape Town, South Africa, 14-15 October 2010.
De Souza GA, **Fortuin S**, Aguilar D, Hernandez-Pando R, McEvoy CRE, Van Helden PD, Koehler CJ, Thiede B, Warren RM, Wiker HG. A label-free method identified differentially abundant proteins in related M. tuberculosis Beijing strains. Poster presented at the ESF 4th conference on Functional Genomics and Disease, Dresden, Germany, 14 – 17 April 2010.
Möller M, Flachsbart F, Till A, Thye T, Horstmann R, Meyer C, Osei I, Van Helden PD, Hoal EG, Schreiber S, Nebel A, Franke A. A functional haplotype in the 3'UTR of TNFRSF1B is associated with TB in two African populations. Poster presented at the EMBO|EMBL Symposium Human Variation: Cause and Consequence, Heidelberg, Germany, 20-23 June 2010.
Hoal EG, Cobat A, Gallant C, Simkin L, Black GF, Stanley K, Hughes J, Doherty T, Hanekom W, Eley B, Beyers N, Jaïs J, Boland-Auge A, Van Helden PD, Casanova J, Abel L, Alcaïs A, Schurr E. A Genome-Wide Linkage Search for Genetic Loci Controlling Resistance to Tuberculosis (1001/W). Poster presented at the 60th Annual Meeting of The American Society of Human Genetics, Washington DC, USA, 2-6 November 2010.
Möller M, De Wit E, Delport W, Harmant C, Rugamika CE, Quach H, Meintjes A, Balanovsky O, Zaporozhchenko V, Bormans C, Van Helden PD, Seioghe C, Behar DM, Hoal EG. The History in our genes: The complex structure of the South African Coloured population. Poster presented at the ASHG 60th Annual Meeting in Washington DC, USA, 2-6 November 2010.
Salie M, Möller M, Van Helden PD, Schreiber S, Hoal EG, Nebel A, Hofmann S. A 3'UTR polymorphism in ANXA11 is associated with pulmonary tuberculosis in the South African coloured population. Poster presented at the ASHG 60th Annual Meeting in Washington DC, USA, 2-6 November 2010.

Other Relevant Outputs (including honours and awards to staff)

Prof. Mizrahi delivered the Division U Mycobacteriology Lecture at the 110th Meeting of the American Society for Microbiology in San Diego, on 24th May 2010. This honour is given to a scientist who has made a significant contribution to the field of mycobacteriology, and is accompanied by the award of a certificate to mark the occasion.
Prof. Mizrahi was appointed to three-year terms on the Board of Directors and the Scientific Advisory Board of K-RITH, UKZN.
Dr. Bavesh Kana was appointed as the new Head of the Wits University node of the CBTBR, effective 1 January 2011.
Eileen Hoal-van Helden received the SU Rector's award for Excellence in Research in November 2010
Warren, RM. Obtained a B2 rating by the NRF (2010)
Gey van Pittius, NC was elected as a member of the Academy of Science of South Africa (ASSAf)

Progress of Students Who Have Qualified or Trained in the CBTBR (2005-2010)

Title	Surname, Initial	Degree / Training	Year graduated/ completed	Current position
Dr	Abrahams, GL	Postdoctoral	2010	Appointed as Research Officer in new UCT node of CBTBR, funded by HIT-TB. Will be seconded for 3 years to Dr. Clifton Barry's lab at the NIAID to work with Dr. H. Boshoff on TB drug discovery

Dr	Babb, C	PhD	2007	Took up a Scientist post with Wits/NHLS
Dr	Bapela, BN	Postdoctoral	2007	Took up a permanent position at the MRC
Mr	Barnard, M	MSc	2005	Unemployed
Dr	Baumann R	Postdoctoral	2006	Returned to Germany, to private company
Ms	Barichievy, S	MSc	2005	Awarded a Sydney Brenner postdoctoral fellowship from ASSAf with Dr. Musa Mhlanga at the CSIR
Ms	Bester, M	MSc	2009	Remained in CBTBR for a PhD degree
Dr	Bezuidenhout, J	PhD	2005	Employed as F/T pathologist at Tygerberg Hospital
Dr	Bintou, AA	PhD	2010	Took up a postdoctoral position with Prof. Bishai, John Hopkins University, USA
Dr	Black, JF	Postdoctoral	2010	Took up a position with Livelihoods Foundation
Ms	Black, P	Hons	2009	Remained in CBTBR for a MSc degree
Ms	Botha, J	MSc	2007	Studying pharmacy at UWC
Ms	Brackin, R	MSc	2005	Took up a PhD position at CSIR
Dr	Brown, N	Postdoctoral	2007	Moved to UK
Ms	Carinus, H	Hons	2005	Moved to Dubai
Dr	Chegou, N	PhD	2009	Remained in CBTBR as postdoctoral fellow
Dr	Conradie E	Postdoctoral	2006	Full-time mother
Dr	de Wit, E	PhD	2009	Housewife
Dr	Djoba, J	PhD	2008	Took up a postdoctoral position in France
Mr	Dudhia, ZE	Hons	2009	Took a MSc studentship at the MRC
Ms	Du Toit, I	Hons	2006	Planning to do forensics through UNISA
Ms	Ehlers, L	Hons	2010	Remained in CBTBR for a MSc degree
Dr	Esterhuyse, M	Postdoctoral	2010	Took up a post in Prof Kaufmann's lab (Germany)
Ms	Falmer, A	MSc	2008	Moved to HIV NGO in Paarl
Dr	Fenhalls, G	Postdoctoral	2005	Now working in husband's company
Ms.	Goosens, V	MSc	2005	Took up PhD studentship in the Netherlands
Dr	Hanekom, M	PhD	2009	Remained in Lecturer's post
Dr	Hayward, D	Postdoctoral	2010	Took up a permanent position at Triclinium
Ms	Heysen, T	Hons	2009	Unknown
Ms	Hoek, K	PhD	2010	Took up a permanent position at the NHLS
Dr	Johnson, R	Postdoctoral	2009	Took up a permanent position at the MRC
Mr	Jennings, G	Hons	2005	Moved to the USA for postgraduate study
Dr	Kleynhans, L	MSc	2009	Remained in CBTBR for a PhD degree
Ms	Kruger, C	PhD	2009	Took up PhD at Water Health Research Unit, JHB
Mr	Laisse, CJM	MSc	2010	Returned to UEM in Mozambique
Mr	Lambrecht, D	Hons	2005	Left CBTBR to do MSc in Chemistry at SU
Dr	Louw, G	PhD	2009	Remained in CBTBR as a postdoctoral fellow
Dr	Loxton, A	PhD	2009	Remained in CBTBR as a postdoctoral fellow
Mr	Lucas, L	Hons	2009	Remained in CBTBR for a MSc degree
Dr	Machowksi, E	Postdoctoral	2006	Employed in CBTBR as P/T Medical Scientist. Emigrated to Austria in July 2010
Ms	Magan, N	Hons	2009	Unknown
Dr	Magwira, C	Postdoctoral	2010	Expected to take up a position at the NTBRL to

				work on an Optimus collaborative project.
Mr.	Mahasha, P	MSc	2007	Moved to Univ. of Pretoria, for family reasons
Dr	Matsoso, LG	PhD	2007	Took a Medical Scientist in the National TB Reference Centre, NICD, Johannesburg
Mr	Mazorodze, JH	MSc	2010	Took up a PhD in Bill Jacobs's lab in USA
Dr	McEvoy, CRE	Postdoctoral	2010	Will return to Australia in March 2010
Dr	Moller, M	PhD	2007	Remained in CBTBR as a postdoctoral fellow
Dr	Mowa, B	PhD	2009	Took up a postdoctoral position with Prof. P. Arbuthnot, Wits
Mr	Mufamadi, S	Internship	2005	Completed MSc at Wits
Ms	Muller, L	Researcher	2006	Full-time mother
Ms	Myburgh, R	Hons	2006	Left the CBTBR to start her family
Ms	Naran, K	MSc	2010	Registered for a PhD in UCT node of the CBTBR
Ms	Ndabambi, S	MSc	2009	Unknown
Mr	Ndong, PE	Hons	2010	Remained in CBTBR for a MSc degree
Dr	Nel, HJ	PhD	2007	Took a postdoctoral at Trinity College Dublin, Ireland
Dr	Nene, N	PhD	2009	Took up a Postdoctoral at LifeLab in Durban
Ms	Newton-Foot, M	MSc	2009	Remained in CBTBR for a PhD degree
Dr	Parsons, S	PhD	2009	Remained in CBTBR as postdoctoral fellow
Ms	Phalane, KG	Hons	2010	Remained in CBTBR for a MSc degree
Dr	Ramburan, A	PhD	2009	Took up a permanent position at NHLS, Durban
Ms	Richardson, M	PhD	2006	Deceased
Dr	Roberts, T	PhD	2008	Took up a permanent position at CPGR, UCT
Ms	Sao Emani, C	Hons	2010	Remained in CBTBR for a MSc degree
Dr	Savvi, S	PhD	2009	Took up a postdoctoral position at UCT with Prof. Brombacher
Dr	Sholto-Douglas-Vernon, C	PhD	2005	Employed at St. George's Hospital, London (currently on 4 months' maternity leave)
Mr	Siame, KK	Hons	2010	Remained in CBTBR for a MSc degree
Ms	Strauss, O	MSc	2009	Moved to Kayaletsha HIV clinic in Cape Town
Ms	Steyn, NL	Hons	2010	Remained in CBTBR for a MSc degree
Dr	Streicher, EM	PhD	2007	Remained in CBTBR as postdoctoral fellow
Ms	Thiart, L	Hons	2010	Remained in CBTBR for a MSc degree
Dr	Van der Spuy, G	PhD	2009	Remained in CBTBR in MRC Post
Dr.	Veenstra, H	PhD	2007	Housewife
Mr	Viljoen, B	Hons	2009	Remained in CBTBR for a MSc degree
Dr.	Warner, DF	Postdoctoral	2007	NHLS Medical Scientist & CBTBR team member
Dr	Williams, M	Postdoctoral	2009	MRC-funded post in MMRU, seconded to SU node.
Dr	Wright, CA	PhD	2009	Remained in University Post

FINANCES

The income statement, balance sheet and cash flow statement for period 1 Jan 2010 to 31 Dec 2010 are currently under review by the external auditors and will be forwarded to the Board as soon as it becomes available.