DST-NRF CENTRE OF EXCELLENCE

ANNUAL PROGRESS REPORT

Reporting Period

1 January 2011 – 31 December 2011

CONTENTS **Page**

Identification

Name of Director	:	Professor Paul D. van Helden
Names of Node Heads	:	Professor Valerie Mizrahi Dr Bavesh Kana
Name of CoE	:	DST/NRF Centre of Excellence for Biomedical TB Research
Abbreviated CoE Name	:	CBTBR
Host institutions	:	University of Stellenbosch, University of the Witwatersrand University of Cape Town
Date completed	:	07/03/2012

EXECUTIVE SUMMARY

1. Financial Information (Funding of the CoE)

Total NRF funding for 2010 (entire year) – CoE only	:	R 8 686 678
CoE-specific Funding from Host institution in 2011 – WITS	:	R 220 000
– UCT	:	R 499 890
– SU	:	R 748 667
Funding from other sources for the CoE in 2011	:	R 32 767 248
Total funding	:	**R 42 922 483**

Funding for 2011 for Wits node: (Total: R 6 066 917)

- CoE funding from NRF: **R 1 956 485**

- Funding from WITS and NHLS: **R 1 583 246**, made up as follows:
  - WITS R 865 500[1]
  - NHLS R 717 746[2]

- Funding from other sources: **R 2 527 186**, made up as follows:
  - MRC Career Development Award R 250 000
  - Friedel Sellschop Research Award R 105 000
  - Various WITS grants and small projects R 40 986
  - Institutional Targeting Talent R 50 000
  - Mellon Funding[3] R 152 134
  - NRF Incentive Funding R 80 000
  - CUSA000 Caprisa Postdoctoral Grant R 130 000
  - WITS Rollover RINC funds R 84 637
  - K/RITH - KwaZulu Natal Research Institute R 58 255
  - Other NRF grants[3] R 427 058
  - MRC Research Grants[3] R 92 482
  - TIA (SATRII) R 1 018 634
  - SATBAT R 38 000

Funding for 2011 for UCT node: (Total: R 10 272 684)

- CoE funding from NRF: **R 1 200 000**

- Funding from UCT and NHLS: **R 2 563,802**, made up as follows:
  - UCT R 1 499,890[4]
  - NHLS R 1 063,912[5]

- Funding from other sources:[6] **R 6 508,882**, made up as follows:
  - MRC Unit (MMRU) R 989 434 (1 Apr 2011 – 31 Mar 2012)
  - MRC (Salary) R 356 725 (1 Jan 2011 – 31 Dec 2011)
  - Swiss/SA Joint Research Programme (Yr 3) R 1 249 499[7] (1 Jan 2011 – 31 Dec 2011)
  - EU FP7 (MM4TB) (Yr 1) R 925 885 (1 Feb 2011 – 31 Dec 2011)[8]
  - FNIH (HIT-TB) R 1 986 839 (1 Mar 2011 – 31 Dec 2011)[9]
  - TIA (SATRII) R 1 000 500 (23 Mar 2011 – 30 Sep 2012)

[1] Salaries and 10% Institutional Commitment to CoE
[2] Salaries and intramural grant funding from the NHLS Research Trust
[3] Combination of current and rollover funds
[4] Salaries and equipment
[5] Salaries
[6] Where applicable, grant awards from external funders include indirect costs (IDC)
[7] Includes cumulative carry-over of unspent funds from Yrs 1 and 2
[8] Year 1 of four-year grant (total €331,667 - including IDC), calculated at exchange rate of R11.1/€
[9] Year 1 of three-year grant from FNIH (sub-contractor on grant from BMGF), calculated at exchange rate of R8.2/$

Funding for 2011 for SU node: (Total: R26 582 882)

- CoE funding from NRF : **R5 530 139**

- Other Funding from SU: (best estimate): **R2 950 000,** incl. some salaries, student bursaries, excl.space, basic infrastructure, secretary, cleaners.

- Funding from other sources (best estima**te):** **R18 103 000**, made up as follows:
  - MRC Centre (estimate of the TB component) R 4 472 743 (incl. salaries)
  - PGWC R 1 850 000 (salaries only)
  - USAID R 600 000
  - EDCTP R 2 700 000
  - BMGF R 4 100 000
  - Welcome Trust R 1 200 000
  - Harry Crossley Foundation R 100 000
  - Optimus Foundation R 500 000
  - WOTRO R 350 000
  - DF (Germany) R 350 000
  - K-RITH R 300 000
  - NIH R 100 000
  - Other NRF funding R 1 480 000

2. Summary of progress against 5 KPAs

(i) Research

The research productivity of the CBTBR remained excellent in 2011 as evidenced by the fact that 4 book chapters, 47 articles in peer-reviewed journals, 4 non-peer-reviewed articles were published, and 69 conference presentations were made, including 3 plenary/ keynote lectures, and numerous invited talks. Of the research articles published, 44 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 2 postdoctoral fellows, 4 PhD students, 4 MSc students and 3 Honours students from the CBTBR graduated or completed their training in 2011. All these postgraduate students completed degrees within their maximum allowable time agreed upon in the SLA. A number of new postdoctoral, PhD and MSc students were enrolled in the nodes of the CBTBR. A number of students were afforded the opportunity to work in international labs (details provided below).
Progress against SLA 4 targets: The total of 77 postgraduate students associated with the CBTBR in 2010 is more than double the SLA target of ≥25. The student breakdown according gender (69% female) and percentage of postdoctoral fellows (15% of total student complement) equalled or exceeded the SLA targets of ≥50% and ≥10%, respectively. The percentage of black students (50%) equalled the SLA target of ≥50%. The percentage of Honours students was 4% in 2011. No honours students were rejected unless they did not meet the entrance requirements set by the university.

(iii) Knowledge Brokerage

On 21 October 2011, Valerie Mizrahi was interviewed by the Wellcome Collection in London as part of the *Exchanges at the Frontier Series*. Prof Mizrahi's interview was broadcast on the 10th and repeated on the 11th December 2011 to a listenership of approximately 40 million people. Her interview is available as a podcast at **www.bbc.co.uk/programmes/p00lzhr6**.

Members of the CBTBR continue to disseminate scientific information at the operational, scientific community and stakeholder levels as far as possible. Apart from enjoying country-wide and sometimes international publicity in various media platforms, the CBTBR was involved in many outreach activities, targeting school teachers and learners, and on science communication in general. A number of meetings/workshops were held with provincial health authorities, MSF and NHLS, particularly regarding drug resistant TB. Meetings with SANParks were held, to advise them with regard to TB in wildlife. CBTBR members were also engaged with the media to develop more accurate and meanginful science communication channels between various media stakholders and the basic researchers. In this regard CBTBR members played an integral role in the *Sense about Science* workshop held at Wits University and in another workshop, *Award winning Health Journalism - lessons from the first three years of the Discovery Health Journalism Awards*, aimed at improving health journalism, hosted by Discovery Health. The CBTBR also participated in the the panel for the Discovery Health Journalism Awards.

For the first time, the CBTBR has prepared an MRC "Policy Brief" concerning recommendations for TB treatment in the case of drug resistant disease. This policy brief was prepared in consultation with the MRC and will be released in early 2012.

(iv) Networking

The creation of the new UCT node has created major new collaborative opportunities with and within UCT. Most importantly, strong ties have been established with the H3-D Drug Discovery Centre led by Prof. Kelly Chibale. This research collaboration has been formalized under the auspices of the South African Tuberculosis Research and Innovation Initiative (SATRII). The SATRII project involved all nodes of the CBRBR and has allowed for further networking and collaboration between the different nodes and other key stakeholders. Other new collaborators include Prof. Sue Harrison (Chemical Engineering) Prof. Jonathan Blackburn (SARChI Chair in Applied Proteomics and Chemical Biology) and Prof. Robert Wilkinson (CIDRI, IIDMM). Linkages with K-RITH were further strengthened by Prof. Mizrahi's continued service on the Board of Directors, Scientific Advisory Board and Search Committee of this new research institute at UKZN. The UCT node is involved in two large international drug discovery consortia, funded by the Bill & Melinda Gates Foundation (HIT-TB) and the EU FP7 (MM4TB), which involve some of the top TB research groups in the world. The SU node continues to be a major partner in various BMGF grants (eg Prof. Walzl via Prof. Kaufmann in Berlin on GC6). Furthermore, Prof Walzl has other BGMF grants:1) Biomarker Discovery with a consortium including Drs. Clif Barry (NIH), David Alland and others, 2) Hazel Dockrell (LSHTM) on surrogate markers. The CBTBR partners extensively in Africa, for example through an EDCTP grant supporting an African/European consortium with 7 African and 5 European sites. The Wits node is playing the leading role in a new international collaboration between Wits University, the USA National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), the University of Leicester, the Perinatal HIV Research Unit (PHRU) and the Aurum Institute. In addition, the Wits node collaborates with Dr. Gilla Kaplan at the Public Health Research Insitute in New Jersey on a collaborative project involving the National TB Reference Laboratory at the National Insitute for Comminicable Diseases in Johannesburg. The Wits node has also initiated new collaborative ventures with Drs. Stoyan Stoychev and Musa Mhlanga at the Council for Scientific and Industrial Research and with Dr. Lesley Scott, Dr. Melinda and Dr. Pierre Durand from the Department of Molecular Medicine and Hematology at Wits University. Furthemore, an MSc student from the SU spent 6 months at the Wits node on an exchange visit to learn new techniques for saturating transposon mutagenesis in mycobacteria, this expertise has now been transferred to the SU node which has resulted in strengthening of collaborative linkages between the Wits and SU nodes

(v) Service rendering

Activities in this area include the provision of technical/ scientific services to the Eastern and 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. We continue to test 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 continues to be given, and to the AACL for companion animals. The SU node continues to assist the MRC (Delft) and SAAVI with TB infection problems in their animals. The Wits node is playing a critical role in the national rollout of the new GeneXpert diagnostic platform which promises to revolutionize TB diagnostics in South Africa. In this regard, the Wits node has been involved in the establishment of the external quality assurance system for this platform, The Wits node has further assisted the Contract Laboratory Services division of the NHLS to establish DNA extraction methodologies for strain typing for samples in the REMOX clinical trial. The SU node developed a new sample collection vial for fine needle aspirates for TB diagnosis. This vial system is being rolled out by NHLS.

3. Gender Impact

From the "Science by Women" perspective, it is important to note that 69% (compared to 64% in 2010) of post graduate students in the CBTBR in 2011 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 change in leadership of the CBTBR
A third node of the CBTBR at UCT was formally established in January 2011. This development resulted in changes in leadership of the CBTBR. Prof. van Helden became the sole director of the Centre, Prof. Mizrahi became Head of the UCT node and Dr. Kana was appointed as Head of the Wits node. . In accordance with UCT's position as a co-hosting institution of this CoE, the DVC (Research), Prof. Danie Visser was appointed as co-Chair of the Board of the CBTBR.

Staffing and Staff Development
The employment contracts of Drs. Streicher and Gey van Pittius are linked to the existence of the CBTBR. In May 2011, Dr. Gey van Pittius was employed part-time on contract as acting Research Director in the Stellenbosch University Health Sciences Faculty. 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, B. Bapela and G. Louw).

PROGRESS REPORT

1. Scientific Research

In a departure from previous report format, we have placed this information in the Appendix, since this part of the document is detailed and extensive. Only the joint research and training activities are outlined below.

Joint Research and Training Activities

Extensive collaboration and integration of research activities has been achieved between the three nodes, as outlined below. Details of the projects joint projects are given in the Scientific Research Report (Appendix).

1. **UCT-Wits-SU.** The project on the biosynthesis and function of molybdopterin in mycobacteria is a strong collaboration between the UCT and Wits nodes. A key researcher in this project is Dr. Monique Williams, whose appointment is in the MRC/NHLS/UCT Molecular Mycobacteriology Research Unit (UCT node of the CBTBR) but is seconded full-time to the SU node.
2. **UCT-Wits.** Two students are being jointly supervised by Team Members from the UCT and Wits nodes – Duduzile Ndwandwe, supervised by Prof. Mizrahi (UCT node) and Dr. Warner (UCT node) and co-supervised Dr. Kana (Wits node); and Nicole Narrandes, supervised by Dr. Kana (Wits node) and co-supervised by Prof. Mizrahi. The second major collaboration between these nodes is on the TIA-funded SATRII project which involves Team Members and students from both the Wits (Dr. Gordhan) and UCT

nodes (Dr. Warner, Krupa Naran and Prof. Mizrahi). Researchers at both nodes together form the Biology component of the SATRII project, with the Wits node providing the biology support for the iThemba medicinal chemistry group led by Dr. Chris Edlin, and the UCT node supporting the UCT medicnal chemistry group led by Prof. Kelly Chibale.

3. **WITS-SU.** Ms. Philippa Black from the SU node spent approximately 6 months at the Wits node working on generation and characterization of transposon mutant libraries in the presence of rifampicin and a reporter construct for an important set of genes involved in energy metabolism. The work has resulted in collaboration between the SU and Wits nodes and coincides with the previous work from the Wits node on the mycobacterial electron transport chain.
4. **UCT-SU.** The UCT node's project on the physiology of drug-resistant mycobacteria forms part of a collaboration between a Team Member (Dr. Warner) and PhD student from the UCT node (Anastasia Koch) and Team Members Prof. Warren and Prof. Gey van Pittius from the SU node.
5. **UCT-SU.** The secondment of UCT node member, Dr. Monique Williams, to the SU node has enabled her to participate in several core SU projects studying drug resistance and efflux. Specifically, Dr. Williams been involved in designing of experiments for transcriptome analysis (MSc project – Melanie Grobbelaar) and has assisted a MSc. student (James Mozorodse) with designing a strategy for generating a deletion mutant in *M. smegmatis*. She is currently training a PhD student (Magareta De Vos) in the procedures involved in making allelic exchange mutants in *M. tuberculosis* and has assisted Philippa Black with the design and construction of a reporter plasmid which she will use in her MSc. Project. She has also assisted Mae Newton-Foot (PhD student) with the procedure for isolating RNA from *M. smegmatis*.

2. Education and Training

Breakdown of postgraduate students and postdoctoral fellows in the CBTBR in 2011

Student category	Number/percentage	Target based on SLA4 (for Performing Phase, 2009-2011)
Total number of students	77	≥ 25
% Postdoctoral fellows	15%	≥10%
% PhD students	43%	N/A
% MSc students	38%	N/A
% BSc (Hons) students	4%	N/A
% Women students	69%	≥ 50%
% Black students	51%	≥ 50%

Degrees conferred and postdoctoral fellowships completed

The CBTBR graduated 2 postdoctoral fellows, 4 PhD, 4 MSc, and 3 Honours students in 2011.

Dissertations and theses

MSc dissertation:

1. Anastasia Koch - "Fitness costs of drug resistance mutations in mycobacteria"
2. Zandile Mlamla - "Improving methods for genotypic drug resistance testing in Mycobacterium tuberculosis"
3. Nokwanda Crystal Ngombane - "Immune profiles of recently exposed household contacts in a Tuberculosis endemic setting in the Western Cape"
4. Prudy Seepe - "Differential expression of genes in clinical strains of mycobacterium tuberculosis in response to isoniazid"

PhD theses:

1. Bintou Ahmadou Ahidjo – "Analysis of the role of VapBC toxin-antitoxin modules in growth, stress tolerance and drug tolerance in mycobacteria"
2. Violet Chihota – "Use of genetic approaches to study the disease dynamics of *Mycobacterium tuberculosis* infection in Southern African regions with high tuberculosis incidence" Promoter: Prof RM Warren, Co-Promoter: Prof P van Helden.

3. Carrie Kirsten – "Nitrogen metabolism and the regulation thereof in *Mycobacterium smegmatis"* Promoter: Prof I Wiid.
4. Laurianne Loebenberg – "Environmental influences on innate and adaptive immune responses against *Mycobacterium tuberculosis"* Promoter: Prof G. Walzl

Research interns (MRC sponsored)

1. Ms NC Ngombane (Research Intern) Registered for 3rd year of MSc degree and graduated in 2011.
2. Ms P Seepe (Research Intern) Registered for 1st year of PhD degree in 2011

Recruitment of new postgraduate students

A number of new students have joined the team already or will do so during the course of 2012. 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, 10 PhD, 12 MSc and 2 Honours students into the CBTBR in 2011. At the UCT node 1 Postdoctoral fellow, 2 PhD students and one Honours student were recruited. At the Wits node 2 Postdoctoral fellows and 4 MSc students were recruited in 2011.

Honours and awards to students

- Mr. Germar Beukes from the Wits node was selected to give an oral presentation at the MRC Research Day and won the prize for best oral presentation by an MSc student.
- Dr. Melissa Chengalroyen from the Wits node won third prize for a poster presentation at the Wits Molecular Biosciences Research Thrust Symposium.
- Ms. Nicole Narrandes from the Wits node was selected to give oral presentations at two local conferences/meetings as testimony to the high quality of their research
- Ms. Duduzile Ndwandwe from the Wits node was awarded a K-RITH Collaborative Travel Award. Ms. Ndwandwe also received a Mellon Postgraduate Mentoring Award and a Postgraduate Merit award from Wits University.
- Dr. Christopher Ealand was awarded a Postdoctoral Fellowship from the Centre for the Aids Program of Research in South Arica.
- Ms. Lusanda Mapela was awarded an NRF prestigious MSc bursary.
- Mr. Sibusiso Senzani was awarded an Innovation NRF MSc bursary.
- Ms. Nabiela Moolla and Ms. Farzanah Hassim, newly appointed MSc students, were both awarded prestigious NRF bursaries
- Dr. Monique Williams and Ms. Atica Moosa were both awarded Keystone Symposia Global Health Travel Awards to attend the Keystone Symposium on Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics, held in Vancouver in January 2011.
- Ms. Anastasia Koch received the Benfara Scholarship from UCT and a DAAD-NRF Joint Scholarship from the NRF.
- Ms. Krupa Naran was awarded a UCT Equity Scholarship and a DAAD-NRF Joint Scholarship from the NRF
- Ms. Zanele Ditse was awarded the Marion Beatrice Waddel Scholarship and an Equity Scholarship from UCT and a DAAD/NRF Joint Scholarship from the NRF.
- Ms. Atica Moosa was awarded a Postgraduate Merit Award from Wits University.
- Stefanie Malan received a three month Novartis internship in drug discovery (1 July 2011 – 30 September 2011).
- Louw G. Visited as a Post Doctoral fellow at Harvard School of public health and Partners Organisation centre for Personalised genetic Medicine, Cambridge, USA.
- Louw G. Won 1st Prize in the Infectious disease session for a presentation by a young researcher under 35 years. Academic year day (August 2011), Stellenbosch University
- A number of Scholarships to attend the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
- Siame K., Basic Sciences Best Poster Prize at the 54th Academic Year day of the Faculty of Health Sc*iences* of Stellenbosch University with a poster presentation entitled "Investigating the genome variation and evolution of Group 1 *M. tuberculosis* strains".
- Bloem L. Second place for PhD poster presentation category at the 4th MRC Medical Research Day, 15th - 15th October 2011.

- Malan S. Novartis internship in drug discovery and clinical research. Basel, Switzerland. 1 July – 30 September 2011.
- Le Roex N. Award to attend EMBO (European Molecular Biology Organisation) computational biology course, entitled "Computational Biology: Genomes, Cells and Systems". Reykjavik, Iceland, 6-13 August 2011.
- Ehlers L. Award for Best Poster Presentation category Basic Sciences for, Effect of TB Treatment on Metabolic Hormone Profiles. Presented at Annual Academic Year Day. Cape Town, South Africa, 17-18 August 2011.
- Kleynhans L. Medroxyprogesterone acetate alters cytokine expression in response to Mycobacterium Bovis BCG in vitro and in PBMCs of contraceptive users. Poster winner at the Infectious Disease session at the Annual Academic Day. Faculty of Health Sciences, Stellenbosch University, Cape Town, 17-18 August 2011.
- Blanckenberg J. DRD Travel Grant: Research Visit Abroad. To attend a GEO-PD meeting. Chicago, USA, 19-21 September 2011.
- Ehlers L. Harry Crossley Foundation Scholarship awarded for, Investigation of the underlying molecular mechanisms of immune modulation by the contraceptive Medroxyprogesterone acetate (MPA) on immune response to mycobacteria. R14 000 awarded for 2010-2011.
- Salie M. (2011) Deutscher Akademischer Austausch Dienst-National Research Foundation (DAAD-NRF) Joint In-Country Scholarship.
- Salie M. (2011) Columbia University-Southern Africa Fogarty AIDS and TB Training and Research Program.
- Salie M. (2011) Harry Crossley Foundation (Project funding).
- Wagman C. (2011) National Research Foundation Scarce Skills Scholarship.
- Wagman C. (2011) Stellenbosch University Postgraduate Merit Bursary.

Hosting of international exchange students

The SU node hosted Mr Rick Henrikson a chemical engineer (Ph.D. candidate at the UC Berkeley/UC San Francisco USA) for 1 month to work on a nanotech device for diagnosis of XTR-TB.

Molecular Epidemiology Course

Prof. Rob Warren ran Molecular Epidemiology courses for African/Asian/South America fellows. In 2011 trainees Lubabalo Macingwana, Carine Kunsevi, Razeenah Hunter, Brigetta Derendiger, D Stanley from CCTR and for postgraduate students at the Honours level from the faculty of Health Sciences. 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.

FIND sponsored a HAIN test training course in Tanzania in 2011

Other Training courses

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

- PanACEA – RUNMC – HR1 protocol; 26 May 2011
- Pfizer B1171003 protocol; 02 August 2011
- REMoxTB Laboratory Manual and Quality Manual update; 18 August 2011

Training courses attended by staff and students

- Dr. Bavesh Kana from the Wits node of the CBTBR attended several courses at the Epidemiology and Population Health Summer Institute at Columbia University (EPIC). Some of the EPIC courses he took included a study of randomized clinical trials, an introduction to observational epidemiology, and statistical analysis of complex data using SAS and SUDAAN. 6-17 June
- Dr. Bhavna Gordhan and Ms. Nicole Narrandes participated in the Basic and Advanced Immunology course hosted by the Faculty of Health Sciences at Wits University from 28th February - 4th March and 4th-8th April 2011.
- Dr. Bhavna Gordhan, Ms. Nicole Narrandes, Ms. Suzanne Nicholson, Mr. Germar Beukes, Mr. Sibusiso Senzani, Ms. Farzanah Hassim and Ms. Nabiela Moola attended a Laboratory and Safety & chemical Grades Seminal at University of Johannesburg, hosted by Merck, 25th January
- Dr. Christopher Ealand, Ms. Nicole Narrandes, Mr. Sibusiso Senzani participated in IEFA Emergency First Aid Level I course at the NHLS in Johannesburg on 11 November 2011.

- Mr Keith Siame, Ms Anzaan Dippenaar, Ms Mae Newton-Foot and Ms Louise Vos attended a Proteomics workshop on Tygerberg Campus presented by Dr. Salome Smit in May 2011.
- Ms Mae Newton-Foot and Ms Nastassja Steyn attended a fluorescent Microscopy course at CAF on Main Campus on 2-3 June 2011.
- Drs André Loxton and Daleen Kriel attended a Conference presentation skills workshop at STIAS, Stellenbosch University on 24 May 2011.
- Prof Rob Warren accessed an On-line Good Clinical Practise course in 2011.
- Ms Marisa Klopper attended the Basic epidemiology course presented by Dr Jo Barnes at SACEMA in April 2011.
- Ms Michelle Daya attended the EBI 2 day workshop at UWC in March 2011.
- Ms Michelle Daya attended the 16th Summer Institute in Statistical Genetics, Seattle, Washington, USA in June 2011.
- Miss Nikki Le Roux attended the EMBO practical course: "Computational Biology: Genomes, Cells and Systems" held in Reykjavik, Iceland, 6-13 August 2011.
- Ms Anzaan Dippenaar attended a course on "High throughput sequencing in disease studies" at the London School of Hygiene and Tropical Medicine (LSHTM), 12-15 September 2011.
- Dr Bienyameen Baker attended an international workshop for postgraduate supervisors at Stellenbosch University in September 2011.

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

- Cedric Werely (PGWC) is a part-time 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 SUN-IRG organized a database, statistics and Excel training workshop for the African-European Tuberculosis Consortium in Addis Ababa in October 2011 for African scientists.
- The SUN-IRG organized a capacity development and networking meeting between EDCTP-funded TB, malaria and HIV research networks in Addis Ababa during the EDCTP Forum in October 2011.

Exchange visits

- Ms. Philippa Black from the SU node of the CBTBR spent ~ 6 months at the Wits node on an exchange visit. Ms. Black was trained at the Wits node on methodology for saturating transposon mutagenesis in *Mycobacterium tuberculosis*. In addition, Ms. Black learnt key new methodologies in gene cloning, gene manipulation and transformation of various mycobacterial species. Upon her return, this expertise has now been transferred to the SU node which has resulted in strengthening of collaborative linkages between the Wits and SU nodes
- Ms. Dudu Ndwandwe, a PhD student currently based at the Wits node with co-spuervision from Dr. Digby Warner and Prof. Valerie Mizrahi at the UCT node, received a K-RITH Collaborative Travel Award to support an exchange visit to the Harvard School of Public Health. The purpose of Ms. Ndwandwe's visit was to conduct a proteomics analysis of mutant strains generated during her PhD studies
- Dr. Monique Williams from the SU node is collaborating with Dr. Bavesh Kana and Ms. Nicole Narrandes at the Wits node on a project aimed further understanding the biosynthetic pathway for molybdopterin cofactor (MoCo) biosynthesis. This is a collaborative project that also involves the UCT node and will soon include Dr. Gilla Kaplan from the Public Health Research Institute in the USA. Dr. Williams has recently been awarded a CU-SA Fogarty Fellowship to spend three months in Dr. Kaplan's laboratory to obtain training on animal models for TB infection. Her work will specifically involve the use of mutant defective for MoCo biosynthesis
- Dr. Garth Abrahams was appointed on 1 April 2011 to the position of Research Officer in the UCT node of the CBTBR, funded by the HIT-TB grant (sub-award from FNIH on a grant from the Bill & Melinda Gates Foundation). This is a three-year contract position. Dr. Abrahams has been seconded, full-time to the laboratory of Dr. Clifton E. Barry III and Dr. Helena Boshoff (Tuberculosis Research Section, NIAID, NIH) for the duration of this contract appointment. He is working on the HIT-TB project and therefore interacts closely with the postdoctoral fellow in the UCT node who is also working on this project
- The IIDMM is a hub of tuberculosis and infectious disease immunology research and has a steady stream of international visitors throughout the year. Many of these visitors who included Prof David Russell (Cornell

University), Prof. Gilla Kaplan (UMDNJ), Prof. Douglas Young (Imperial College), Prof. William Bishai (K-RITH & JHU), and Dr. Ken Duncan (Bill & Melinda Gates Foundation) send time with staff and students in the UCT node

- Dudu Ndwandwe and Dr. Digby Warner received a K-RITH Collaborative Travel Award to support an exchange visit by Ms. Ndwandwe to the laboratory of Dr. Sarah Fortune at the Harvard School of Public Health. The purpose of this visit was to continue the proteomics work that Dudu had initiated in Dr. Fortune's lab during her Fogarty AITRP Pre-doctoral training fellowship in 2010. Ms. Ndwandwe's visit was unfortunately cut short owing to illness in her family. The visit was nonetheless reasonably productive and generated some new data as part of Ms. Ndwandwe's doctoral project
- Louw G. Visited as a Post Doctoral fellow at Harvard School of public health and Partners Organisation centre for Personalised Genetic Medicine, Cambridge, USA
- Doctoral student Andile Ngwane received a Fogarty Fellowship for a research training visit for 3 months (January-March 2011) at the Public Health Research Institute (PHRI) in New Jersey, USA.
- PhD student Melanie Grobbelaar visited the lab of Prof WR Bishai for Microarray training at the Johns Hopkins University, Baltimore, Maryland, USA in May – July 2011
- Tom Evans of AERAS visited on 16 Feb 2011 for discussions on future collaborations
- Jill Winter of the Catalysis Foundation spent time with the SUN-IRG at the field sites and laboratory for the ongoing study
- Prof Cliff Barry of the NIH visited on the 28 July 2011 as part of the ongoing collaboration
- Dr Leander Grode of the vaccine company, VPM , Germany visited several times to discuss the ongoing vaccine trial
- Prof Henry Boom of Cleveland University, USA, visited on 7-8 November 2011 for the TBRU meeting and the ongoing proteogenomics collaborative study
- Alex Lastovich, senior manager at BD, USA visited for the kick-off meeting of the BDFACSCAP study on 5 December 2011
- Prof Eileen Hoal visited Prof Erwin Schurr at McGill University, Montreal in October to discuss ongoing collaboration
- Profs Eileen Hoal and Paul van Helden visited Prof Vic Rutten and Dr Ad Koets at Utrecht Veterinary School, Netherlands, in June to discuss ongoing collaboration.
- Dr David Hain (Hain Lifescience, Germany), Prof Christie Jeon (Dept Epidemiology, Harvard University), Prof Eric Bottger (Institute of Medical Microbiology, University of Zurich), Prof Goerge Huber (University of Pittsburgh, USA), Prof William MacKenzie (Centers for Disease Control, USA), Dr Patrice Matchaba (Novartis, USA) and Dr Bernd Eisele (Vakzine Projekt Management, Germany) all visited to discuss collaborative projects with the SU node
- Dr C de Chastellier, INSERM, France – November - Discussions on EM and Mycothiol pathway

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 the 2011 training course on Drug resistance: Dezemon Zingue from Burkina Faso, Aristid Herve Ekollo Mbange and Pride Mbuh Teyim from Cameroon, Dieudonne Ouassa Timothee from Cote d'Ivoire, Samuel Kudzawu from Ghana, Imane Chaoui from Morocco, Ruth Umoh and Emmanuel Chibuike Wenah from Nigeria, Muataz Eldirdery from Sudan, Leila Jeljeli from Tunisia and Patrick Kaonga from Zambia.

For the past 11 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 35 students were trained between 2004 and 2011. The technologies and experience have helped other countries in Africa to get a better understanding of tuberculosis, and the value of these initiatives are shown in collaborative papers.

3. Knowledge Brokerage

The operational environment

All three 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 the three 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. 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 advice to international organisations, such as GATB and WHO.

Knowledge translation to stakeholder groups

CBTBR members were involved in numerous public awareness activities countrywide in 2011:

Public awareness, public engagement, and publicity

- Prof. Mizrahi was interviewed by the Wellcome Collection in London as part of the *Exchanges at the Frontier* program. Her interview was broadcast by BBC World Radio on 10 and 11 December 2011 to an estimated international audience of >40 million listeners. The interview is available as a podcast from www.bbc.co.uk/programmes/p00lzhr6.
- Prof. Warren gave a talk on Tuberculosis to members of the SANDF at Youngsfield Military Base
- Prof. Warren presented two lectures in the MBChB module on Infections and Clinical Immunology in 2011. Title: Molecular Epidemiology of Drug Resistant TB in South Africa.
- Numerous radio, TV and newspaper interviews locally and abroad. Owing to extreme administrative burden, opportunistic interviews, no accurate records were kept.
- Du Plessis N. Presentation: Worms and Tuberculosis: Do co-infections shape disease outcome? New Voices in Science colloquium. 2 December 2011.
- Kleynhans L. Presentation: Injectable contraceptives: Good or bad? New Voices in Science colloquium. 2 December 2011.
- In November 2011, Dr. Kana from the Wits node served on an expert panel at a workshop run by Discovery Health entitled: Award winning Health Journalism - lessons from the first three years of the Discovery Health Journalism Awards. This workshop was held at the Discovery Health headquarters in Sandton, Johannesburg and was facilitated by Prof. Harry Dugmore, interim director for the Discovery Centre for Health Journalism, School of Journalism and Media Studies, Rhodes University. The workshop was attended by several health journalists with video conferencing to participants in other areas of South Africa.
- Dr. Bavesh Kana served as a judge for the 2011 Discovery Health Journalism Awards.

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; since 1998, she has received support and encouragement for this work from different stake holders and has actively encouraged the participation of others in these events. Many of these activities have been undertaken with “outreach” funding from the CMCB or with the Community Liaison Office/Research Translation Office of the MRC, previously with Mrs Khalipha Ramahlape and more recently with Ms Benita Mayosi.

A highlight of 2011 has been the completion of phase three of the Wellcome Trust International Engagement WTIE grant awarded to Prof Corfield (Principal awardee) in partnership with the MTN Sciencentre, Cape Town. The first two phases of the project entitled “Catalysing partnerships: the role of science centres as intermediaries between the public and scientists in engagement with biomedical sciences in South Africa” brought science centres and scientists together to make biomedical science issues more assessable to the general public. Regional workshops held in 2010 had brought together 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.

Consequently, a successful outcome of the project has been the planned “seeding effect” with independent development of existing and new public engagement activities across the country. Thus, in 2011, the

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"* (all developed originally by Prof Corfield) have been presented occasionally by Prof Corfield but generally by others at, *inter alia*, The Cape Town Science Centre, the Giyani Science Centre [Limpopo], the Gateway Science Centre and the Science Centres at the Universities of Pretoria, Limpopo, Kwa-Zulu Natal and Zululand.

The final phase three of the project was completed in December 2011 with the production of a "Handbook for South African Science Centre Communicators" and a website containing an electronic version of the handbook and supporting material (http://www.saastec.co.za/scibiolosa.htm) which has achieved the goal of empowering others to become involved in furthering public awareness and engagement in biomedicine and in examining the ethical and societal issues raised by new technologies.

Another highlight of science engagement activities in 2011 was Prof Corfield's participation at the Sixth Annual Science Centre World Conference, held in Cape Town. She was invited to participate in two panel discussions; for the first one she presented a talk entitled "Can 'Science and Society' be squeezed into a one-size fits all science centre experience?" in which she examined the need to tailor activities for culturally/ linguistically different target audiences. The title of the second contribution "Promoting women in science centres across cultures; the contribution of science centres" examined the ways in which science centres can change help change mindset and raise awareness among young girl learners about careers in science and technology. The two posters presented by Prof Corfield outlined the role of SAWISE and the goals of the WTIE award and were entitled "SAWISE: South African sisters in science and engineering network with science centres" and "Catalysing partnerships: can science centres bridge the gap and promote dialogue between scientists and the public in biomedicine?", respectively

An interesting spinoff of the WTIE workshops conducted in phase two has been the further development by Prof Corfield in 2011 of the use of the Murder Mystery genre to engage the general public in the science underpinning DNA forensics and in the ethical issues that this technology raises. Prof Corfield has written a number of "who-dun-it" scenarios which have been used in public engagement activities, viz., at Scifest Africa 2011, the University of Limpopo Science Centre schools' programme and at the AGM of South African Women in Science and Engineering (SAWISE), an organisation to strengthen the role of women in science and engineering.

During 2011, Prof Corfield was involved in other activities that furthered public awareness of various aspects of science. One of these is the continued rollout of the DNA Project, an organisation which seeks to raise awareness of the importance of DNA forensic evidence through many activities. During 2011, Prof Corfield has presented lectures for the DNA Project to the University of the Third Age, to the National Prosecuting Authority and to the company responsible for developing the Project's advertising material. She has also been involved with a workshop, *DNA CSI*, which she has helped develop and has presented to the South African Police, Neighbourhood Watch groups, Rape and Women's Abuse Centres and schools. Prof Corfield has lead further training projects with the Public Understanding of Biotechnology programme (PUB), viz., facilitating and assessing the Basic Biotechnology programme of the University of Limpopo Science Centre and she undertaken an assessment of media reports of biotechnology for SAASTA (SA Association for Science and Technology Advancement).

In 2011, in response to the need to raise awareness of the range of health-related careers available to school learners, Prof Corfield updated a presentation she had previously prepared entitled "Careers in Health Care". She has presented this talk to the University of Stellenbosch's bridging programme and made it available to the MRC's Research Translation Office – who presented it at the Limpopo's 2011 Eding Science Festival. She also devised SAWISE's "meet-a-female-scientist" event for school learners.

The oral presentations skills workshop previously developed by Prof Corfield has also been updated and in 2011 was presented twice as part of the University of Stellenbosch's Research Capacity Development office's programme for lecturing staff and senior students and to the CMCB's BSc Honours students. In addition, a shorter version entitled "101 tips for a pitch perfect presentation" was given at an SAWISE workshop and at the Biannual Conference for Women in Engineering (Gauteng).

In summary, the activities in which Prof Corfield and members of CMCB/MRC were involved in 2011 are detailed below:

1 February: “Sci-speak to street-speak; How to de-jargonise genetics” workshop presented to Genetic Councillors UCT

9 February: *DNA:CSI* workshop Pinelands High School and Tamboerskloof Neighbourhood Watch

15-17 February Basic Biotechnology training workshop University of Limpopo Science Centre

18 February *DNA:CSI* workshop Rape Crisis Centre Khayelitsha

1 March *DNA:CSI* workshop Saartjie Bartman Centre Athlone

10 March “*DNA:CSI* what’s happening in South Africa?” seminar University of Stellenbosch

14-18 March Eding Fest, Polokwane. Part of MRC team, “HIV comes to the party” workshop and “Careers in Health Care”

24 March TB drama workshop at Delft clinic with Dr Gill Black

April 7 and 8 DNA forensics lecture to University of Third Age Hermanus and *DNA:CSI* workshop to Hermanus Neighbourhood Watch

May 4-6 Scifest Africa, Grahamstown. *DNA:CSI* workshop (award for best workshop); “The DNA Detective: what’s in your genes?” workshop. Basic Biotechnology workshop and DVD for teachers; Murder Mystery and part of MRC team.

June 1 and 2 “TIK’s TRICKS” teachers’ workshop, Primary Science Programme, Phillipi

June 21 SAWISE “101 tips for a pitch perfect presentation” CMCB

July 18 “Careers in Health Care” University of Stellenbosch bridging programme

July 20 and 21 DNA Project *DNA:CSI* workshop further development of a “train the trainers” programme

August 4 DNA forensics lecture for National Prosecuting Authority

August 10 *DNA:CSI* workshop Steenberg SAPs

August 18 Guest speaker University of Stellenbosch Faculty of Health Sciences Academic Year Day “The Science Communication Scene in South Africa: what’s happening and who *really* cares?”

August 22 SAWISE celebration of women in science – “Meet-a-female-scientist” schools’ career morning

September 4-8 Sixth Science Centre World Conference. Presented two orals and two posters.

September 22 *DNA:CSI* workshop Claremont SAPs

October 11-13 Facilitate and assess University of Limpopo Science Centre’s Murder Mystery and DNA forensics schools’ programme

November 18 “101 tips for a pitch perfect presentation” given at 2nd biannual women in Engineering conference

December 5 SAWISE “Murder in the ‘bos” Murder Mystery event Kirstenbosch

4. Networking

New networks and linkages

- The Wits node is playing the leading role in a new international collaboration between Wits University, the USA National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), the University of Leicester, the Perinatal HIV Research Unit (PHRU) and the Aurum Institute. This new collaborative project is led by Dr. Bavesh Kana involves the detection and characterization of non-replicating bacterial populations in the sputum of patients with active TB disease.
- Dr. Bavesh Kana from the Wits node collaborates with Dr. Gilla Kaplan and Dr. Dorothy Fallows from the Public Health Research Institute, USA, on a project to aimed at further understanding hetero-resistance to the first and second line antibiotics used to treat TB. This joint project, which is funded by the US National Institutes of Health, also involves participation of various members of the National TB Reference Laboratory and Siswe Clinic in Johannesburg.

- The Wits node has initiated a new collaborative venture with Drs. Stoyan Stoychev and Musa Mhlanga at the Council for Scientific and Industrial Research. This collaboration involves the use mass spectroscopy and super-resolution microscopy to determine the specific changes that occur in the cell wall of mycobacteria during different stages of TB disease and under stress conditions. Members of the Wits node, together with the CSIR, are currently in the process of developing key methodologies for this project. This particular collaboration promises to strengthen the ties between the CBTBR and a key industrial partner in SA.
- Dr. Bavesh Kana from the Wits node has initiated a new collaboration with Dr. Lesley Scott from the Department of Molecular Medicine and Hematology at Wits University. The ultimate aim of this partnership is to develop a robust external quality assurance system for rollout of the new GeneXpert TB diagnostic system. The Wits node is playing a critical role in this venture which will help to bolster the national TB control program further testifying to the impact of the CBTBR on TB control in South Africa.
- Further collaborative links at the Department of Molecular Medicine and Hematology at Wits University, through the Wits of the CBTBR includes a new collaboration with Dr. Melinda Suchard on characterization of the immune response in humans when challenged with mutant mycobacterial strains, defective in cell wall remodeling. The Wits node is also collaborating with Dr. Pierre Durand on the phylogenetic and evolutionary analysis of cell wall remodeling enzymes in various mycobacterial species.
- The Mycobacterial Referral Laboratory at the National Health Laboratory Service (Johannesburg, Central branch) has initiated a new collaboration with the Wits node which involves the characterization of discordant rifampicin resistant mutants in the Gauteng region. The strains selected for this study would comprise those samples that give inconsistent phenotypic resistance data when compared to the rifampicin resistance genotype provided by the line probe assay. The project represents an important partnership between the CBTBR and the national diagnostic platform for TB in South Africa.
- The SU node group of Prof Eileen Hoal, working on host genetics of TB, collaborates with the Computational Biology Group at UCT's IIDMM, headed by Prof Nicola Mulder. This project brings the bioinformatic power and expertise of Computational Biology to bear on the analysis of the SNP chip genetic data generated at the CBTBR.

Existing networks and linkages

The three 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.

NAME	INSTITUTION	NATURE/ PURPOSE, OUTPUTS AND FUTURE DIRECTION OF COLLABORATION
Dr. William Mac Kenzie	Centers for Disease Control and Prevention, USA	Collaboration on the detection and characterization of Rpf-dependent bacterial populations in sputum. Project funded by the NIH.
Prof. Michael Barer and Dr. Galina Mukamolova	University of Leicester, UK	Collaboration on the detection and characterization of Rpf-dependent bacterial populations in sputum. Project funded by the NIH
Dr. Gavin Churchyard	The Aurum Institute	Collaboration on the detection and characterization of Rpf-dependent bacterial populations in sputum. Project funded by the NIH. The Wits node also collaborating with Dr. Churchyard on several other ventures under the auspices of the Wits-Aurum Coalition.
Prof. Gilla Kaplan and Dr. Dorothy Fallows	Public Health Research Institute, International Center for Public Health,	Prof. Kaplan serves as the international member on the Board of the CBTBR. She and Dr. Kana serve on the CU-SA Fogarty AITRP Advisory Board. Furthermore, she visited the Wits node in November 2011. Dr. Kana

	Newark, NJ	collaborates with Dr. Kaplan and Dr. Fallows on an NIH funded project to study hetero-resistance in TB patients with active disease
Prof. Lesley Scott	University of the Witwatersrand	Ongoing collaboration on the rollout of the GeneXpert diagnostic test and establishment of an external quality assurance system.
Dr. Melinda Suchard	University of the Witwatersrand	Ongoing collaboration of immunological characterization of mutants defective for cell wall turnover/remodeling
Dr. Musa Mhlanga and Dr. Stoyan Stoychev	Council for Scientific and Industrial Research	Ongoing collaboration to develop methods for super-resolution microscopy in mycobacteria and to establish and optimize the technology for cell wall analysis in various mycobacterial strains.
Dr. Chris Edlin and Dr. Garreth Morgans	iThemba Pharmaceuticals	Ongoing collaboration on SATRII initiative for TB drug discovery
Dr. William Mac Kenzie	Centers for Disease Control and Prevention, USA	Collaboration on the detection and characterization of Rpf-dependent bacterial populations in sputum. Project funded by the NIH.
Prof. John D. McKinney	École Polytechnique Fédérale de Lausanne (EPFL), Switzerland	Collaboration on the mechanisms of propionate catabolism, funded by a grant from Swiss/ SA Joint Research Programme.
Dr. Clifton E. Barry III and Dr. Helena Boshoff	Tuberculosis Research Section, Laboratory of Host Defenses, National Institute of Allergy & Infectious Diseases, NIH, MD	Ongoing collaboration on the IMTB project, and new collaboration on the HIT-TB and SATRII projects
Prof. Česlovas Venclovas	Institute of Biotechnology, Vilnius, Lithuania	Ongoing collaboration on the structure and function of a novel mutagenic complex in mycobacteria.
Prof. Eric Rubin	Microbiology and Immunology, Harvard Medical School, USA	Collaborating member of IMTB Consortium
Prof. David Sherman	Seattle Biomed, USA	Ongoing collaboration on TB drug discovery under the auspices of the “IM TB” Consortium funded by the Bill & Melinda Gates Foundation
Prof. James Sacchettini & Dr. Tom Ioerger	Biochemistry & Biophysics, Texas A&M University, College Station, TX	Collaborating members of the “IM TB” Consortium. Also collaborating on whole-genome sequence analysis of strains of *M. tuberculosis*.
Prof. Sir Tom Blundell and Prof. Chris Abell	Cambridge University, UK	Collaborating members of the IMTB, HIT-TB and MM4TB Consortia
Prof. Chris Sassetti	University of Massachusetts, USA	Collaboration on carbon metabolism in *M. tuberculosis.* Co-authored one paper (in press). Also collaborating partner in IMTB consortium
Prof. Tanya Parish	Barts and the London, UK & Infectious Diseases Research Institute (IDRI), Seattle, USA	Collaborating member of IMTB COnsortium
Prof. Stewart Cole	EPFL, Lausanne, Switzerland	PI of the MM4TB Consortium

Prof. Vickery Arcus	AgResearch, University of Waikato, New Zealand	Collaboration on the role of VapBC toxin-antitoxin modules in the physiology of *M. tuberculosis*. Co-authored paper
Prof. Jonathan Blackburn	IIDMM, UCT	New collaboration on lipidomic analysis of *M. tuberculosis* strains
Prof. Susan Harrison	Chemical Engineering, UCT	New collaboration on TB drug discovery
Prof. Kelly Chibale	H3-D Drug Discovery Centre, UCT	Nre collaboration on SATRII and other TB drug discovery projects
A/Prof. Nicola Mulder	CBIO, IIDMM, UCT	New collaboration on bioinformatic analysis of mycobacterial genomes
Prof. Robert Wilkinson	CIDRI, IIDMM	Co-applicant on several new grant applications
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	K-RITH and University of Alabama, Birmingham	The ESAT-6 secretion system interactome (2007-present).
Prof. VPMG Rutten, Dr. I. van Rhijn, Dr. A.P. Koets	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 and Identification of ofloxacin and amikacin heteroresistance.
Prof D. van Soolingen	RIVM The Netherlands	Evolution of the Beijing genotype Lineage
Prof D. van Soolingen	RIVM The Netherlands	Evaluation of the MIRU-VNTR typing method
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 Bottger	University of Zurich	Development and evaluation of novel genetic based diagnostics for drug resistance; 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 Alkes Price	Harvard School of Public Health, Boston, USA	New collaboration. Analysis of admixture mapping.
Dr Brenna Henn	Stanford University, San Francisco, USA	Population Ancestry genetic determinations
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 K Jacobson	Harvard University	1) GIS of drug resistant TB in the Western Cape 2) MDR treatment outcome in Brewelskloof Hospital Treatment outcome of M(X)DR-TB in Khayelitsha
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 and Dr G de Souza	Bergen University and Oslo 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 Anita Schurch	RIVM, The Netherlands	Ongoing collaboration on *M. tuberculosis* genome evolution.
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	Collaboration on drug resistance in Khayelitsha, Western Cape. Impact of mixed infection on treatment outcome.
Prof. Tom Alber	Berkeley	Collaboration on the *M. tuberculosis* lipidome

Prof. Brigitte Gicquel	Pasteur Institute	Collaboration on mutation in *M. tuberculosis* DNA repair genes
Prof K Dheda	UCT	Molecular epidemiology of XDR-TB; Collaboration in diagnostic/biomarker project.
Prof R McNerney	LSTHM	Whole genome sequencing of drug resistant M. tuberculosis strains
Dr. Kim Mallard	LSTHM	Whole genome sequencing of *M. tuberculosis* strains
Prof Anab Pain	KAUST	Whole Genome Sequencing of Mycobacterial Species
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	LondonSchool 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 and haring of technology (multicolour FACS, Luminex machine), sharing of samples (manuscript accepted for publication.
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	Collaboration 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 N. Beyers, Dr A. Hesseling, Dr S. Tonkin, Prof B. Marais	DTTC, 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.
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.
Dr Else Marais	Wits/NHLS	Ongoing collaboration on the molecular epidemiology of drug resistant TB in Gauteng.
Prof C. Reinecke and 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. 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.
Dr. Anna Mandalakas	Case Western Reserve University, USA	Collaboration of diagnostic studies in paediatric TB.
Dr Jurgen Seier & Ms Charon de Villiers	MRC animal unit & MRC Delft Animal Facility	The implementation of TB testing of vervet monkeys, rhesus macaques and baboons. Dr Parsons also advise the MRC on the management of TB cases and outbreaks in these units.
Dr Lize van der Merwe	Biostatistics Unit, Medical Research Council, Cape Town	Statistical analysis of genetic data and studies such as gene-gene interaction.
Mrs. Lungi Kwitshana	MRC, Durban	Collaboration in project on worm-HIV co-infection.

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. Kana examined an MSc dissertation submitted to the University of KwaZulu Natal and several MSc/PhD project proposals submitted to the Faculty of Health Sciences, Wits University. Dr. Bhavna Gordhan examined protocols submitted for various degrees and further examined MSc dissertations submitted to UCT and Wits University.
- Dr. Warner examined a PhD thesis submitted to the University of the Witwatersrand, a MSc dissertation submitted to Stellenbosch University and Honours projects submitted to UCT.
- 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. Details are not kept.

Journal editing and reviews

- Dr. Kana reviewed manuscripts submitted *to FEMS Immunology and Medical Microbiology, Journal of Bacteriology and Antimicrobial Agents and Chemotherapy*.
- Dr. Gordhan reviewed a manuscript submitted to *Tuberculosis Research and Treatment*.
- Prof. Mizrahi served on the Editorial Advisory Boards of the *Biochemical Journal, Tuberculosis*, and *Cellular Microbiology,* and was appointed to the Editorial Board of *Emerging Microbes and Infection*. In 2011, she also reviewed manuscripts submitted to the following journals: *Cell, Cell Host & Microbe, Microbiology, Tuberculosis, mBio, EMBO Molecular Medicine, Journal of Bacteriology, PLoS Pathogens* and *Science Signalling*.
- Dr. Warner reviewed manuscripts submitted to *PLoS Pathogens, International Journal of Tuberculosis and Lung Disease, Molecular Microbiology, Nature Communications, Journal of Clinical Microbiology, Journal of Infectious Diseases, Journal of Bacteriology* and *Tuberculosis*.
- Dr. Evans reviewed manuscripts for *Clinical & Developmental Immunology* and the *International Journal of Tuberculosis and Lung Disease.*
- Dr. Gopinath reviewed manuscripts for *Archives of Clinical Microbiology*, and the *Journal of AIDS and HIV Research.*
- 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

- Dr. Kana serves on the Advisory Committee of the CU-SA Fogarty AITRP.
- Dr. Kana serves on the Steering Committee for the Wits-Aurum Coalition and chairs the Projects Committee for this venture
- Dr. Kana has been elected to serve on the University Research Council (URC) at Wits University
- Dr. Kana and Dr. Gordhan serve on the Faculty Research Council (FRC), Faculty of Health Sciences Wits University
- Dr. Kana and Dr. Gordhan serve on the Postgraduate Committee, Faculty of Health Sciences Wits University
- Dr. Gordhan serves on the Research Entity Review Task Group, Faculty of Health Sciences Wits University
- Dr. Kana chairs the Wits Health Sciences Research Day Committee (2012)
- Dr. Kana was elected to serve on the FRC Budget task group, Faculty of Health Sciences Wits University
- Dr. Kana serves on the Faculty Advisory Board, Faculty of Health Sciences Wits University

- Dr. Kana serves on the Executive Committee of the School of Pathology, Faculty of Health Sciences Wits University
- Dr. Kana serves on the Research Entity Forum, Faculty of Health Sciences Wits University
- Dr. Kana serves on the Faculty of Health Sciences Research Equipment Review Committee, Wits University
- Dr. Kana serves on the URC major and minor Equipment Review Committees, Wits University
- Dr. Kana serves on the Research Coordinators Committee, Faculty of Health Sciences, Wits University
- Dr. Kana serves on the Faculty of Health Sciences Imaging Committee, Wits University
- Dr. Kana serves on the Faculty Graduate Studies Committee Working Group on Research Output Guidelines, Faculty of Health Sciences, Wits University
- Dr. Kana serves on the Molecular Biosciences Thrust Committee, Faculty Health Sciences, Wits University
- In November 2011, Dr. Kana served on an expert panel at a workshop run by Discovery Health entitled: Award winning Health Journalism - lessons from the first three years of the Discovery Health Journalism Awards. This workshop was held at the Discovery Health headquarters in Sandton, Johannesburg and was facilitated by Prof. Harry Dugmore, interim director for the Discovery Centre for Health Journalism, School of Journalism and Media Studies, Rhodes University. The workshop was attended by several health journalists with video conferencing to participants in other areas of South Africa.
- On the 9th September, Bavesh Kana from the Wits node co-chaired the Tuberculosis session at the 4th Congress of the Federation of Infectious Diseases Societies of Southern Africa, held at the Elangeni Hotel in Durban. This session was particularly significant for the CBTBR since representatives of all three nodes were present at delivered presentations on ongoing work in their respective laboratories.
- Dr. Bavesh Kana served on a panel for the Sense about Science workshop held at Wits University aimed at assisting researchers with media interactions. At the workshop, Dr. Kana gave a short talk on his interest in science communication and provided some suggestions for improving interactions with journalists for a mutually beneficial outcome. The workshop was attended by several researchers within the Faculty of Health Sciences including Dr. Bhavna Gordhan from the Wits node of the CBTBR.
- Dr. Bavesh Kana served as a judge for the Discovery Health Journalism Awards.
- Prof. Mizrahi served as a member of the Board of Directors, KwaZulu Natal Research Institute for TB and HIV (K-RITH), University of KwaZulu Natal, South Africa
- Prof. Mizrahi served on the Council of Scientific Advisors of the International Centre for Genetic Engineering and Biotechnology (Trieste, Italy)
- Prof. Mizrahi served as a member of the Scientific Advisory Board of K-RITH, University of KwaZulu Natal
- Prof. Mizrahi served on the Search Committee of K-RITH, University of KwaZulu Natal, and also served as a member of the Stakeholders' Committee of K-RITH
- Prof. Mizrahi served as a member of the Scientific Advisory Committee of the Global Alliance for TB Drug Development (TB Alliance, New York)
- Prof. Mizrahi served as a member of the SACEMA Trust and also as a member of the SACEMA Management Board, Stellenbosch University
- Prof. Mizrahi chaired the Executive Committee and Membership Committee of the IIDMM, UCT
- Prof. Mizrahi served on the Management Committee of the IMTB Project, Seattle Biomed, USA
- Dr. Warner served as a member of the Steering Committee, iThemba Pharmaceuticals, Johannesburg
- Dr. Warner served as a member of the Institutional Biosafety Committee, UCT
- Dr. Warner served as a member of the GMO Committee, UCT
- Dr. Warner served as Lead Academic in charge of core Biological Safety Level III laboratory, IIDMM, UCT
- Dr. Warner served on the Administration and Operations Committee of the IIDMM, UCT
- Dr. Evans served on the IIDMM Health & Safety Committee of the IIDMM
- Dr. Evans chaired the UCT Health Sciences Postdoctoral Association and Dr. Gopinath served as a member of this committee
- Profs van Helden and Walzl served on MSF, GATB, WHO and Stop TB Partnership.
- 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, 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

- Dr. Kana and Dr. Gordhan participated in the review of several research/equipment/travel proposals in 2011. Dr. Kana served as a reviewer for the NRF Rating Program (for several applications), the NRF Competitive Grants for Rated Researchers Program and for several grants submitted to the KwaZulu –Natal Research Institute for TB and HIV. Dr. Kana also served as a reviewer on grants submitted to Wits University. Dr. Gordhan serves on the NRF Postdoctoral Review Panel and further assessed an SA/Swiss grant application for the NRF. Dr. Gordhan also serves as an international reviewer for the Welcome Trust/DBT India Alliance.
- MMRU staff participated actively in reviewing proposals submitted to international and local funding agencies/ institutions. Prof. Mizrahi served as a reviewer of proposals for the Institut Pasteur (Paris, France), the Wellcome Trust, the Bill & Melinda Gates Foundation and the NRF. She also served as a reviewer for promotion at the University of Massachusetts, McGill University, and Cornell University. Dr. Warner reviewed grant applications submitted to TWAS (2011 TWAS Research and Advanced Training Fellowship Program) (Trieste, Italy), the NRF, the Technology Innovation Agency (TIA), and K-RITH (Collaborative Grants Program).
- Many of the SU node members are either on editorial boards or act as regular reviewers for many journals. Again, a list is not provided, since we have so many of these we do not keep record.

Other services rendered

- Speciation of Non Tuberculous Mycobacteria (NTM) for 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 2011
- 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
- Weekly tutorials/practicals to Clinical Genetics registrars in molecular genetics by CMCB staff during 2011 (Prof Corfield and Mrs de Villiers).
- 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.
- Vakzine Project Management (VPM): phase IIa vaccine trial on tuberculosis
- Cellestis: Evaluation of new peptide to diagnose TB

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.

"Science by Women" (the participation by women in the research programme)
Five out of the 13 Core Team Members of the CBTBR are women. In 2011, the CBTBR has also maintained a high percentage of female students (69% of all students and 42% of postdoctoral fellows), which is in line with demographic norms for the Life and Health Sciences at a national level. All three 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	UCT/NHLS	F	W	100
Dr.	Gordhan	SA	Wits	F	B	100
Dr.	Kana	SA	Wits	M	B	100
Dr.	Warner	SA	UCT	M	W	100
Prof.	Gey van Pittius	SA	US	M	W	40[a]
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

a. Appointed as part-time Director of Research in May 2011

2. Postdoctoral Fellows

Title	Surname	Citizenship	Institution	Gender	Race	% Time spent in CBTBR
Dr.	Chegou	Cameroonian	SU	Male	Black	100
Dr.	Chengalroyen	South African	Wits	Female	Black	100
Dr.	Ealand	South African	Wits	Male	White	80[a]
Dr.	Evans	South African	UCT	Female	White	100
Dr.	Gopinath	Indian	UCT	Male	Black	100
Dr	Louw	South African	SU	Female	Black	100
Dr.	Loxton	South African	SU	Male	Black	100
Dr.	Möller	South African	SU	Female	White	100
Dr.	Muller	Swiss	SU	Male	White	50[b]
Dr.	Parsons	South African	SU	Male	White	100
Dr	Roetz	South African	SU	Female	White	100
Dr	Singh	Indian	UCT	Male	Black	65[c]

b. Commenced March 2011
c. Left the SU node in July 2011
d. Commenced May 2011

3. Students

Title	First Name	Surname	Degree	Institution	Race	Gender	Nationality	Status
Ms	Juanelle	Du Plessis	Hons	SU	White	Female	South African	Complete
Ms	Jade	Hotchkiss	Hons	UCT	White	Female	South African	Complete
Ms	Vuyiseka	Mpongoshe	Hons	SU	Black	Female	South African	Complete
Ms	Amanda	Axcell	MSc	Wits	White	Female	South African	Incomplete
Mr	Germar	Beukes	MSc	Wits	White	Male	South African	Incomplete

Ms	Philippa	Black	MSc	SU	White	Female	South African	Incomplete
Ms	Michelle	Daya	MSc	SU	White	Female	South African	Incomplete
Ms	Lizaan	Ehlers	MSc	SU	White	Female	South African	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	Farzanah	Hassim	MSc	Wits	Black	Female	South African	Incomplete
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
Ms	Angela Maria	Menezes	MSc	SU	White	Female	South African	Incomplete
Ms	Nabiela	Moolla	MSc	Wits	Black	Female	South African	Incomplete
Ms	Nicole	Narrandes	MSc	Wits (co-sup UCT)	Black	Female	South African	Incomplete
Mr	Paulin Essone	Ndong	MSc	SU	Black	Male	Gabonese	Incomplete
Mrs	Nokwanda Crysta	Ngombane	MSc	SU	Black	Female	South African	Complete
Ms	Khutso Germina	Phalane	MSc	SU	Black	Female	South African	Incomplete
Ms	Carine	Sao Emani	MSc	SU	Black	Female	Cameroonian	Incomplete
Mr	Sibusiso	Senzani	MSc	Wits	Black	Male	South African	Incomplete
Mr	Kabengele Keith	Siame	MSc	SU	Black	Male	Zambian	Incomplete
Ms	Nastassja Lise	Steyn	MSc	SU	White	Female	South African	Incomplete
Ms	Marisa	Tait	MSc	SU	White	Female	South African	Incomplete
Ms	Leani	Thiart	MSc	SU	White	Female	South African	Incomplete
Mr	Albertus	Viljoen	MSc	SU	White	Male	South African	Incomplete
Ms	Louise	Vos	MSc	SU	White	Female	South African	Incomplete
Ms	Chandré Kim	Wagman	MSc	SU	Black	Female	South African	Incomplete
Ms	Danicke	Willemse	MSc	SU	White	Female	South African	Incomplete
Ms	Bintou	Ahmadou Ahidjo	PhD	Wits (sup UCT)	Black	Female	Cameroonian	Complete
Mr	Marinus	Barnard	PhD	SU	White	Male	South African	Incomplete
Dr	Adane Mihret	Bekele	PhD	SU	Black	Male	Ethiopian	Incomplete
Ms	Maria Magdalene	Botha	PhD	CSIR	White	Male	South African	Incomplete
Ms	Natalie	Bruiners	PhD	SU	Black	Female	South African	Incomplete
Ms	Marieta	Burger	PhD	SU	White	Female	South African	Incomplete
Mrs	Violet	Chihota	PhD	SU	Black	Female	South African	Complete
Ms	Margaretha	de Vos	PhD	SU	White	Female	South African	Incomplete
Ms	Zanele	Ditse	PhD	UCT	Black	Female	South African	Incomplete
Ms	Nelita	Du Plessis	PhD	SU	White	Female	South African	Incomplete
Mr	Zhuo	Fang	PhD	SU	Black	Male	Chinese	Incomplete
Ms	Catriona Jane	Kirsten	PhD	SU	White	Female	South African	Complete
Ms	Leanie	Kleynhans	PhD	SU	White	Female	South African	Incomplete
Ms	Anastasia	Koch	PhD	UCT	White	Female	South African	Incomplete
Mrs	Lungile	Kwitshana	PhD	U Kwazulu Natal	Black	Female	South African	Incomplete
Ms	Nikki	Le Roux	PhD	SU	White	Female	South African	Incomplete
Ms	Laurianne	Loebenberg	PhD	SU	White	Female	South African	Complete
Ms	Charmaine	Mlambo	PhD	SU/Wits	Black	Female	South African	Incomplete
Ms	Zandile	Mlamla	PhD	SU	Black	Female	South African	Incomplete
Ms	Atica	Moosa	PhD	Wits (sup UCT)	Black	Female	South African	Incomplete

Ms	Matsie	Mphahlele	PhD	SU	Black	Female	South African	Incomplete
Ms	Krupa	Naran	PhD	UCT	Black	Female	South African	Incomplete
Ms	Duduzile	Ndwandwe	PhD	Wits (sup UCT	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
Mr	Muneeb	Salie	PhD	SU	Black	Male	South African	Incomplete
Ms	Prudy Mashika	Seepe	PhD	SU	Black	Female	South African	Incomplete
Ms	Michelle	Smit	PhD	SU	White	Female	South African	Incomplete
Mrs	Anzaan	Steenkamp	PhD	SU	White	Female	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

4. Administrative and Other Staff

Title	Surname	Position	Based at	Gender	Race
Dr	Abrahams[a]	Scientific Officer	UCT	M	B
Dr	Baker	Project Manager	SU	M	B
Mrs	Hull-Conrad[b]	Part-time admin clerk	UCT	F	B
Ms	Peachy	Bookkeeper/ Admin. Assistant	WITS	F	B
Ms	Moremi[c]	Research Assistant	WITS	F	B
Ms	Nicholson[c]	Research Assistant	WITS	F	W
Ms	Ramchandra[c]	Research Assistant	WITS	F	B
Dr	Williams[d]	Senior Scientist	UCT	F	B

a. Appointed in Jan 2011. Seconded full-time to NIAID,NIH
b. Appointed in Jan 2011. Employed by NHLS.
c. Appointed in Jan 2011
d. Seconded full-time to SU node

OUTPUTS

* The Names in bold are CBTBR staff

Books / Chapters in Books (Total: 4)

***Muller B**, **Warren, RM**, **Williams M**, Bottger EC, **Gey van Pittius NC**, **Victor TC**. 2011. Acquisition, transmission and amplification of drug-resistant tuberculosis. In: Antituberculosis chemotherapy. Progress in Respiratory Research, volume 40, Karger, pp. 96–104.
Donald PR, **van Helden PD**. Progress in Respiratory Research – Vol 40, Antituberculosis Chemotherapy**.** Edited by Peter R Donald, and Paul D van Helden. Published by S Karger AG, Basel, Switzerland, 2011. ISBN 978-3805-596275.
Williams M, Müller B, Uys P, Victor TC, Warren RM, Gey van Pittius NC. (2011) Tuberculosis Recurrence: Exogenous or Endogenous? In: Antituberculosis chemotherapy. Progress in Respiratory Research, volume 40, Karger, pp 73-80.
Diacon AH, Maritz JS, Donald PR. (2011) Early bactericidal activity of antituberculosis agents. In: Antituberculosis chemotherapy. Progress in Respiratory Research, volume 40, Karger, pp 213-219.

Articles in Peer-Reviewed Journals (Total: 47)

Scott LE, Gous N, Cunningham BE, **Kana BD**, Perovic O, Erasmus L, Coetzee G, Koornhof H, Stevens W. 2011. Dried Culture Spots for Xpert MTB/RIF External Quality Assessment: Results of phase 1 pilot study from South Africa. J Clin Microbiol. **49:**4356-4360. [IF=4.220]
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Carugati M, Zanini F, Schiroli C, Gori A, Franzetti F, **Hanekom M, Van der Spuy GD, Gey van Pittius NC**, McEvoy CR, **Ndabambi SL**, **Victor TC, Hoal EG**, **van Helden PD**, **Warren RM**. (2011) Mycobacterial Interspersed Repetitive-Unit-Variable-Number Tandem-Repeat Analysis and Beijing/W Family of Mycobacterium tuberculosis. J Clin Microbiol. 49(7): 2780-2781. . [IF=4.220]
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De Souza GA, Arntzen MO, **Fortuin S**, Schürch AC, Malen H, McEvoy CR, Van Soolingen D, Thiede B, **Warren RM**, Wiker HG. (2011) Proteogenomic analysis of polymorphisms and gene annotation divergences in prokaryotes using a clustered mass spectrometry-friendly database. Mol & Cell Proteomics. 10: 10.1074/mcp.M110.002527 1-10. [IF=8.354]
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Jacobsen M, Repsilber D, Kleinsteuber K, **Gutschmidt A**, Schommer-Leitner S, **Black G, Walzl G**, Kaufmann SHE. (2011) Suppressor of cytokine signaling-3 is affected in T cells from TuberculosisTB patients. Clin Microbiol Infect. 17(9): 1323-1331. [IF=4.784]
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Schürch AC, Kremer K, **Warren RM,** Hung NV, Zhao Y, Wan K, Boeree MJ, Siezen RJ, Smith NH, Van Soolingen D. (2011) Mutations in the regulatory network that underlie the recent clonal expansion of a dominant subclone of the *Mycobcterium tuberculosis* Beijing genotype. Infection, Genetics and Evolution. 11: 587-597. [IF=3.086]
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Van der Spuy DA, Cader S, **Van der Spuy GD**, Westwood AT. (2011) Improving nutritional status of children with cystic fibrosis at Red Cross War Memorial Children's Hospital. J Paediatr Child Health. 47: 282-286. [IF=1.122]
Van Wyk AC, Marais BJ, **Warren RM**, Van Wyk SS, Wright CA. The use of light-emitting diode fluorescence to diagnose mycobacterial lymphadenitis in fine-needle aspirates from children. Int J Tuberc Lung Dis. 15(1): 56-60. [IF=2.557]

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Walzl G, Ronacher K, Hanekom W, Scriba TJ, Zumla A. (2011) Immunological biomarkers of Tuberculosis. Nature Rev Immunol. 11(5): 343-354. [IF=35.196]
Warren RM, Hoek K, **Sirgel F, Tait M, Gey van Pittius NC, Müller B, Streicher EM, Victor TC, Van Helden PD.** (2011) Emergence and treatment of drug resistant tuberculosis: A comedy of errors. South African Respiratory J. 16(4): 112-116. [IF=Not Available]

Non Peer-Reviewed Articles (Total: 4)

Van Helden PD. (2011) Adaptation, redundancy or resilience. EMBO Reports. 12(9): 872.
Van Helden PD. (2011) Algorithms and surrogate markers in translational research. EMBO Reports. 12(12): 1206.
Van Helden PD. (2011) Treasure our rarities. EMBO Reports. 12(7): 611.
Van Helden PD. (2011) Water. EMBO Reports. 12(1): 2.

Published Abstracts (Total: 0)

Technical Reports (Total: 0)

Products / Artefacts / Patents (Total: 0)

Conferences/Meetings Attended & Invited Talks/Seminars Presented (Total: 69)

Kana B. Mycobacterial heterogeneity in TB Infection - Extreme makeover for the cell wall. Howard Hughes Medical Institute Early Career Scientist Competition. Janelia Farm Research Campus, Ashburn, Virginia, USA, 7 November.
Kana B. Mycobacterial heterogeneity in TB Infection - Extreme makeover for the cell wall. Invited Plenary Lecture. MRC Research Day, MRC Conference Centre, Parow, Cape Town, 19-20 October.
Kana B. Remodelling the mycobacterial cell wall for better TB drugs. Invited oral presention. 4th Federation of Infectious Diseases Societies of Southern Africa, Durban, 8-11 September.
Beukes G, Mapela L, Kana B. The role of resuscitation promoting factors in peptidoglycan hydrolysis and reactivation from dormancy in *Mycobacterium smegmatis*. Oral Presentation. MRC Research Day, MRC Conference Centre, Parrow, Cape Town, 19-20 October.
Narrandes N, Mizrahi V., Kana B. Functional characterization of molybdopterin synthase encoding genes in mycobacteria. Oral Presentation. MRC Research Day, MRC Conference Centre, Parow, Cape Town, 19-20 October.
Gordhan B. The role of DNA glycosylases in mutagenesis and adaptation to stress in mycobacteria. Oral Presentation. MRC Research Day, MRC Conference Centre, Parrow, Cape Town, 19-20 October.
Williams M, Kana B, Mizrahi V. Phenotypic characterization of Δ*mobA* mutants of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Talk presented at the South African Society of Microbiology (SASM) Conference, Cape Town, 6-9 November 2011.
Mizrahi V. New tools for tuberculosis drug discovery: the development of novel screening strains of *M. tuberculosis* and application in target-based approaches. Invited lecture presented at the CIDRI and Imperial College Wellcome Centre for Clinical Tropical Medicine Joint Annual Meeting, University of Cape Town, 12-14 March 2011.
Abrahams GL, Savvi S, Kumar A, Hung A, Ciulli A, Sherman DR, Barry CE III, Boshoff HIM, **Mizrahi V**. The development of novel screening Strains of M. tuberculosis for use in target-led approaches to tuberculosis drug discovery. Plenary lecture delivered at the Eighth International Conference on the Pathogenesis of

Mycobacterial Infections, Saltsjöbaden, Sweden, 29 June – 3 July, 2011.
Mizrahi V. The development of novel screening strains of M. tuberculosis for use in target-led approaches to tuberculosis drug discovery. Plenary lecture delivered at the K-RITH Groundbreaking Symposium, Durban, 13-14 July 2011.
Gopinath K, Mizrahi V, Warner DF. Identification of genes required for vitamin B_{12} transport in M. tuberculosis. Oral presentation delivered at the Fourth Postgraduate Research Day, Faculty of Health Sciences, University of Cape Town. 7 September 2011.
Warner DF. Mycobacterial metabolism and human disease. Plenary lecture presented at the FIDSSA Congress, Durban, South Africa, 8-11 September 2011.
Gopinath K, Mizrahi, V., and Warner, D.F. Identification of genes required for vitamin B_{12} transport in *M. tuberculosis*. UCT Postdoctoral Retreat, Cape Town, 12 -13 September 2011.
Walzl G. Biomarkers for different forms of tuberculosis. Keystone Symposia. Vancouver, Canada. 16-20 January 2011 (Co-organiser and speaker at conference)
Hoal EG. Association of toll-like receptor 1, 2, 4, 8 and 9 genes with susceptibility to pulmonary tuberculosis. Invited talk presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Warren RM. Stellenbosch University, South Africa: New molecular epidemiology insights of tuberculosis in Southern Africa. Invited talk presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Le Roex N. Host genetics and disease susceptibility. Invited talk presented at WOTRO Midterm Symposium. Utrecht, Netherlands, 27-29 June 2011.
Victor TC. High infection pressure: implications on the TB epidemic in South Africa. Invited talk presented at the University of Antwerp, Belgium, 23rd September 2011.
Essone P, Kalsdorf B, Chegou NN, Loxton A, Kriel D, Schöllhorn V, Ernst M, Lange C, and Walzl G. Bifunctional T-cell derived cytokines allow for discrimination between latent and active tuberculosis. Invited talk presented at the ERS meeting during September 2011 in Amsterdam.
Warren RM. Characterizing Drug-Resistant Epidemics in South Africa. Invited talk presented at the Center for Communicable Disease Dynamics 2nd Annual Symposium. Boston, USA. 3-4 October 2011.
Walz G. Biomarkers of treatment response. Chronic Lung Disease Conference, Cape Town. November 2011.
Van Helden PD. Progress in TB vaccine development for humans and animals. Invited talk presented at the 30th World Veterinary Congress 2011. Cape Town International Convention Centre, Cape Town, South Africa. 10-14 October 2011.
Van Helden PD. Burden of disease from a medical perspective. Invited talk presented at the Coloquium on Zoonoses and neglected infectious diseases of Africa. Johannesburg, South Africa. 1-4 November 2011.
Van Helden PD. Diagnostic Development. Invited talk presented at the Coloquium on Zoonoses and neglected infectious diseases of Africa. Johannesburg, South Africa. 1
Van Helden PD. The African tuberculosis situation including Mycobacteria outside the TB complex species. Invited talk presented at the 46th Annual Meeting of The American Society for Veterinary Clinical Pathology. Nashville, Tennessee, USA, 3
Warren RM. Seminar presented on Drug-resistant TB for World TB Day. Youngsfield Military Base, Cape Town, 24 March 2011.
Warren RM. Emergence of Drug resistant Tuberculosis: A comedy of errors. Invited talk presented at the 4th FIDSSA Congress. Durban, South Africa, 8-11 September 2011.
Willemse D, Williams MJ, Victor TC. Regulation of efflux in rifampicin resistant mutants of Mycobacterium tuberculosis. Invited talk presented at the 55th Annual Faculty of Health Sciences Academic Year Day. Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Corfield V. Promoting women in science across cultures: the contribution of science centers. Talk presented at the 6th Science Centre World Congress. Cape Town, South Africa, September 2011.
Corfield V. "Science and Society" be squeezed into a one-size-fits-all Science Centre experience? Talk presented at the 6th Science Centre World Congress. Cape Town, South Africa, September 2011.
Mapela L, Beukes G, Gordhan B, **Kana B**. Resuscitation promoting factors in bacterial growth,

peptidoglycan remodelling and muropeptide production. Poster presentation. Gordon Research Conference on TB Drug Development. Il Ciocco Hotel and Resort, Lucca (Barga), Italy, 3-8 July.
Chengalroyen M, Kana B. The role of resuscitation promoting factor in the reactivation of *Mycobacterium tuberculosis* in sputum. Poster Presentation. MRC Research Day, MRC Conference Centre, Parow, Cape Town, 19-20 October.
Chengalroyen M, Kana B. The role of resuscitation promoting factor in the reactivation of *Mycobacterium tuberculosis* in sputum. Poster Presentation. Wits Molecular Biosciences Research Thrust Symposium, 7 December.
Williams M, Kana B, Mizrahi V. Analysis of molybdopterin cofactor biosynthetic genes in mycobateria. Poster presented at the Keystone Symposium on Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics (J4), Vancouver, Canada, January 5-20, 2011
Ndwandwe DE, Venclovas C, Mizrahi V, Warner DF A novel mutasome in *Mycobacterium tuberculosis***.** Poster presented at Keystone Symposiun on Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics. Vancouver, Canada, January 15 - 20, 2011.
Ndwandwe DE, Venclovas C, Mizrahi, V, Warner, DF A novel mutasome in *Mycobacterium tuberculosis***.** Poster presented at Fifth Annual New England TB Symposium on Vaccine Development: from Basic Research to Clinical Trials. June 23, 2011. Broad Institute, Cambridge, USA.
Williams M, Kana BD, Mizrahi V. Phenotypic characterization of Δ*mobA* mutants of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Poster presented at the MRC Research Conference, Cape Town, 21 October 2011.
Moosa A, Mizrahi V, Warner D F. A Mycobacterium-specific protein family member required for vitamin B_{12} biosynthesis in *M. tuberculosis*. Poster presented at Keystone Symposiun on Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics. Vancouver, Canada, January 15 - 20, 2011.
Koch A, Mizrahi V, Warner DF. The physiological implications of drug resistance mutations in mycobacteria. Poster presented at the 4th Postgraduate Research Day, Faculty of Health Sciences, University of Cape Town, 7 September 2011.
Naran K, Fenner C, Harrison STL, Mizrahi V, Warner DF. Characterization of the Antimycobacterial Effect of a Pseudomonas-Derived Activity. Poster presented at the 4th Postgraduate Research Day, Faculty of Health Sciences, University of Cape Town, 7 September 2011.
Naran K, Fenner C, Harrison STL, Mizrahi V, Warner DF. Characterization of the Antimycobacterial Effect of a Pseudomonas-Derived Activity. Poster presented at the South African Society of Microbiology (SASM) Conference, Cape Town, 6-9 November 2011.
Kleynhans L, Walzl G, Ronacher K. Medroxyprogesterone acetate alters mycobacterium bovis BCG induced cytokine expression in vitro and in PBMCS of contraceptive users**.** Poster presented at Tuberculosis: Immunology, Cell Biology and Novel Vaccination Strategies (Keystone meeting), Faimont Hotel, Vancouver, British Columbia, Canada, 15-20 January 2011.
Macingwana L. Investigation of a synergistic effect of Sulfamethoxazole and Trimethoprim in combination with first-line TB drugs as potential first-line combination drug regime against Mycobacterium tuberculosis. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Victor T. XDR-TB is evolving towards TDR-TB in the Eastern Cape, South Africa. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Gey van Pittius NC. A metabolomics approach to exploring the function of the ESX-3 type VII secretion system of M. smegmatis. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Louw GE. Rifampicin reduces susceptibility to ofloxacin in rifampicin resistant Mycobacterium tuberculosis through efflux. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Parsons S. Adaptation pf the QuantiFERON-TB gold (in tube) assay for the diagnoses of bovine tuberculosis in African buffaloes (Syncerus caffer). Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
van der Merwe R. The isolation, characterization and comparative genomics of novel South African

mycobacteriophages from soil samples. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Wiid I. Elucidation of the physiological roles of Glutamine synthesas and Glutamate dehydrogenase in *Mycobacterium smegmatis*. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Svenson S. Differential activation of macrophages and dendritic cells by MDR and Non-MDR Mycobacterium tuberculosis strains. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Källenius G. Beijing *Mycobacterium tuberculosis* genotype is emerging in Mozambique in association with HIV co-infection. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Källenius G. Interferon inducible protein-10 (IP10) as a marker for *Mycobacterium tuberculosis* infection. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Källenius G. Mycobacterium tuberculosis strains causing the TB epidemic in Sweden a century ago. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Källenius G. A protective role for CD103+ lung dendritic cells during pulmonary tuberculosis. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Källenius G. Bystander effects of mycobacteria-infected macrophages on dendritic cells and their ability to mediate HIV-1 trans-infection. Poster presented at the 8th International Conference on the Pathogenesis of Mycobacterial Infections. Stockholm, Sweden, 30 June – 3 July 2011.
Möller M, Adams LA, Nebel A, Schreiber S, van der Merwe L, van Helden PD, Hoal EG. Polymorphisms in MC3R promoter and CTSZ 3`UTR are associated with tuberculosis susceptibility. Poster presented at the 12th International Congress of Human Genetics (ICHG) and the 61st ASHG Annual Meeting, Montréal, Canada, 11-15 October 2011.
Möller M, Quintana-Murci L, de Wit E, Delport W, Harmant C, Rugamika CE, Meintjes A, Balanovsky O, Zaporozhchenko V, Bormans C, van Helden PD, Seoighe C, Behar DM, Hoal EG. The History in our Genes: the Complex Structure of the South African Coloured Population. Poster presented at the Joint International Conference of the African and Southern Africa Societies of Human Genetics, Cape Town, South Africa, 6-9 March 2011.
Salie M, Möller M, Duh F-M, Martin MP, Hoal EG, Carrington M. The role of killer cell immunoglobulin-like receptors and their human leukocyte antigen class-I ligands in susceptibility to tuberculosis. Poster presented at the Joint International Conference of the African and Southern African Societies of Human Genetics. Cape Town, South Africa, 6-9 March 2011.
Le Roex N. Solid SNPs in Syncerus: Novel SNP detection in African buffalo. Poster presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Ehlers L, Chegou NN, Walzl G, van Helden PD, Ronacher K. Effect of TB Treatment on Metabolic Hormone Profiles. Poster presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Möller M, Adams LA, Nebel A, Schreiber S, van der Merwe L, van Helden PD, Hoal EG. Polymorphisms in MC3R promoter and CTSZ 3`UTR are associated with tuberculosis susceptibility. Poster presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Siame KK. A phylogenomic and Proteomic Investigation into the Evolution and Biological Characteristics of the Principal Genetic Group 1 Members of *Mycobacterium tuberculosis*. Poster presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Steyn NL. Stellenbosch University, South Africa. Investigating the localization of ESX-3 secretion system components in Mycobacterium smegmatis. Poster presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Van der Merwe C. An investigation into the role of mitochondrial dysfunction in Parkinson's disease. Poster

presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Wagman C, Möller M, van der Merwe L, Hoal EG. Investigating single nucleotide polymorphisms and interactions of candidate genes involved in susceptibility to tuberculosis in a South African population. Poster presented at the 55th Annual Faculty of Health Sciences Academic Year Day, Stellenbosch University, Cape Town, South Africa, 17-18 August 2011.
Corfield V. Catalysing partnerships: can science centres bridge the gap and promote dialogue between scientists and the public in biomedicine? Poster presented at the 6th Science Centre World Congress. Cape Town, South Africa, September 2011.
Corfield V. SA WISE: South African sisters in science and engineering network with science centres. Poster presented at the 6th Science Centre World Congress. Cape Town, South Africa, September 2011.
Ehlers L. Effect of TB Treatment on Metabolic Hormone Profiles. Poster presented at the Medical Research Council Research Day. Cape Town, South Africa, 19-20 October 2011.
Willemse D, Victor TC, Williams MJ. Regulation of efflux in rifampicin resistant mutants of *Mycobacterium tuberculosis.* Poster presented at the Medical Research Council Research Day. Cape Town, South Africa, 19-20 October 2011.

Other Relevant Outputs (including honours and awards to staff)

In October 2011, Dr. Bavesh Kana from the Wits node was invited to give the opening plenary lecture at the MRC Research Day, held at the MRC Conference Centre in Parrow. Dr. Kana's lecture was attended by all participants of the Research Day, the acting president of the MRC and some members from the MRC Board. Dr. Kana was invited on the basis of being an MRC Career Awardee.
Dr. Kana from the Wits node received a renewal for his MRC Career Development Award. This was based on a competitive renewal process for this program and provides a further two years of funding to the Wits node.
Dr. Bhavna Gordhan from the Wits node was selected to give oral presentations at two local conferences/meetings as testimony to the high quality of their research.
Prof. Mizrahi was invited to London for an interview under the auspices of the *Exchanges at the Frontier* program of the UK-based Wellcome Collection. The interview, which took place at the Wellcome Collection on 21 October 2011. An abridged version of the interview was broadcast by BBC World on 9 Dec 2011 to an estimated audience of 40-50 million listeners worldwide. The podcast is available at http://www.bbc.co.uk/iplayer/episode/p00lzhr6/Exchanges_At_The_Frontier_Episode_3_Valerie_Mizrahi/ and can be downloaded from http://www.bbc.co.uk/podcasts/series/discovery
Dr. Digby Warner was the overall winner of the prestigious 2011 BioVision-Lilly Award in conjunction with the Academy of Sciences for the Developing World (TWAS). The award recognises young researchers from developing countries for outstanding scientific achievements in tuberculosis-related research. The winners were announced at a special ceremony at the 7th Biovision World Life Sciences Forum
Dr. Digby Warner awarded a K-RITH Collaborative Travel Award.
Prof. Mizrahi was appointed to the Editorial Board of the international journal, *Emerging Microbes & Infection*
Dr. Digby Warner was appointed as an Associate Member of the IIDMM
Gey van Pittius, NC was elected as a member of the Academy of Science of South Africa (ASSAf)
Hoal, EG. Obtained a B2 rating by the NRF (2011)
Walzl G. Invited to the AERAS vaccine research workshop
Walzl G. Workgroup member of Endpoints and Biomarker Working Groups of the Critical Path to New TB Drug Regimens
Walzl G. A member of the core writing team of the International Roadmap for Tuberculosis Research

Progress of Students Who Have Qualified or Trained in the CBTBR (2005-2011)

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. 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	Sydney Brenner postdoctoral fellow in the lab of 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	2011	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	Chihota, V	PhD	2011	Unknown
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	Kirsten, C	PhD	2011	Remained in CBTBR as a postdoctoral fellow
Dr	Kleynhans, L	MSc	2009	Remained in CBTBR for a PhD degree
Ms	Koch, A	MSc	2011	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	Loebenberg, L	PhD	2011	Employed by AFRIPLEX in Paarl
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	Emigrated to Austria in July 2010
Ms	Magan, N	Hons	2009	Unknown

Dr	Magwira, C	Postdoctoral	2010	Took up a second postdoctoral fellowship in the RMPRU, Wits
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, and then to an position in a TB-focusd NGO in 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
Ms	Mlamla, Z	MSc	2011	Remained in CBTBR for a PhD degree
Dr	Moller, M	PhD	2007	Remained in CBTBR as a postdoctoral fellow
Dr	Mowa, B	PhD	2009	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	Remained in the CBTBR for a PhD degree
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
Ms	Ngombane, NC	MSc	2011	Returned to MRC
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	Completed first postdoctoral fellowship with prof. Brombacher and took up a second in Chemical Engineering at UCT
Ms	Seepe, P	MSc	2011	Remained in CBTBR for a PhD degree
Dr	Sholto-Douglas-Vernon, C	PhD	2005	Employed at St. George's Hospital, London
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	Moved from NHLS to UCT as CBTBR Team Member
Dr	Williams, M	Postdoctoral	2009	Given an MRC-funded post in the MMRU (Wits, then UCT node), seconded to SU node in 2010 for 3 years.
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 2011 are currently under review by the external auditors and will be forwarded to the Board as soon as it becomes available.

APPENDIX: Detailed Scientific Research Report

THEME I: FUNDAMENTAL RESEARCH ON ASPECTS OF THE PHYSIOLOGY AND METABOLISM OF MYCOBACTERIA OF RELEVANCE TO TUBERCULOSIS DRUG RESISTANCE AND DRUG DISCOVERY

The biosynthesis, transport and function of vitamin B_{12} in mycobacteria. The first project under this theme forms part of Swiss/SA Joint Research Program (SSAJRP)-funded study with Prof. John McKinney (EPFL, Lausanne) on the mechanisms of propionate catabolism in *M. tuberculosis*. Vitamin B_{12} is synthesized exclusively by prokaryotes, and only a few of them possess the complete machinery for *de novo* biosynthesis. Included among these organisms is *M. tuberculosis*. Prior work from the Wits node of the CBTBR has demonstrated the function of B_{12}-dependent enzymes in core metabolic pathways and the ability to utilize exogenous B_{12} and precursors (Dawes *et al*. 2003; Warner *et al.* 2007; Savvi *et al.* 2008). A key question in this regard is: does *M. tuberculosis* synthesize B_{12} during infection or does it satisfy its B_{12} requirements from the host sources? This knowledge is crucial to understand the potential role of B_{12}-utilizing pathways in *M. tuberculosis* pathogenesis, but is complicated by the absence of an obvious B_{12} transporter in the M. tuberculosis genome. To address this question, whole-genome random transposon mutagenesis was applied in an initial screen to identify genes whose disruption restored the ability of a B_{12}-sensitive (Δ*metH*) strain to grow on solid medium supplemented with B_{12} at high concentrations. Subsequently, the sensitivity of these "B_{12}-resistant" mutants to propionate toxicity was then assayed in a secondary screen, which reduced the number of isolates predicted to contain insertions in B_{12}-transport or assimilatory genes from 612 to 84. Of the 37 identified transposon insertions, 35 were in one gene, Rv1819c, which encodes a homologue of the *bacA* gene that has been characterized in other organisms. This finding was significant as this gene had previously been characterized in the laboratory of Dr. Clifton Barry (NIAID) as an ABC-type transporter of unknown function, but which is essential for chronic infection *in vivo* (Domenech *et al.* 2009). On testing the B_{12} transport capability of a targeted *bacA* deletion mutant of *M. tuberculosis* and its complemented derivative, Dr. Gopinath confirmed that BacA is indeed a major B_{12} transporter in *M. tuberculosis*. Previously, it was demonstrated that a small proportion of B_{12}-resistant suppressor mutants (B_{12}^R) of the Δ*metH* strain of *M. tuberculosis* contained mutations in the highly conserved B_{12} riboswitch upstream of the *metE* gene, which encodes the B_{12}-independent methionine synthase enzyme. Based on the transposon screening results, it was reasoned that the remaining suppressor mutants might carry mutations in *bacA*, which would allow the Δ*metH* strain to grow in the presence of B_{12}. As predicted, of 8 B_{12}^R mutants, 3 carried mutations (non-synonymous SNPs) in *bacA,* thereby providing strong supporting evidence for the proposed role of *bacA* in B_{12} transport. In their previous study, Domenech *et al.* (2009) reported that deletion of *bacA* resulted in increased resistance to bleomycin. Consistent with this result, both Δ*metH:Tn bacA* and ∆*metH* B_{12}^R mutants also showed resistance to bleomycin. To validate this result, spontaneous bleomycin resistant mutants of the ∆*metH* and ∆*prpDC* mutants were isolated by plating on supra-inhibitory concentrations of bleomycin. Importantly, all of the resulting mutants were unable to transport B_{12} thereby confirming that *M. tuberculosis* BacA has dual functionality in the transport of both vitamin B_{12} and bleomycin. In summary, BacA has been identified as a major vitamin B_{12} transporter in *M. tuberculosis*. The identification of a vitamin B_{12} transporter in *M. tuberculosis* represents the major highlight from the UCT node in 2011**.**

The second SSAJRP-funded project forms the basis of Ms. Atica Moosa's PhD. PPE family proteins are limited to the genus *Mycobacterium*, and are present in elevated numbers in pathogenic mycobacteria, yet their functional characterization remains elusive. Of the more than sixty *PPE* genes in *M. tuberculosis*, *PPE2* is unusual in possessing directly upstream of its predicted start codon one of only two B_{12}-dependent riboswitches in the *M. tuberculosis* genome. By generating a panel of mutant *M. tuberculosis* strains containing disruptions in *PPE2* and/or related vitamin B_{12} biosynthetic (*cobK, cobU*) or B_{12}-dependent genes (*metE*), Ms. Moosa demonstrated that *PPE2* does not function in vitamin B_{12} transport in *M. tuberculosis*. Instead, work carried out over the past year has revealed that *M. tuberculosis* can synthesize vitamin B_{12} *de novo* if liquid media is supplemented with cobalt, the metal ion which lies at the heart of the vitamin B_{12} co-factor. Moreover, the ability to utilise exogenous cobalt is dependent on a functional *PPE2*. In combination, these observations implicate this PPE family protein in the assimilation of cobalt in *M. tuberculosis*. As the first confirmed assignment of a biochemical function to a PPE protein, this work represents another research highlight from the UCT node. Structurally, vitamin B_{12} comprises a central corrin ring co-ordinated by α- and β- axial ligands. In the active form, the β ligand consists of 5'-deoxyadenosine or a methyl group, with 5,6-dimethylbenzimidazole (DMB) at α; substituting DMB with adenine yields pseudovitamin B_{12}. Through a panel of mutant strains, the capacity of mycobacteria to synthesise pseudovitamin B_{12} and utilise this alternate

cofactor to support B_{12}-dependent metabolism was investigated. The findings indicated that *M. tuberculosis* requires exogenous cobinamide (Cbi) plus DMB to synthesize vitamin B_{12} aerobically, and also demonstrated the apparent ability of *M. tuberculosis* to synthesize and utilize pseudo-B_{12} when provided with adenine (AD) and Cbi. However, pseudo-B_{12} biosynthesis could not be claimed with certainty owing to the ability of the mutant (Δ*metE*) to grow in media supplemented with Cbi only. Moreover, unlike *M. smegmatis*, an adenine titration in *M. tuberculosis* did not provide adequate resolution of this issue. Finally, recent studies from another group have provided insight into the synthesis of the α-ligand, in particular the critical role of *bluB* in the formation of DMB (Taga *et al.,* 2007). It was hypothesized that deletion of the putative *M. tuberculosis bluB* homologue (Rv0306) would enable the definitive assessment of the ability of *M. tuberculosis* to synthesize and utilize pseudo-B_{12}.This hypothesis is currently being addressed. While completing the experimental part of her PhD over the past year, Ms. Moosa also made good progress on her thesis, which will be submitted by March 2012.

Molybdopterin cofactor biosynthesis in mycobacteria. This study is being carried out by Dr. Monique Williams (UCT - SU nodes) and is part of a collaboration with the Wits node (Dr. Bavesh Kana and Ms. Nicole Narrandes). Dr. Williams' part of the project is aimed at investigating the role of molybdopterin cofactor (MoCo) in pathogenesis of *M. tuberculosis* by generating a panel of mutants harboring deletions in genes involved in various steps in the MoCo biosynthetic pathway. In the past year, two groups of mutants were generated and phenotypically characterized. Firstly, Δ*mobA* mutants of *M. smegmatis* and *M. tuberculosis* were constructed and shown to be defective for the synthesis of *bis*-molybdopterin guanine dinucleotide (*bis*-MGD), which is the form of the cofactor required by the majority of the MoCo-dependent enzymes identified in *M. tuberculosis* by bioinformatic analysis. The absence of the cofactor in these mutants was confirmed by their inability to assimilate nitrate, a function requiring the activity of *bis*-MGD-dependent enzymes. The second group of mutants harbored in-frame deletions in one of the three *moaD* homologues in the virulent, PDIM-producing laboratory strain, H37RvMA, namely, Δ*moaD1*, Δ*moaD2* and Δ*moaX*. These strains are currently being used to generate double mutants which lack various combinations of these homologues, and will ultimately be used to generate a strain that lack all three *moaD* homologues. The strains will be used to assess the contribution of each of the homologues to the capacity of *M. tuberculosis* to synthesize MoCo. In order to do this, Dr. Williams is currently developing a mass spectrometry-based method for the direct quantification of the cofactor. Dr. Williams has been awarded a three-month CU-SA Fogarty AITRP fellowship which will enable her to carry out in vivo phenotyping of selected MoCo-deficient strains in the lab of Prof. Gilla Kaplan (PHRI, UMDNJ). The second component of this project is being conducted by Ms. Nicole Narrandes (MSc student) at the Wits node. Characterization of the MoCo biosynthesis pathway in *M. tuberculosis* by Dr. Williams revealed that there is genetic redundancy for the MPT-synthase encoding genes (*moaD1*, *moaD2*, *moaE1* and *moaE2*) and further identified a fused molybdopterin synthase, MoaX. The functional consequences of this were studied by Ms. Narrandes in a heterologous expression system, which involved the expression of these genes in mutants of *M. smegmatis* – a non-pathogenic mycobacterial species – defective for MPT-synthase. The above mentioned genes were cloned into integrative vectors followed by expression of different gene combinations in *M. smegmatis* and the functionality of the reconstituted MPT-synthase was measured using conditions that required a MoCo dependent enzyme, nitrate reductase, for growth. Analysis of these strains revealed that a single, integrative copy of the gene of interest was not adequate to achieve sufficient expression levels for heterologous complementation. To obtain a higher gene dosage, multi-copy episomal vectors carrying different combinations of the *M. tuberculosis moaD1*, *moaD2*, *moaE1* and *moaE2* genes, under the control of a constitutive promoter, were constructed. These vectors were introduced into *M. smegmatis* and growth assays indicated that only the *moaD2*+*moaE2* combination resulted in the formation of a functional MPT-synthase in this strain. Biochemical analyses in other organisms have demonstrated that the mature MPT synthase is a multimeric enzyme comprising of 2 MoaD and 2 MoaE subunits, suggesting that cleavage of MoaX, which retains distinct MoaD and MoaE domains, is required to generate a functional multimeric enzyme. To determine if post-translational cleavage of MoaX occurs, the protein was FLAG-tagged and its relative mobility was assessed by western blot analysis. Preliminary data confirm that MoaX is indeed cleaved into its constituent MoaD and MoaE subunits. Cleavage of MoaX is now being further investigated through site directed mutagenesis of key residues in the predicted cleavage site. The heterologous complementation assay used in this study invokes growth on nitrate using the nitrate assimilation pathway and nitrate reductase. It has been hypothesized that nitrate assimilation in *M. smegmatis* is due to the function of a MoCo-dependent enzyme, NarB. To confirm this, the *narB* gene was deleted in the organism however, the resulting mutant was still able to assimilate nitrate. This suggested that another enzyme may play a role in nitrate assimilation, possibly the *narGHI*-encoded respiratory nitrate reductase which plays a central role in nitrate assimilation in

M. tuberculosis. To test this hypothesis, the *narGHJI* operon was deleted from wild type *M. smegmatis* and in the Δ*narB* mutant background. The resulting mutants were genotypically confirmed by PCR and southern blot analysis and assessed for nitrate assimilation. Surprisingly, both the Δ*narGHJI* single mutant and the Δ*narB* Δ*narGHJI* double mutant were still able to assimilate nitrate, implicating yet another or several other enzymes in nitrate assimilation in *M. smegmatis*.

Mechanisms of DNA repair, replication and mutagenesis in mycobacteria. Research over the past year has focused on developing tools for studying the protein interaction network involving the mutasome components, Rv3394c and Rv3395c, *in vivo*, and on generating recombinant forms of the proteins suitable for structural work. The *M. smegmatis* homologues of Rv3394c (MSMEG_1622) and Rv3395c (MSMEG_1620) were epitope-tagged with the TAP tag and functionally assessed by determining the ability to rescue the DNA damage hypersensitivity phenotype of mutant strains of *M. smegmatis* lacking one or both genes. C-terminal tagging of MSMEG_1622 was found to abrogate functionality of the protein, underscoring the importance of the C-terminal domain of this protein for DNA damage tolerance. N-terminal tagging of MSMEG_1622 with a 3XFLAG tag also disrupted the function of this protein. However, N-terminal, 3XFLAG tagging of MSMEG_1620 was well tolerated as evidenced by functional complementation of the DNA damage tolerance and induced mutagenesis phenotypes of the ∆1620 deletion mutant of *M. smegmatis*. This complemented ∆1620 strain carrying the tagged MSMEG_1620 protein is being used for pull-down experiments that are aimed at identifying interacting partners of this components of the mutasomal complex under conditions of genotoxic stress. Dr. Warner and Ms. Ndwandwe were granted a K-RITH travel award which enabled Ms. Ndwandwe to spend a month working in the laboratory of Dr. Sarah Fortune at the Harvard School of Public Health in June 2011. The purpose of this award was enable Ms. Ndwandwe to further the work that she had begun in 2010, as a CU-SA Fogarty AITRP pre-doctoral fellow in Dr. Fortune's lab, on comparative proteomic analysis of wild type, ∆*1622*, ∆*1620* and ∆*dnaE2* deletion mutants of *M. smegmatis* alongside complemented controls using state-of-the-art mass spectrometry-based proteomics. Analysis of the data generated from this set of experiments is currently underway. The second focus of this project has been on investigating systems for expression of recombinant forms of the Rv3394c and Rv3395c proteins in *E. coli*. This work has proven to be extremely challenging, with recombinants being either insoluble, or partly soluble, and refractory to purification.

A new project was initiated in 2011 which aims to determine key structure-function relations of the mycobacterial DNA replication machinery, with a special focus on the roles of the *dnaE1*- and *dnaE2*-encoded DNA polymerase III alpha subunits under conditions of genotoxic stress. A primary focus of this project is to establish whether the intrinsic proofreading domain in DnaE1 is a critical determinant of replication fidelity in *M. tuberculosis*. To investigate this possibility, the exonuclease proofreading activity of DnaE1 will be disrupted by targeted mutagenesis of key amino acids that have been shown previously to be required for the intrinsic exonuclease proofreading activity in both Gram-positive and Gram-negative bacteria. Work to date has focused on generating a set of molecular tools required to address this question. Ongoing work in the Wits node has focused on elucidating the role of certain DNA glycoslases in DNA repair and mutagenesis. The Base Excision Repair (BER) pathway is important for DNA repair during bacterial pathogenesis and DNA glycosylases are central to this function since they identify and excise damaged DNA bases. Endonuclease VIII (Nei) and formamidopyrimidine DNA glycosylase (Fpg) are members of the Fpg/Nei family of glycosylases. *M. tuberculosis* and its non-pathogenic relative *M. smegmatis* both encode two homologs each for *fpg*- and *nei*-like genes. The exact function(s) of these multiple glycosylases during genotoxic stress is not well defined in mycobacteria. In a previous study at the Wits node, combinatorial mutants lacking Fpg/Nei homologs were generated and phenotypically characterized. Mutants lacking the entire Fpg/Nei family of DNA glycosylases did not show reduced survival under normal culture conditions and displayed no significant increase in mutator phenotypes as assessed by the fluctuation assay. There was only a marginal increase in mutation rates for the mutants under oxygenic stress conditions as generated by hydrogen peroxide. These data suggested that in the absence of a functional Fpg/Nei family, other DNA glycosylases in the BER pathway are able to deal with damaged DNA lesions. The Nth DNA glycosylase in the BER repair system shares functionality with Nei, hence it is likely that this enzyme is able to deal with DNA damage in the absence of the Fpg/Nei family of DNA glycosylsases. Furthermore, in *E. coli* which has a single Fpg glycosylase, an increase in mutation prevalence is seen only when the *nth* gene is inactivated in combination with the Fpg DNA glycosylase. Currently, an MSc student, Ms. Nabiela Moolla has inactivated the Nth DNA glycosylase in the parental *M. smegmatis* strain and in mutants lacking the Fpg/Nei family of DNA glycosylases to elucidate the specific and/or combined role(s) of the various DNA glycosylases in *M. smegmatis* under genotoxic stress conditions. Mycobacterial genomes are G+C rich and the guanine base is chemically more susceptible to oxidative damage resulting in the formation

of 7, 8-dihhydro-8-oxoguanine lesions which induces G to T transversions within the chromosomal DNA. This damage is repaired by the GO repair system at three levels involving the enzymes MutT, Fpg (MutM) and MutY. MutT is responsible for repairing the guanines in the nucleotide pool, Fpg (MutM) excises the 8-oxoG and initiates the BER pathway to repair the incorporated damaged bases and MutY removes misincorporated adenines or guanines that are base paired to 8-oxoG thus preventing mutations in the genome during replication. Studies in *E. coli* showed increased levels of G to T mutation rates in a *mutY*/*mutM* double mutant compared to the respective single mutants. *M. smegmatis* mutants lacking the Fpg/Nei family of DNA glycosylases displayed no increase in mutator phenotype or survival to oxidative damage suggesting that MutY may also play an important role in mycobacterial DNA maintenance under oxidative stress conditions. Using homologous recombination, Ms. Farzanah Hassim (MSc student) has inactivated the *mutY* gene in the parental *M. smegmatis* strain, as well as in mutants lacking the Fpg/Nei family of DNA glycosylases. Currently, the various mutant strains together with the parental strains are being assessed in vitro for phenotypic differences under normal and oxidative stress conditions, to better understand the role of MutY during DNA repair.

Physiology of drug-resistant mycobacteria: implications for pathogenesis. This is a new project that was initiated in 2011 and forms the basis of Ms. Koch's PhD. The primary objective for the first year of this project was to initiate the construction of a panel of *M. tuberculosis* strains containing rifampicin resistance (Rif^R)-associated mutations in the absence of Rif selection in order to evaluate the impact of individual *rpoB* mutations – without second site or compensatory mutations – on the physiology of *M. tuberculosis*. Information from the TB Drug Resistance Mutation Database (http://www.tbdreamdb.com/), coupled with an extensive analysis of the published literature, informed the initial selection of *rpoB* mutations for the study. Site-directed mutagenesis was used to introduce point mutations into the *M. tuberculosis rpoB* gene which have been incorporated into to appropriate suicide vectors for use in allelic exchange mutagenesis. A second part of this study is aimed at asking whether mutations that confer resistance to distinct drugs are epistatically related. Fluctuation assays were applied to determine the mutation rate to resistance to a second drug [streptomycin (Str) or ciprofloxacin (Cip)] in a mutant strain of *M. smegmatis* carrying the $rpoB^{S531L}$ allele constructed previously by allelic exchange mutagenesis without Rif selection during. Sequencing of resistance determining regions of mutant strains, generated under conditions of a fluctuation assay, is underway. This analysis will reveal whether particular Cip^R- or Str^R-associated alleles occur more frequently in the $rpoB^{S531L}$ strain, and are thus epistatically linked to the S531L mutation.

Tuberculosis drug discovery research. The UCT node has established a major thrust in TB drug discovery research which has grown out of the work conducted under the *Integrated Methods for Tuberculosis Drug Discovery* (IMTB) project funded by the Bill & Melinda Gates Foundation (BMGF) from 2007-2010. Three new projects were initiated in 2011, all of which are based within international and local TB drug discovery research consortia. The first, three-year project, funded by the BMGF, forms part of the *Identification of High-Quality Hits for Tuberculosis* (HIT-TB) consortium led by Dr. Clifton Barry (NIAID, NIH) under the Foundation's TB Drug Accelerator program. Dr. Joanna Evans initiated this project in February 2011 and has made exceptionally good progress. The project aims to use conditional mutagenesis to identify vulnerable targets within the *M. tuberculosis* coenzyme A pathway. The methodology being employed is based on that developed by Dr. Garth Abrahams under the IMTB project. In order to interrogate the vulnerability of individual steps in the coenzyme A biosynthetic pathway, promoter replacement mutants in *panB*, *panE*, *panK*, *coaBC*, *coaD* and *coaE* were generated in *M. tuberculosis* H37RvMA. In all cases, site-specific recombination was confirmed using both PCR screening assays and Southern hybridization. Growth and stability of these knockdown mutants in response to varying concentrations of inducer, anhydrotetracycline (ATc), is being investigated in both the Tet-ON and Tet-OFF configurations. All mutants are currently being assessed for dose-dependence of growth as a function of ATc concentration on solid and in liquid media. In all cases, the *panB* and *panE* SCOs are additionally supplemented with pantothenate in order to minimise the risk of loss of Tet regulation by mutation. In addition, supplementation with exogenous pantethine was shown to facilitate reversal of growth inhibition of the *coaBC* knockdown mutants, thereby providing a means for maintaining stability of AHT responsiveness in these mutants. Results obtained to date suggest that the *coaBC* and *coaE* promoter replacement mutants may be sensitive to ATc regulation in both the Tet-ON_M and Tet-OFF configurations. Some AHT-dependent regulation of growth was also seen in the case of the *panB* and *coaD* Tet-ON_M mutants, although to a lesser extent. The lack of growth of the *panB*, *coaBC*, *coaD* and *coaE* promoter replacement mutants when TetR is strongly expressed suggests that these targets are relatively vulnerable. Conversely, neither the *panE* nor *panK* knockdown mutants show any ATc responsiveness of growth in the Tet-ON_S , Tet-ON_M or Tet-OFF

configurations, suggesting that these targets are either invulnerable or are expressed at very low levels *in vitro*. Characterization of the other mutants in both solid and liquid media is currently underway.

The second, four-year EU FP7-funded project was initiated in May 2011, following the recruitment, from India, of a new postdoctoral fellow with considerable experience in molecular mycobacteriology. The project is aimed at identifying and validating novel drug targets, and developing novel screening strains for hit identification. Excellent progress has been made to date. Initial work focused on constructing promoter replacement mutants of *M. tuberculosis* H37Rv in *thyA* and *thyX*, which encode two different forms of thymidylate synthase (TS). Analysis of conditional knockdown mutants produced in the Tet-ON and Tet-OFF configuration in terms of AHT dependence for growth suggested that *thyX* is essential for the growth of M. tuberculosis, whereas *thyA* is not; however, *thyA* knockdown results in an early-stage growth defect which argues against functional redundancy of the TS enzymes. These conditional knockdowns are being used as tools to investigate the mechanism of anti-mycobacterial action of 5-fluorouracil (5FU) and related compounds. *In silico* analysis of the complements of *de novo* pyrimidine biosynthesis and salvage genes have identified potential pathways for the metabolism of 5FU to 5FdUMP – a potent inhibitor of both ThyA and ThyX forms of TS. Mutants of *M. tuberculosis* and *M. smegmatis* resistant to 5FU were isolated by selecting on plates containing the inhibitor at 5× and 10× MIC and genotypically confirmed by re-plating at supra-inhibitory concentrations of 5FU. In an important new development, selected sequencing of candidate genes identified by the pathway analysis has revealed two major mechanisms of resistance, mediated by blocking the first step in 5FU metabolism. Ongoing work is aimed at using this information to uncover the molecular target(s) of 5FU metabolites in *M. tuberculosis*.

The third project under this theme is aimed at biochemically analyzing the anti-mycobacterial activity produced by a strain of *Pseudomonas* strain, αMB, the preliminary characterization of which was done as part of Ms. Naran's MSc at Wits. The standard extraction protocol utilizing plate-grown *Pseudomonas* αMB was found to yield ~2-3mg dry mass of crude extract. Owing to the resource-intensive nature of this protocol, an alternative was sought. To this end, the potential use of bioreactor technology was explored in collaboration with Prof. Sue Harrison (Centre for Bioprocess Engineering Research (CeBER), UCT) with experiments in 4L batch culture resulting in a 100-fold dry-mass yield of crude extract. Initial structural characterization was initiated in collaboration with Dr. Stefan Louw (Chibale Laboratory). Since preliminary semi-preparative-scale HPLC purifications yielded no inhibitory activity in any of the fractions, an alternative HPLC method is currently being explored together with an alternative extraction procedure aimed at separating polar and non-polar constituents.

In addition to the abovementioned activities, the CBTBR has started a new project aimed at exploring structure-activity relationship (SAR) studies of novel compound series with potential anti-tubercular activity. This study, which falls within SATRII, is aimed at providing high-level biology (mycobacteriology) support for this TIA-funded TB drug discovery project which involves researchers from all three nodes of the CBTBR (Dr. Bhavna Gordhan, Wits node; Prof. Paul van Helden, SU node; Prof. V. Mizrahi, Dr. Digby Warner and Ms Krupa Naran, UCT node) as well as collaborators from other institutions in South Africa (iThemba Pharmaceuticals, Johannesburg; H3-D/Chibale Lab, UCT) and the USA (Drs. Barry and Boshoff, NIAID). Dr. Gordhan has made excellent progress in this project. The first set of compounds for the Wits node from iThemba Pharmaceuticals were received in May 2011 and were tested for activity against *M. tuberculosis* strains, JHB and H37RvMA to ascertain whether there was a difference in general susceptibility between the two strains. The H37RvMA strain retains the cell wall associated lipid Phthiocerol Dimycocerosate (PDIM) a key virulence factor whereas the JHB strain has lost production of this lipid. There was a 2-4 fold difference between the two strains for some compounds and overall analyses of the data suggested that H37RvMA would be the better strain for future work. All future MIC assessment of compounds was done using this strain. Next, the stability of the compounds after freeze-thaw from -20^{0}C was tested at least three independent times. For some compounds, there was a change in potency which correlated to the compound losing chemical stability during the freeze-thaw process. Consequently, a standard practice was introduced which involved keeping all compounds lyophilized until testing the initial MIC, after which the compound is frozen at -20^{0}C. Using the established methodologies, 256 iThemba compounds and 56 compounds from UCT have been tested for activity against *M. tuberculosis* between May 2011 to November 2011. Analysis of the iThemba compounds showed that 8/256 (3.1%) of the compounds have MIC ≤ 0.625μM; 9/256 (3.5%) compounds have MIC ≤ 1.25μM; 4/256 (1.6%) have MIC ≤ 2.5μM and 18/256 (7.0%) have MIC = 5 -10 μM. 5 compounds have been selected for mutant generation and further target identification/biological studies. At the UCT node, the initial focus in 2011 was on establishing the appropriate assay platforms for MIC determination (broth microdilution

assay with visual or Alamar blue readout) and mutant generation. An initial batch of 53 compounds from a series provided by Prof. Chibale's group was assessed for activity against *M. tuberculosis* H37Rv. Several were found to display MIC values at or near 10 μM, the initial cut-off set for compound progression. The generation of mutants resistant to these compounds is currently underway.

Resuscitation promoting factors in mycobacteria. The success of *M. tuberculosis* lies in its intrinsic ability to cause a spectrum of disease ranging from subclinical, latent TB infection (LTBI) to chronic progressive granulomatous disease. The majority of infected individuals, approximately 2 billion people, harbor LTBI and carry a defined life time risk of developing active disease, a risk which is significantly enhanced in the presence of HIV infection. Latent infection is difficult to treat effectively since most anti-tubercular agents are efficacious at treating active disease. Hence, new treatments for this form of infection are urgently required. The lack of culturable tubercle bacilli during LTBI suggests that the bacteria are characterized by a metabolically quiescent/dormant-like state highlighting the importance of bacterial factors that enhance culturability and the possible role that these play during recrudescent disease. The resuscitation promoting factor (Rpf) is able to enhance the culturability of dormant bacteria, when added at picomolar concentrations, possibly through muralytic digestion of the β-1,4-glycosidic bond in bacterial peptidoglycan (PG). Consequently, these factors may play an important role in reactivation disease with latently infected individuals and as such constitute novel drug targets for LTBI. Furthermore, we postulate that these factors are involved in the production of bioactive muropeptides that play an important role in bacterial and host immune signalling. *M. tuberculosis* encodes five *rpf* homologues (designated *rpfA*-*E*) which all contain the conserved Rpf domain, and their roles in bacterial growth, signaling and virulence have been the subject of intense study at the CBTBR. However, further study of the biological roles of Rpfs in cell wall remodeling, cell growth and signalling was hampered by the pathogenic nature of *M. tuberculosis* and need for specialized equipment under Biosafety Level III conditions. Hence, Lusanda Mapela and Germar Beukes, MSc students, from the Wits node, have expanded the study of Rpfs to the non-pathogenic *Mycobacterium smegmatis* which also encodes a multiplicity of *rpf* genes. During 2011, a panel of mutants that lack between one and all four *rpf* genes in *M. smegmatis* was generated along with complemented derivatives of some mutant strains. This represents a significant advancement in our study of Rpfs since these mutants are a valuable resource which can now be used to further study the roles of these proteins in growth and reactivation from dormancy. The ability to generate a strain of *M. smegmatis* that was deficient for all the *rpf* genes indicated that these genes were collectively dispensable for growth and survival in vitro, as previously observed in *M. tuberculosis* and *Corynebacterium glutamicum*. Mutants defective in *rpfA* and *rpfB*, individually or in combination, were characterized extensively for growth defects and the ability to form biofilms. Strains defective for *rpfA*, individually or in combination with *rpfB*, *rpfC* or *rpfE*, displayed significant clumping in broth culture suggesting changes in cell surface hydrophobicity or overall structure. Further analysis of *rpf* deficient mutants in *M. smegmatis* indicated that the Δ*rpfA* Δ*rpfB* and Δ*rpfA* Δ*rpfB* Δ*rpfC* combinatorial deletion mutants were defective for biofilm formation and displayed altered colony morphologies when plated on solid media. Transmission electron microscopy of these mutant strains revealed striking differences in cell size, surface and overall morphology. The *rpfA* deletion mutant displayed ruffling of the cell surface, bulging of the cell poles and abnormal placement of the septum for cell division. The Δ*rpfA* Δ*rpfB* double mutant displayed an exacerbation of the phenotypes seen for the *rpfA* mutant with dramatic shortening of cell size and severe bending/twisting of cells. In contrast, no significant defects in cell shape/size was seen with the *rpfB* deletion mutant suggesting a hierarchy of *rpf* gene function, as previously noted for *M. tuberculosis*. Analysis of growth suggests that these mutant strains do not display defects for exit from extended stationary phase and are not attenuated for growth when inoculated at very low bacterial numbers in axenic culture. These and other phenotypes are currently being investigated. Several studies have confirmed that the Rpfs play an important role in PG degradation through interaction with various partnering proteins and that their catalytic activity may be dynamically regulated through these protein interactions. The recent demonstration of increased drug permeability in *rpf* quintuple mutants further suggests that the PG remodelling mediated by Rpfs plays an important role on cell wall stability and turnover during growth and survival under stress. In other organisms, PG degradation and remodelling is mediated by a complex set of enzymes that act in synergy, and in many case, in multi-protein complexes. Hence, in 2011 further effort was placed in identifying other interacting partners for the Rpfs through the production of C-terminally FLAG-tagged derivatives of these proteins. In this case, the FLAG-tag can be used in co-immune precipitation assays coupled with peptide mass fingerprinting to identify new interacting partners. The approach involved the construction of these derivatives in an inducible vector system to allow for conditional over-expression of the C-terminally FLAG-tagged *rpf* genes. Increased expression was required to obtained sufficient quantities of protein complexes for further processing and a constitutive expression system was found to be toxic for these

genes. To achieve this, the tetracycline regulated gene expression system for mycobacteria was used. This system utilizes the gene of interest cloned under the control of the tetracycline operator (tetO) and a strong mycobacterial promoter in one vector, and the cognate repressor (tetR) cloned into another vector. After some modification and confirmation of this vector system to allow for affinity tagging, the 5 *rpf* genes from *M. tuberculosis* were cloned into this vector system and the resulting recombinant vectors were confirmed by restriction analysis and sequences. These clones were eletroporated into *M. smegmatis* in combination with repressors of different strengths, to create a panel of strains that had the 5 *rpf* genes under the inducible control of different repressors. The genomic integrity of these recombinant strains was confirmed by rescue of the plasmid from *M. smegmatis* transformants, followed by subsequent restriction analysis or by direct confirmation using PCR. With the exception of RpfA, all the other strains produced the expected genotypes. Strains carrying the RpfA were subjected to genomic rearrangements, loss of the entire gene or mutations in the TetO operator region, resulting in loss of inducible gene expression. Further analysis of these strains with western blotting indicated that, with the exception of RpfB, all the remaining Rpfs were secreted into the media and could not be detected. Affinity pull down studies with the FLAG-tagged RpfB will be attempted to identify new interaction partners, if any.

Detection and characterization of Rpf-dependent bacteria in sputum. The mechanism of the demonstrated growth stimulatory effect conferred by Rpfs remains elusive and is expected to be related to the ability of Rpfs to bind externally to the cell wall to directly initiate growth, as mentioned above. Alternatively, the predicted ability of this group of enzymes to cleave glycosidic bonds in bacterial peptidoglycan will result in the production of signaling molecules that reactivate dormant bacteria through the activity of serine-threonine protein kinases (STPKs). There is extensive evidence from *Bacillus subtilis* which confirms an essential role for a STPK, PrkC, in the sensing of peptidoglycan fragments through an external PASTA (penicillin-binding protein and serine/threonine kinase associated) domain. The binding of unlinked peptidoglycan to this domain triggers a co-ordinated series of events that results in the reactivation of bacterial growth from dormancy. *M. tuberculosis* encodes a homologue of *prkC*, designated *pknB*, which is expected to play a similar role in growth regulation as demonstrated by a recent study implicating PknB in the phosphorylating a kinase-like domain in MviN, a protein essential for the early steps of PG biosynthesis. The demonstrated ability of Rpfs to cleave bacterial peptidoglycan suggests that they could play an important role in this signalling process. Furthermore, the Rpfs may be involved in host immune signalling as evidenced by the observation that this group of proteins can serve as potent vaccine antigens. In this regard, it is noteworthy that strains of *M. tuberculosis* defective for multiple *rpf* genes can confer significant protective efficacy when used as vaccines in mice. The emerging picture from these data suggests that Rpf-dependent signalling can strongly influence bacterial physiology during infection. In this regard, it has been shown that sputa from TB patients are characterized by a heterogeneous population of mycobacteria, carrying different amounts of lipid inclusion bodies. This is further corroborated by a separate study which demonstrated that sputum from TB patients, before the initiation of drug treatment, contains a subpopulation of bacteria that are non-culturable and can be stimulated to grow by supplementation with Rpfs, suggesting that these enzymes may be critical in controlling the ratio of non-culturable bacilli present in sputum. These data indicate that there is a significant degree of phenotypic heterogeneity among infecting tubercle bacilli and the consequences of this on disease progression, transmission and response to treatment have not been investigated extensively. The presence of this occult population of dormant, Rpf-dependent (RPFd) organisms would also have significant implications for the culture-based diagnostic tests currently used for TB which would initially detect only those organisms that can commence and sustain growth under standard conditions with no external stimulus. It is possible that Rpfs derived from actively growing organisms retain the capacity to stimulate the growth of dormant bacteria in the same culture, resulting in a declining proportion of dormant mycobacteria in culture over time. However, it is noteworthy that the addition of recombinant RpfB (Rv1009) from *M. tuberculosis* to Bactec 960 culture media, significantly reduced the time to detection of positive growth confirming that stimulation of dormant bacteria could drastically shorten the duration of culture for diagnosis of TB disease and for drug susceptibility testing, thereby reducing the time for starting the most appropriate treatment. This would have significant implications for disease management and would reduce the emergence of drug resistant strains. In 2011, the Wits node initiated a new project, led by Dr. Melissa Chengalroyen (Postdoctoral fellow), to independently demonstrate the presence of RPFd bacteria in sputum samples from a South African patient cohort to confirm that phenotypic bacteria heterogeneity indeed exists in patients with active TB disease. As indicated above, this study is based on the premise that exogenous supplementation of sputum samples with Rpfs will result in reactivation of these RPFd organisms thereby unmasking them for detection by conventional microbiological methods. Given that all the Rpfs in *M. tuberculosis* are secreted or cell wall associated, Rpf supplementation is

achieved by addition of used culture filtrate from an axenic culture of wild type *M. tuberculosis* and the first stage of the pilot study involved the production of culture filtrate as per published protocols. Culture filtrates from a laboratory strain H37Rv (JHB) were generated by standard axenic growth to mid-logarithmic phase followed by centrifugation and filtration. Sterility was determined by plating various aliquots on 7H11 Middlebrook agar followed by immediate use in growth stimulation assays. Growth stimulation was measured by the Most Probably Number (MPN) and standard Colony Forming Unit (CFU) assays. The sputum material for this study came from a random sampling of sputum from the Mycobacterial Referral Laboratory at the NHLS in Johannesburg. An initial sample size of 6 sputa was used for optimization/standardization of the methodology and assessment of growth stimulatory potency of culture filtrate from wild type *M. tuberculosis*. Subsequently, approximately 50 samples have been analysed using the standardized methodology. Growth stimulation with culture filtrate has been seen in ~ 20% of the samples, most of the remaining samples have not produced any detectable mycobacterial growth. This is consistent with the fact that the majority of samples from the Mycobacterial Referral Laboratory come from TB suspects rather than clinical or smear confirmed TB cases. Our preliminary analyses on the samples that do exhibit growth confirm that stimulation of sputum samples with culture filtrate unmasks a significant population of non-culturable bacteria in patients from our cohort and demonstrates promise for larger, more complex studies. It should be noted that a consistent growth stimulatory effect was not seen in all samples tested during our preliminary analyses, suggesting that the degree of phenotypic heterogeneity may vary in different patients depending on disease severity and treatment program, a hypothesis that will be studied further. This project forms part of an international collaboration that involves key stakeholders at the CDC, NIH and University of Leicester.

Analysis of DD-Carboxypeptidase and *N*-Acetylmuramoyl-L-alanine amidase function in mycobacteria. Previous studies on characterization of Rpfs in *M. tuberculosis* at the Wits node involved the generation of a quintuple *rpf* deletion mutant which was devoid of all *rpf*-like gene function. To further understand the changes in PG structure that occur upon deletion of *rpf* genes, the susceptibility of the quintuple mutant to a panel of drugs, which included lipophilic compounds and antibiotics that target cell wall biogenesis, was assessed. Increased susceptibility to vancomycin and erythromycin was observed with no significant differences in susceptibility to other drugs tested. The increased susceptibility to a limited number of drugs suggested that in addition to the Rpfs, there are other enzymes involved in remodelling and maintenance of PG structure and stability. There are several classes of enzymes, which degrade different components of PG to produce muropeptides, which possibly serve different functions during growth and survival under stress conditions or non-replicating persistence. These enzymes would also play a critical role in remodeling of the PG for growth and cell division. The synergistic role of RpfB with an endopeptidase, RipA, has already been demonstrated but the possible combinatorial activity of Rpfs with other PG degrading enzymes remains to be investigated. In 2011, the Wits node initiated a new project, led by Dr. Christopher Ealand (Postdoctoral fellow) and Mr. Sibusiso Senzani (MSc student) to study the role of *N*-Acetylmuramoyl-L-alanine amidases (amidases) and DD-Carboxypeptidases (DD-CPases) in PG remodeling and muropeptide production either individually or in combination with the Rpfs. Amidases cleave the amide bond between the glycan chain and the stem peptide while DD-CPases cleave the terminal D-alanine in the stem peptide. Our initial analyses revealed three possible homologues for amidases in *M. smegmatis*, *amiA2* (MSMEG_2432), *amiB2* (MSMEG_2497) and *amiC* (MSMEG_2521). However, further analyses of the genome suggested that these genes did not encode specific PG degrading amidases since they lacked the classic PG degrading domain as identified by Pfam analyses. Additional bioinformatics revealed a distinct set of three putative amidase-encoding genes designated MSMEG_6406, MSMEG_6935, MSMEG_6281, these genes were chosen for further study through regulated gene knockdown. For gene knockdown, the tetracycline inducible gene expression system was used for creating site-specific promoter replacements. This involved cloning of homologous sequences for the three genes into an integrating vector for site-specific incorporation into the mycobacterial genome to create promoter replacements. These vectors were successfully created and eletroporated into *M. smegmatis*. This resulted in the construction of various strains with promoter replacements, each of them having repressors of different strength. The genomic integrity of these vectors is currently being confirmed by southern blot. Preliminary phenotypic characterization of these clones revealed significant differences in growth and recovery from stationary phase. The second component of the study of amidases was to create gene knockouts of PG amidase-encoding genes. For this, MSMEG_6935 and MSMEG_6281 were chosen since they displayed the highest homology to PG degrading amidases from other organisms. For construction of knockout vectors, the relevant upstream and downstream regions were amplified by PCR and cloned into a suicide vector to create in-frame unmarked deletions, followed by cloning of selectable and counter selectable marker genes. These vectors were successfully constructed and are currently being used to generate knockout mutants for

MSMEG_6935 and MSMEG_6281. Bioinformatics analysis of DD-CPase-encoding genes in *M. tuberculosis* and *M. smegmatis* resulted in the identification of five DD-CPases homologues in *M. smegmatis*, MSMEG_1661, MSMEG_2432, MSMEG_2433, MSMEG_6133 (*dacB*), and MSMEG_1900. With the exception of MSMEG_1900, gene knockdown constructs, as described above, for all the DD-CPases were constructed in 2011, followed by electroporation and selection of knockdown strains. The genotypes for these strains are currently being confirmed and further analysis on expression of these genes is underway.

Analysis of the respiratory chain in mycobacteria. Bacteria display a remarkable ability to rapidly adapt to varying environments and the availability of different substrates due to their complex, branched electron transport chains (ETC). The ETC of *M. tuberculosis* which, as in other bacteria, consists of several different dehydrogenases that channel electrons to menaquinone, a lipid soluble electron carrier, and several different terminal cytochrome oxidases and reductases. The bacterium is thus able to modulate the use of different components of this system for growth and survival depending on the availability of electron acceptors such as oxygen, nitrate and fumarate. The *cydAB* operon encodes a specialized cytochrome *bd* quinol oxidase which has been shown to be essential for microaerobic respiration and hypoxic growth of *M. smegmatis* and *E. coli*, and is further postulated to be important under conditions of non-replicating persistence. The Wits node is running a project aimed at further characterization of the microaerobic and anaerobic components of the mycobacterial electron transport chain with the underlying hypothesis that *M. tuberculosis* is dependent on these respiratory complexes for optimal growth and colonization of the human host during pathogenesis due to the reduced oxygen tensions found within granulomas and the increased availability of nitrate in these lesions. Furthermore, inhibition of the main branch of aerobic respiration through cytochrome *c* oxidase by nitric oxide probably renders the tubercle bacillus reliant on its ability to switch between using alternate electron acceptors for virulence, as has been observed in other organisms. Transcriptional analysis of genes involved in energy metabolism in the murine model of tuberculosis infection revealed that expression of the *cydAB* genes is increased upon onset of the adaptive immune response and is later repressed with the concomitant induction of expression of the nitrate reductase-encoding *narGHJI* operon during chronic infection. These observations suggest that respiration using alternate electron acceptors is intrinsically related to the ability of the organism to adapt and survive under stressful conditions both in vitro and in vivo. During 2011, Mrs. Prathna Ramchandra (research assistant) further studied the phenotype of a mutant of *M. smegmatis* defective for cytochrome *bd* oxidase and assessed the sensitivity of this strain to nitric oxide which is expected to poison the cytochrome caa_3 oxidase branch of the aerobic mycobacterial electron transport chain thus rendering the organism solely reliant on the use of cytochrome *bd* oxidase. Knockout of cytochrome *bd* oxidase results in enhanced growth of *M. smegmatis* under aerobic conditions, possibly through increased utilization of the energetically favourable cytochrome caa_3 oxidase complex. Exposure to varying concentrations of nitric oxide generated from the acidification of sodium nitrite resulted in a dose-dependent increase in bactericidal activity with no significant differences in susceptibility between the wild type and a cytochrome *bd* oxidase deficient strain. In addition, under acidic conditions the mutant showed mild resistance when compared to the wild type strain. Collectively, these data suggest that knockout of cytochrome *bd* oxidase results in the re-routing of electron flow through alternate branches of the electron transport chain resulting in enhanced growth. Furthermore, the loss of this particular respiratory complex results in changes in pH homeostasis, presumably due to changes in the proton motive force. In addition, deletion of the genes encoding the structural components of cytochrome *bd* oxidase in *M. tuberculosis* results in attenuated growth and survival in activated J774 macrophages. These and other possible defects are currently being explored further.

THEME II: BRIDGING THE GAP BETWEEN BASIC AND CLINICAL RESEARCH

Mycobactomics

Many studies were completed during 2010-2011, 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 even on an annual basis.

Molecular Epidemiology Databases: As part of an ongoing molecular epidemiological investigation into the disease dynamics of TB in a high incidence urban setting, six molecular epidemiological databases has been constructed to record clinical, epidemiological and molecular data from patients diagnosed with TB.

1. This molecular epidemiology database of TB cases in an epidemiological field site in Cape Town 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. In order to understand the evolution of drug resistance in this community we have sequenced the genes conferring resistance to both first- and second-line anti-TB drugs from the diagnostic sample from each patient and their respective episodes. The data generated from this study will be used to inform policy guidelines on inadequacies in the diagnostic and treatment algorithms.
2. Our second molecular epidemiological study focuses 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.
3. Our 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.
4. Our fourth molecular epidemiological study was initiated to investigate the XDR-TB epidemic in the Western Cape. This involves the genotypic characterization of XDR-TB isolates with the view to determine whether the epidemic develops through acquisition or transmission. In addition we aim to describe the repertoire of mutations conferring resistance and determine whether standardized DNA fingerprinting methods are able to accurately delineate transmission chains. Initial analysis of the database indicates that MDR-TB is mainly due to transmission and that XDR-TB is mainly due to acquisition and amplification of resistance in patients infected with MDR strains. Transmission may in part directly be related to diagnostic delay by current routine drug resistant testing and amplification of resistance can be interpreted as a function of a poor functional control program. Both these two issues have to be seriously addressed by the control program to curb the increasingly problem with drug resistant TB.
5. Our fifth molecular epidemiological study was initiated to collect and genotypically characterize all drug resistant isolates (> 17 000) cultured from 10 000 patients resident in the Western Cape and Boland Overberg Karoo regions. 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.
6. Our 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 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.

Mycobacterial Evolution

***Mycobacterium tuberculosis* Beijing genotype:** The diverse clinico- and histopathological features, frequency of transmission and treatment outcome of *Mycobacterium tuberculosis* have been associated with several environmental, host and bacterial factors. Many *Mycobacterium tuberculosis* genotypes have been studied in an attempt to understand the genetic variations among the different genotypes and to clarify their contribution to phenotypic differences. Strains of the Beijing genotype have been extensively investigated due to their increased ability to spread and cause disease. In this study we reviewed the evidence of hypervirulence of the Beijing genotype as well as other Beijing-associated phenotypic characteristics such as alternate host immune modulation, clinical and pathological features, drug resistance, resistance to BCG vaccination and other epidemiological features to enhance our understanding of the contribution of pathogenic factors. From the data collected it is clear that the genetic background of *Mycobacterium tuberculosis* may influence the differential induction of the immune response, drug resistance patterns and clinical, epidemiological and pathogenic features which define disease progression following infection. This highlights the importance of ongoing research into the genetic mechanisms underlying the phenotypic and genotypic characteristics of different *Mycobacterium tuberculosis* genotype strains. Furthermore, these findings could help to direct future drug, vaccine and diagnostic test development towards targeting critical virulence factors and to identify persons at risk for developing active disease thereby limiting transmission and the perpetuation of the tuberculosis epidemic.

Phylogeny of *Mycobacterium tuberculosis* Beijing strains: The Beijing family is a successful group of *M. tuberculosis* strains, often associated with drug resistance and widely distributed throughout the world. Polymorphic genetic markers have been used to type particular *M. tuberculosis* strains. In this study we identified a group of polymorphic DNA repair replication and recombination (3R) genes. It was shown that evolution of *M. tuberculosis* complex strains can be studied using 3R SNPs and a high-resolution tool for strain discrimination was developed. We investigated the genetic diversity and propose a phylogeny for Beijing strains by analyzing polymorphisms in 3R genes. A group of 3R genes was sequenced in a collection of Beijing strains from different geographic origins. Sequence analysis and comparison with the ones of non-Beijing strains identified several SNPs. These SNPs were used to type a larger collection of Beijing strains and allowed identification of 26 different sequence types for which a phylogeny was constructed. Phylogenetic relationships established by sequence types were in agreement with evolutionary pathways suggested by other genetic markers, such as Large Sequence Polymorphisms (LSPs). A recent Beijing genotype (Bmyc10), which included 60% of strains from distinct parts of the world, appeared to be predominant. We found SNPs in 3R genes associated with the Beijing family, which enabled discrimination of different groups and the proposal of a phylogeny. The Beijing family can be divided into different groups characterized by particular genetic polymorphisms that may reflect pathogenic features. These SNPs are new, potential genetic markers that may contribute to better understand the success of the Beijing family.

SNP/RD Typing of *Mycobacterium tuberculosis* Beijing Strains: To type Beijing strains reliably we developed a robust typing scheme using single nucleotide polymorphisms (SNPs) and regions of difference (RDs) derived from whole-genome sequencing data of eight Beijing strains. SNP/RD typing of 259 *M. tuberculosis* isolates originating from 45 countries worldwide discriminated 27 clonal complexes within the Beijing genotype family. A total of 16 Beijing clonal complexes contained more than one isolate of known origin, of which two clonal complexes were strongly associated with South African origin. The remaining 14 clonal complexes encompassed isolates from different countries. Even highly resolved clonal complexes comprised isolates from distinct geographical sites. Our results suggest that Beijing strains spread globally on multiple occasions and that the tuberculosis epidemic caused by the Beijing genotype is at least partially driven by modern migration patterns. The SNPs and RDs presented in this study will facilitate future molecular epidemiological and phylogenetic studies on Beijing strains.

Whole Genome DNA sequencing of *M. tuberculosis*: As part of collaboration with Prof R McNerney (LSHTM) and Prof Dheda (UCT) we have submitted purified DNA extracted from over 150 clinical isolates (ranging from fully susceptible to XDR-TB) for whole genome sequence analysis. The sequencing results will be available for analysis in early 2012. This study has a number of objectives;

1) To describe the repertoire of mutations conferring drug resistance.
2) To phylogenetically map evolutionary events which lead to the evolution of XDR-TB.
3) To describe the mechanisms involved in the evolution of drug resistance.
4) To link clinical and treatment data to whole genome sequence data.
5) To link immunological responses to whole genome sequence data.
6) To describe the phylogeny of *M. tuberculosis.*

This study will be expanded in 2012 to include:

1) Members of the *M. tuberculosis* complex
2) Strains representative of the different *M. tuberculosis* clades
3) Clinical isolates of non-tuberculosis mycobacteria.
4) Non-tuberculosis mycobacteria which span the phylogenetic nodes which differentiate fast and slow growing species.
5) Totally Drug Resistant (TDR) isolates from the Eastern Cape.

In addition we have agreed to expand our sequencing efforts to include:

1) Analysis of small RNA.
2) Mycobacterial transcriptomics.

Mutations in the regulatory network underlie the recent clonal expansion of a dominant subclone of the Mycobacterium tuberculosis Beijing genotype: Evidence suggests that the emerging and most frequently isolated "Typical Beijing" lineage has the ability to circumvent BCG-induced immunity. To investigate the phylogeny of the Beijing genotype of *M. tuberculosis*, the genome of six Beijing strains from three different countries was sequenced with next-generation sequencing. The phylogeny of these strains was established using single nucleotide polymorphisms (SNPs). The three Typical Beijing strains clustered very tightly in the Beijing phylogeny suggesting that Typical Beijing strains represent a monophyletic lineage and resulted from recent diversification. Typing of 150 *M. tuberculosis* strains with a subset of the SNPs and comparison of the IS*6110* restriction-fragment length polymorphism (RFLP) patterns of these strains to a database of 1522 Beijing RFLP patterns revealed that about 80% of all Beijing strains belong to the Typical Beijing subclone, which indicates clonal expansion. To identify the genomic changes that are characteristic for all Typical Beijing strains and to reconstruct their most recent common ancestor, the presence of SNPs was assayed in other Beijing strains. We identified 51 SNPs that define the minimal set of polymorphisms for all Typical Beijing strains. Nonsynonymous polymorphisms in genes coding for the regulatory network were over-represented in this set of mutations. We suggest that alterations in the response to environmental signals may have enabled Typical Beijing strains to develop the emerging phenotype.

Mutations that defines the level of Rifampicin resistance in clinical isolates

It was previously shown that the levels of resistance in clinical isolates varies significantly and in most instances exceed the peak serum concentrations reached after treatment. In an attempt to understand this phenomenon, *in vitro* Rifampicin resistant mutants were generated from a pan susceptible clinical isolate and whole genome sequencing were performed on a single colony from the progenitor and colonies with different levels of Rifampicin resistance. Initial analysis indicates that the mutants gain additional mutations when progressed to a higher level of resistance. Both in silico analysis and in vitro gene knockout studies are in progress to understand the mechanism of the genomic changes that takes place when a clone (in this case generated from a common progenitor and mimic the observation in clinical isolates) becomes hyper-resistant to an anti-TB drug.

RNA transcriptomics

RT-qPCR demonstrated that rifampicin induced differential expression of efflux/transporter genes in MDR-TB isolates. The same *in vitro* generated clones (described above) and paired clinical isolates (RiF monoresistant) with similar levels of RIF resistance have been exposed to Rifampicin for different time intervals to investigate the temporal changes in the abundance of RNA transcripts after RIF exposure. This project has been designed to characterize the transcriptional response and regulatory proteins which induces tolerance/cross-resistance in *Mycobacterium tuberculosis* RIF resistant isolates in response to RIF exposure. This knowledge will be valuable for the future design of anti-TB drugs to limit their induction of cross-resistance, as well as providing new targets for rational drug design.

Mycobacterium tuberculosis* proteomics*:

The Orbitrap Velos mass Spectrometry instrument was commissioned in January 2011 and has been fully functional since February 2011. The impact of this instrument on the research field of proteomics in South Africa is demonstrated by the fact that time on the instrument is now booked more than a month in advance despite the fact that the instrument is running 24/7. The major advantages of this instrument are its sensitivity to detect peptides. Currently it is possible to accurately analyse 2500 *M. tuberculosis* proteins in a single experiment which is an order of magnitude higher than what has been reported using 2D technology. This implies that it is now possible to design experiments to understand the physiology of the pathogen in fine detail. Research questions that are being investigated include:

1) How does protein abundance differ between members of the *M. tuberculosis* complex. It is envisaged that this study will provide novel insights into host pathogen interactions and factors which define host specificity.
2) Evolution of drug resistance. Three projects have been initiated:
   a. To describe how the evolution of resistance (rifampicin and ofloxacin) could influence the abundance of whole cell lysate proteins thereby defining a "resistance phenotype".
   b. To determine the difference in the abundance of proteins in closely related strains which show different levels of rifampicin resistance.
   c. To determine how exposure to rifampicin could influence protein abundance in rifampicin resistant strains.
3) Phyloproteomics. A study is being conducted to determine how different lineages of *M. tuberculosis* may have evolved different phenotypes.
4) Phosphorylome: This study aims to identify which proteins are phosphorylated and how this may influence their biological activity and in so doing the pathogenicity of different strains of *M. tuberculosis*.
5) Proteomics of gene knockouts. The first study aim to understand

Genotypic Drug-Susceptibility Testing: Current diagnosis of TB in low-income, high-burden regions relies on smear microscopy and clinical signs and symptoms. However, this smear-centered approach has many pitfalls, including low sensitivity in HIV patients and children, the inability of smear to reveal drug-resistance patterns, and the need for sampling on consecutive days. In order to address these limitations, efforts have been made to expand access to *Mycobacterium tuberculosis* culture and drug susceptibility testing. However, the slow growth rate of the causative agent, *M. tuberculosis*, contributes to significant diagnostic delay. Molecular-based diagnostic methods, targeting mutations that are known to confirm drug resistance, are capable of significantly reducing diagnostic delay. Two such methods, the line-probe assay and the real-time PCR-based Xpert® MTB/RIF assay, have been described. The latter test shows particular promise for smear-negative Publication 42 (Theron et al.) and extrapulmonary specimens Publication 23 (Lichthelm et al). This may prove especially useful in settings where co-infection rates with HIV are high. However, since most research focuses on the performance of both of these assays, further investigations need to be done regarding the impact of the routine implementation of these assays on TB control programs and the cost effectiveness thereof.

Emergence and treatment of multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in South Africa: Drug resistant tuberculosis (TB) has reached alarming proportions in South Africa, draining valuable resources that are needed to fight drug susceptible TB. It is currently estimated that 9.6% of all TB cases have multi-drug resistant (MDR)-TB, thereby ranking South Africa as one of the highest MDR-TB burden countries in the world. Molecular epidemiological studies have demonstrated the complexity of the epidemic and have clearly shown that the epidemic is driven by transmission as a consequence of low cases detection and diagnostic delay. The latter has in turn fuelled the amplification of drug resistance, ultimately leading to the emergence of extensively drug resistant (XDR)-TB. Despite the introduction of new drugs to combat this scourge, culture conversion rates for XDR-TB remain below 20%. Failure to achieve cure may be explained from DNA sequencing results which have demonstrated mutations in 7 genes encoding resistance to at least 8 anti-TB drugs. We have reviewed how molecular epidemiology has provided novel insights into the MDR-TB epidemic in South Africa and thereby has highlighted the challenges that need to be addressed regarding the diagnosis and treatment of MDR-TB. An important step towards for curbing this epidemic will be collaboration between clinicians, laboratories and researchers to establish scientific knowledge and medical expertise to more efficiently guide public health policy.

***inhA* promoter mutations:** To assess the potential association between the evolution of extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* and mutations in the *inhA* promoter or the *katG* gene. We analysed the frequency distribution of isoniazid (INH) resistance conferring mutations in a population sample of drug-resistant isolates of *M. tuberculosis*. In the Western Cape and Eastern Cape Provinces, the percentage of isolates exhibiting *inhA* promoter mutations increased significantly from respectively 48.4% and 62.4% in multidrug-resistant tuberculosis (MDR-TB) isolates to 85.5% and 91.9% in XDR isolates. Data from the Western Cape revealed that significantly more XDR-TB isolates showed mutations in the *inhA* promoter than in katG (85.5% vs. 60.9%, $P < 0.01$), while the respective proportions were equal for INH-resistant non-MDR-TB isolates (~30%). We suggest that this is due to the dual resistance to ethionamide and (low-dose) INH conferred by *inhA* promoter mutations. The use of molecular probe assays such as the GenoType® MTBDRplus assay, which allows the detection of inhA promoter mutations, could enable treatment regimens to be adjusted depending on the pharmacogenetic properties of the mutations detected.

***Mycobacterium tuberculosis* population structure determines the outcome of genetic based second-line drug resistance testing:** The global emergence of multidrug-resistant tuberculosis has highlighted the need for the development of rapid tests to identify resistance to second-line anti-tuberculosis drugs. Resistance to fluoroquinolones and aminoglycosides develops through non-synonymous single nucleotide polymorphisms in the *gyrA/gyrB* and rrs genes, respectively. Using DNA sequencing, as the gold standard for the detection of mutations conferring resistance, in conjunction with spoligotyping, we demonstrated heteroresistance (mixed population of a genetically homogeneous drug-resistant and drug-susceptible strain in the same clinical isolate) in 25% and 16.3% of Mycobacterium tuberculosis isolates resistant to ofloxacin or /amikacin, respectively. Characterization of follow-up isolates from those patients showed that the population structure of clones may change during treatment, suggesting different phases in the emergence of resistance. The presence of underlying mutant clones was identified in isolates which failed to show a correlation between phenotypic resistance and mutation in the *gyrA* or *rrs* genes. These clones harboured previously described mutations in either the *gyrA* or *rrs* genes, suggesting that rare mutations conferring resistance to ofloxacin or kanamycin/amikacin may not be as important as was previously thought. We conclude that the absence of a correlation between genotypic and phenotypic resistance implies an early phase in the emergence of resistance within the patient. Thus the diagnostic utility of genetic-based drug susceptibility tests will depend on the proportion of patients whose bacilli are in the process of acquiring resistance in the study setting. These data have implications for the interpretation of molecular and microbiological diagnostic tests in patients with drug-susceptible and drug-resistant TB who fail to respond to treatment, and those with discordant results.

Accurate Drug susceptibility tests (DST)

Drug resistance testing in *M.tuberculosis* is difficult and worldwide this is specifically true for second line drug resistance testing. In our centre we have established and use the BACTEC MGIT 960 instrument, equipped with TBeXiST and EpiCentre^{TM}V5.75A software (BD Bioscience, Erebodegem, Belgium) to accurately detect phenotypic resistance to first and all second line drugs. The TBeXiST is particularly aimed at testing for 2nd line drug resistance. DST is usually routinely done at only one critical drug concentration which discriminates between susceptible or resistant bacteria but it does not provide information on the level of resistance (MIC) of the bacterium to a particular drug. The MGIT 960 instrument, TBeXiST and EpiCentre machine is automated and technically less demanding and has a much shorter turnaround time than the standard DST tests. It has an important additional advantage in that quantitative drug susceptibility testing (QDST) at different drug concentrations can be determined very accurately and thereby provide information on the level of resistance to the test drug. It therefore provides a tool which helps to better interpret DST results and to differentiate between low level and high level drug resistance which is important for the management of patients. Based on the shortcomings and unreliability of the currently used routine methods, we recommend that use of this technology should be standard to manage MDR and XDR-TB patients. It has data storage capabilities and can also generate results and reports.

Due to our publications (using this technology), reports at conferences and the expertise that exists in this centre (Dr Frik Sirgel, MRC appointment) we are frequently requested locally (NHLS) and internationally (GCDD drug resistant consortium between 5 different countries; University of California San Diego [UCSD]), Manila [Philippines], Mumbai [India], Port Elizabeth [South Africa] and Chisinau [Moldova] to provide reference services on accurate QDST.

Treatment of Drug-Resistant TB:

Treatment outcomes of isoniazid-resistant tuberculosis patients: We showed that 16% of patient with isoniazid monoresistance had poor outcomes, 61% of whom progressed to multidrug-resistant tuberculosis. These data reveal the need for early identification and aggressive follow-up of isoniazid monoresistance to increase treatment success.

Use of fluoroquinolone antibiotics leads to tuberculosis treatment delay: We examined the impact of FQ use on TB outcomes, including smear status, treatment delay and FQ resistance, through a retrospective cohort study of 440 FQ-exposed and 511 non-exposed patients in a gold mining community in South Africa. We considered both recent (≤ 100 days before sputum collection) and distant exposure (≤ 1 year). We examined 201 and 180 isolates from FQ-exposed and non-exposed individuals for the presence of gyrA mutations. Patients recently exposed to ≥ 5 days of FQ were less likely to be smear-positive (OR 0.27, 95%CI 0.11-0.63), with an increased time to treatment (time ratio 2.02, 95%CI 1.19-3.44). The strength of association decreased when we considered distant exposure. Adjusting for smear status nullified the effect of FQ exposure on treatment delay. We detected a *gyrA* mutation in one isolate (0.5%) taken from an individual exposed to FQ for 8 days. FQ exposure is associated with treatment delay, mediated by negative smear status. Short exposures to FQ do not routinely lead to resistance encoded by *gyrA* mutations. We recommend prudent use of FQ in settings with a high burden of human immunodeficiency virus and TB.

Rifampicin reduces susceptibility to ofloxacin in rifampicin-resistant *Mycobacterium tuberculosis*: This study aimed to determine whether rifampicin induces efflux pumps activation in rifampicin resistant *M. tuberculosis* strains thereby defining rifampicin resistance levels and reducing ofloxacin susceptibility. This ground breaking study showed that rifampicin MICs varied independently of *rpoB* mutation and genetic background. Addition reserpine and verapamil significantly restored rifampicin susceptibility ($p = 0.0000$). RT-qPCR demonstrated that rifampicin induced differential expression of efflux/transporter genes in MDR-TB isolates. Incubation of rifampicin mono-resistant strains in rifampicin (2 µg/ml) for 7 days induced ofloxacin resistance (MIC > 2 µg/ml) in strains with an rpoB531 mutation. Ofloxacin susceptibility was restored by exposure to efflux pump inhibitors. Studies in BALB/c mice showed that verapamil in combination with first-line drugs significantly reduced pulmonary CFUs after 1 and 2 months treatment ($p < 0.05$). We concluded that exposure of rifampicin resistant *M. tuberculosis* strains to rifampicin can potentially compromise the efficacy of the second-line treatment regimens containing ofloxacin, thereby emphasising the need for rapid diagnostics to guide treatment. In addition we show for the first time that efflux pump inhibitors have the potential to improve the efficacy of anti-tuberculosis drug treatment

Mutations in the *rrs* A1401G gene confer phenotypic resistance to amikacin and capreomycin: The aminoglycosides amikacin (AMK)/kanamycin (KAN) and the cyclic polypeptide capreomycin (CAP) are important injectable drugs in the treatment of multidrug-resistant tuberculosis. Isolates from the Eastern Cape Province of South Africa were subjected to DNA sequencing of the *rrs* (1400-1500 region) and tlyA genes. Sequencing data were compared with (i) conventional susceptibility testing at standard critical concentrations (CCs) on Middlebrook 7H11 agar and (ii) MGIT 960-based MIC determinations to assess the presence of AMK- and CAP-resistant mutants. Isolates with an *rrs* A1401G mutation showed high-level resistance to AMK (>20 mg/L) and decreased phenotypic susceptibility to CAP (MICs 10-15 mg/L). The MICs of CAP were below the bioavailability of the drug, which suggests that it may still be effective against multi- or extensively drug resistant tuberculosis [M(X)DR-TB]. Agar-based CC testing was found to be unreliable for resistance recognition of CAP in particular.

Evaluation of new diagnostic tools:

Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis: In this study Xpert MTB/RIF was evaluated using single archived spot-sputum samples from 496 South African patients with suspected TB. Mycobacterium tuberculosis culture positivity and phenotypic resistance to rifampicin served as reference standards. Overall, Xpert MTB/RIF detected 95% (95% confidence interval [CI], 88-98%; 89 of 94) of smear-positive culture-positive cases and the specificity was 94% (91-96%; 320 of 339). The sensitivity in smear-negative cases was 55% (35-73%; 12 of 22). In patients infected with HIV compared with patients uninfected with HIV Xpert MTB/RIF showed a trend to reduced sensitivity (P=0.09) and significantly reduced negative predictive value (P=0.01). The negative predictive value for rifampicin resistance was 99.4%.

The use of light-emitting diode fluorescence to diagnose mycobacterial lymphadenitis in fine-needle aspirates from children: Fine-needle aspiration biopsy (FNAB) is a simple, safe and effective method for investigating suspected mycobacterial lymphadenitis in children. Fluorescence microscopy can provide rapid mycobacterial confirmation. Light-emitting diodes (LEDs) provide a cheap and robust excitation light source, making fluorescence microscopy feasible in resource-limited settings. To compare the diagnostic performance of LED fluorescence microscopy on Papanicolaou (PAP) stained smears with the conventional mercury vapour lamp (MVL). FNAB smears routinely collected from palpable lymph nodes in children with suspected mycobacterial disease were PAP-stained and evaluated by two independent microscopists using different excitatory light sources (MVL and LED). Mycobacterial culture results provided the reference standard. A manually rechargeable battery-powered LED power source was evaluated in a random subset. We evaluated 182 FNAB smears from 121 children (median age 31 months, interquartile range 10-67). Mycobacterial cultures were positive in 84 of 121 (69%) children. The mean sensitivity with LED (mains-powered), LED (rechargeable battery-powered) and MVL was respectively 48.2%, 50.0% and 51.8% (specificity 78.4%, 86.7% and 78.4%). Inter-observer variation was similar for LED and MVL ($\kappa = 0.5$). LED fluorescence microscopy provides a reliable alternative to conventional methods and has many favourable attributes that would facilitate improved, decentralised diagnostic services.

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 extracted directly from sputum specimens or cultures. We are currently investigating the reasons for discordance between the MTBDRsl assay and routine drug susceptibility testing.

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. At the request of Prof Erik Bottger we are in the process of collecting an additional 2000 sputum isolates. Each isolate will be stored as decontaminated sputum, crude DNA and MGIT culture.

Evaluation of the Seegene Magiplex assay: This project is done in collaboration with Seegen (Korea), Inqaba Biotech (SA) and the NHLS (SA). Approximately 450 specimens have been tested, of which 80 showed discordance with the Hain line probe assay. The reasons for discordance were 1) invalid negative controls and 2) no Hain line probe result due to the sample being smear negative. Seegen has developed a second version of the assay which will be tested in 2012.

Xpert MTB/RIF for Rapid Diagnosis of Tuberculous Lymphadenitis from Fine-Needle-Aspiration Biopsy Specimens: This study demonstrated the excellent diagnostic accuracy of the Xpert MTB/RIF test in patients with tuberculous lymphadenitis. The test sensitivity and specificity were 96.7% (95% confidence interval [CI], 86.6 to 100%) and 88.9% (95% CI, 69.6 to 100%), respectively, and it correctly identified 6/6 (100%) of the cytology smear-negative/culture-positive cases and 1 of 2 (50%) rifampin-resistant cases.

Evaluation of the Xpert MTB/RIF assay for the diagnosis of mycobacterial Lymphadenopathy. This project is done in collaboration with UCT and Red Cross Childrens Hospital and aims to determine the performance of the GenExpert in the diagnosis of *M. tuberculosis* complex in fine needle aspirate biopsies taken from children suspected of having extrapulmonary tuberculosis. Over 300 transport bottles have been prepared and distributed for the collection of fine needle aspirate biopsies. It is envisaged that the results of this study will be available in 2012. We are currently formulating guidelines for the diagnosis of mycobacterial lymphadenopathy using the Xpert MTB/RIF assay which will be submitted to the Department of Health for approval and distribution.

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.

Rare and Novel *M. tuberculosis* complex Species: *Mycobacterium 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 *Mycobacterium 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. 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.

A second novel mycobacterial strain identified in our laboratory in 2011, and belonging to the MtbC was an organism known to cause TB in the suricate (meerkat; *Suricata suricatta*) but which had previously been incorrectly classified as *M. bovis*. Preliminary genetic analysis of this organism has shown it to be closely related to the dassie bacillus (which occurs almost exclusively in the rock hyrax; *Procavia capensis*) and *M. mungi* which has only been isolated from the banded mongoose (*Mungos mungo*). It is of interest that these closely related strains of the pathogen ("ecotypes") appear to have limited ecological niches (i.e. are host-specific). Ongoing genetic analysis of these strains is planned in 2012 with the aim of elucidating the adaptive genetic changes of these MtbC strains which appear to be dependent on host physiology and ecology. Such analysis will contribute to the identification of components of mycobacterial physiology which are essential for survival in the host, and which might be suitable as novel drug targets.

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.

Bioinformatics

Mycobacterial Proteomics:

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 CMCB 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 CMCB. 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 CMCB 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.

A biostatistical investigation of gene-gene interactions associated with the host genetics of *Mycobacterium tuberculosis*:

Increasing evidence show that host genetic factors determine susceptibility to developing active infection. These factors are complex, and examining them in isolation may result in the effect of factors that do not act independently being missed. It is therefore important to understand the role of gene-gene interactions, commonly referred to as epistasis, in susceptibility to tuberculosis infection.

The community of Ravensmead and Uitsig has been studied extensively in the Centre for Molecular and Cellular Biology at the Faculty of Health Sciences of Stellenbosch University. Over the last 10 years, DNA samples of affected individuals and of healthy controls have been collected and genotyped in a number of candidate gene studies. In addition, 856 cases and 94 controls were genotyped using the Affymetrix 500 000 SNP chip. This data offers a unique opportunity to examine the role of epistasis in the etiology of Mycobacterium tuberculosis.

If traditional statistical methods are used to examine data with many potentially interacting variables, the estimation of a large number of parameters would be required. Using regression based methods to find gene-gene interactions would therefore be computationally unfeasible. A large number of samples would also be required to have sufficient power to detect effects. As data mining and machine learning methods do not perform exhaustive searches on data, these methods do not suffer from such limitations. For this reason, data mining and machine learning methods, including multifactor-dimensionality reduction (MDR), random forests and neural networks, are being used to find combinations of genetic polymorphisms that may interact and alter disease susceptibility.

The effect of the candidate polymorphisms found are assessed using logistic regression, adjusting for other disease susceptibility covariates such as age and sex. In addition, population stratification is a well-known confounder in association studies and is corrected for using principal components.

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 where experiments were performed in the BSL3 animal unit. It was found that *N. brasiliensis* infection, followed 5 days later by BCG infection leads to decreased BCG growth in the lungs of co-infected mice compared to 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. For this work, 2 PhD students from our centre successfully completed their theses. One student has graduated 2007 and one will graduate in March 2012.

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. The group has recruited and followed up (two years) more than 1200 household contacts of TB patients as well as 180 HIV infected people for this study
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, MLPA and Interferon-gamma ELISA assay) and laboratory standard operating procedures issues were developed and are compatible with the other GC6-74 partners. The consortium has received additional funding for the 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 and they are followed up for a two year period. 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. The SUN-IRG group has been tasked with the identification of the incident TB cases across the consortium and has been responsible for the development of the TB case definitions.

Database design
Prof Gian van der Spuy has designed and created a unified database for the consortium 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.

Specific sub-studies in the GC6-74 project:
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 infection 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. Data from the study were published in Clin Microbiol Infect. (see publication 19).

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$ 1.5 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.tb 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-inflammatory 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 randomized 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 M.tb 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 Day1, Day3, Week 8 and Week 12 of treatment. Bacterial ribosomal RNA appears to be a sensitive treatment response marker. This work was published in the Journal of Clinical Microbiology (see publication 17).

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.
c) Evaluation of the dual-colour ELISPOT assay for discrimination between latent and active TB disease: In collaboration with Borstel Research Centre and a private company Autoimmun Diagnostika GmbH we are evaluating the potential of cells producing Interferon-gamma and Interleukin-2 simultaneusly to distinguish between the latent and active form of TB disease. We are recruiting new active pulmonary TB cases and people with latent TB (IGRA positive)/ TB suspects. This study is ongoing.

5) The evaluation of *Mycobacterium 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 SUN-IRG 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 *M.tb* 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 increases 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 *M.tb* 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 *M.tb* antigen coated tubes (either the currently used QFT test tubes or similar tubes coated with newly identified *M.tb* 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 *M.tb* 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.

In 2011, the consortium completed the antigen-screening of the study. The antigens inducing the most prominent immune response across the different countries were selected for further analysis. Stellenbosch as the PI of the study also led the second annual AE-TBC meeting in Addis Ababa. A workshop on databases was led by Dr Gian van der Spuy.

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 24 normal adult volunteers. To date, no safety concerns were found. The analysis of the immunogenicity data is ongoing. The VPM1002 vaccine induced comparable results to that of BCG in the groups tested, The VPM1002 with the same dose as BCG was moved into a phase IIa vaccine trial. Thus far, four newborns were recruited, vaccinated with VPM1002 and are being followed up over a period of 182 days post vaccination.

The Immunology laboratory in collaboration with Prof Mark Cotton is involved in an NIH sponsored IMPAACT (P1073) study. For the study, infants who develop an immune reconstitution syndrome following the start of ARVs are monitored. All the specialised vaccine based assays are performed in the immunology 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. Accreditation documents were submitted in 2011 and a date for assessment was scheduled for February 2012.

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 M.tb 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 M.tb, 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 M.tb. One PhD student and one BSc honours student (who will continue to pursue an MSc on this research 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. The MPA work was published in the PLOS ONE journal (see publication 21).
We have also established a collaboration with Dr Muazzam Jacobs at the University of Cape Town as indicated in the previous report. 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 (starting in 2011) will investigate whether MPA causes reactivation of latent TB in this mouse model similarly to classical glucocorticoids. A PhD student has completed her studies on this work and will graduate in March 2012.

Host Genetics
Over 90% 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. The most obvious and challenging change is the generation of very large amounts of data, such that traditional methods of analysis need to be replaced by methods previously used in the field of Bioinformatics. The expertise to work with this data is very scarce in South Africa.

Research projects
a) 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). 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.

To date a variety of genes such as *NRAMP1* and *HLA* have been implicated in influencing the host response to TB, albeit with varying effects in different populations. Some of the more recently implicated genes are the pattern recognition receptors, the Toll-like receptors (*TLRs*). Genetic variation in these genes has been associated with a myriad of different diseases, including those of an infectious nature, such as TB. In the case of TB, *TLR2* is the most prominent candidate with *TLR8* and *9* more recently implicated. One of the better known genes implicated with TB is the vitamin D receptor (*VDR*), as the antimicrobial gene cathelicidin (*CAMP*), one of the most important agents of mycobacterial killing, has a VDR response element in its promoter. *TLR2*, *VDR* and *CAMP* are all connected in a complex pathway essential for the host defence against *M. tuberculosis.*

Nine single nucleotide polymorphisms (SNPs) in three TLR genes (*TLR2,8 & 9*) were investigated via a case-control approach to determine their potential role in human genetic susceptiblity to TB in the Coloured population of South Africa. The effect of the VDR polymorphism *Cdx2* on the expression of cathelicidin mRNA and protein expression was also investigated. Three genes were found to contribute significantly to genetic host susceptibility in the Coloured population of South Africa. An allelic association (P = 0.031) was observed for the *TLR8* SNP rs3761624, with the A-allele being more prominent in females. Four haplotypes of *TLR8* were found to be significantly linked to TB susceptibility with the three SNP haplotype rs3761624-rs3764879-rs3764880, specifically the allelic combination of G/C/A [P = 0.004, OR = 2.67(95% CI: 1.90-3.74)], showing a marked association (P = 0.001). The *TLR9* intron-exon2 boundary SNP rs352139 was significantly associated with TB susceptibility on a genotypic (P = 0.02) and allelic scale [P = 0.05, OR=0.70; (95% CI: 0.55–0.90)], with the T allele more frequent in controls. The TLR9 two SNP haplotype consisting of rs5743836 and rs352139 was linked (P = 0.037) to TB susceptibility, specifically the combination of the alleles A/T [P = 0.013, OR=0.71; (95% CI: 0.55–0.92)]. No gene-gene interaction between *TLR2*, *TLR8* and *TLR9* was observed. No significant conclusions could be drawn from the analysis of the mRNA and protein expression of *CAMP* in samples harbouring the different genotypes of the *VDR* polymorphism *Cdx2.*

The role of ten polymorphisms from Surfactant protein D (SFTPD), and Macrophage receptor with collagenous structure (MARCO) within a South African Coloured (SAC) population was investigated. A case-control study design was used and polymorphisms were genotyped with Taqman® genotyping assays and amplification refractory mutation system polymerase chain reaction (ARMS-PCR). The results were analysed for association to disease, linkage disequilibrium, haplotypes and gene-gene interactions. Allele and genotype frequencies were also determined which allowed for comparisons to other populations.

Five SNPs were associated with TB: two in SFTPD (rs1923537; rs2255326) and three in MARCO (rs1318645; rs3943679; rs2119112). The associated SNPs were located in regions other than exons and the effects of polymorphisms in these regions are not well understood but studies in other genes have shown them to play a functional role. Gene-gene interaction analysis showed that polymorphisms interacted with each other within and between genes, illustrating the importance of epistasis and the complexity of the genetic influences on TB.

In addition to the case-control association studies, the role of the rs2569190 promoter SNP in CD14 was assessed. Gene-expression analysis was conducted with qPCR and a reporter gene assay and results from both of these approaches showed that individuals with the TT genotype had a twofold greater expression level than individuals with the CC genotype. Previously, the TT genotype has been associated with stronger promoter activity and expression of soluble CD14 in serum. Since the TT genotype was present at a higher frequency in the control group, we speculate that greater expression of CD14 may contribute to a more TB resistant phenotype.

These studies added substantially to the existing work on the above genes and polymorphisms, and introduced new candidate genes to the field.

b) Admixture mapping: This approach to disease gene discovery has elements of both 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 are applying 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, it was essential 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). This work used the publicly available databases of Hapmap and the hgdp population database at Stanford. We 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. Previously we had collaborated with Lluis Quintana-Murci at the Institut Pasteur in Paris, an international expert on mtDNA in population and evolutionary genetics, and 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. Again we found exceptionally high Khoisan contribution from the maternal side, providing much historical insight (Quintana-Murci et al 2010). Work on the fine tuning of these estimates, using a larger number of individuals of Khoisan ancestry, is in progress. This population complexity means that none of the previously established algorithms to do admixture mapping (in African Americans) are applicable to our population, and this is the subject of further investigation of appropriate methodology.

c) 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. A previous study of over 800 cases and controls investigated epistasis between 11 polymorphisms in 9 genes, and found eight instances of statistically significant gene-gene interactions (de Wit 2010b). Further study of epiatasis using the 500k SNP chip data is being carried out using a bioinformatics approach, as the number of potential interactions in this data is too large for traditional approaches.

Targets and new drug development

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 sources 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, namely *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 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 regulators. 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 were undertaken in order to compare the difference (if 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 present in *C. glutamicum* did not result in marked changes in growth in *M. smegmatis;* however there were some modest effects on the transcriptional regulation of a few genes. These same knockout strains were used to infect murine derived bone macrophages (MDBMs) in order to determine the effect of the genetic deletions on the ability of the bacteria to survive *in vivo.* It was found that the *M. smegmatis* strain harbouring the deletion of the regulator similar to the one found in *S.coelicolor* was compromised in its ability to survive within the macrophage. This result could indicate the importance of the aforementioned regulator and therefore, the regulation of the nitrogen metabolic pathway in mycobacterial survival.
In addition to studies done at a transcriptional level, an investigation into the function of the effector enzymes of nitrogen metabolism were 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 is 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 *M. bovis BCG*, it was found that it functions in a very different manner in comparison with *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, however we have shown that an additional nitrogen assimilatory route is possible via glutamate dehydrogenase.
In order to assess the importance of GDH, as an alternate pathway for nitrogen assimilation, to the survival of slow growing pathogenic mycobacteria (e.g. M. tuberculosis) in various environments (e.g. in the macrophage or during latent infection) a GDH gene deletion mutant of *M. bovis BCG* was generated. The nitrogen assimilatory machinery of non-pathogenic *M. bovis BCG* is highly similar to that of *M. tuberculosis* making *M. bovis BCG* an excellent safer model organism for the pathogen. Results from this experiment are still pending, but preliminary findings using the GDH deletion mutant to infect MDBMs with show that GDH is important to the intracellular survival of the organism. Additional experimentation will be undertaken to establish whether GDH is also important to the dormant state that tubercle bacilli persist on in during latent infection as well as to elucidate possible mechanisms that control the activity and function of GDH in slow growing mycobacteria.
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.

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 logsin 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 at 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 analyze the data: 1) what are the genes that were differentially regulated between the control and the treated and 2) which genes are coordinately regulated. Our microarray data has been shown to be reproducible and reliable. When we used the latter paradigm, genes that were coordinately regulated are involved in iron acquisition. The iron acquisition pathway forms part of the gene signature profile of F1082 and their involvement in the mechanism of action of the compound was further tested using knock-out studies. We observed that F1082 is more active in *M. tuberculosis* that was grown in iron rich medium than in low iron. Mutants (irtAB) deficient in iron transport were protected from F1082 activity showing that F1082 requires intracellular iron for most activity. We proposed that F1082 may be generating oxidative stress since abundance of Fe(II) may participate in Fenton reaction thereby generating oxidative stress. We evaluated the activity of F1082 in mutants deficient in protection against oxidative stress and indeed we found that F1082 was more active in these strains (mshA and sodA mutants).We concluded that the F1082 mechanism of action involves the generation of oxidative stress. Our findings are in line with the published data that furanones are mutagenic and cause DNA damage in human cell-line. The cytotoxicity observed in THP1 cell-line and Chines hamster ovary cells hampers the use of F1082 as an anti-TB candidate drug. However F1082 can be used directly to identify new M. tuberculosis targets through studying the mechanism of action and identifying the target enzyme/s or as a scaffold from which analogues can be synthesized. About 42 analogues were synthesized and activity testing was performed against M. tuberculosis. Four compounds were identified to be promising. These compounds had improved activity and were less toxic on Chinese ovary cells. With improved activity and less toxicity, these compounds were thought to warrant testing in animal models for development of TB drug candidate. The future of this project is to provide compounds that will be developed into TB drug and establish a platform for TB drug screening.

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

We evaluated 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 regimens used against *Mycobacterium tuberculosis*.

The M.tb two component system: Regulation of dormancy in human macrophages.

The investigation was aimed to improve the understanding of the binding interactions between DevS and DevR that are implicated in the regulation of the dormancy response in *Mycobacterium tuberculosis*. These binding interactions could possibly provide good drug targets for the treatment of persistent tuberculosis, and the mechanistic understanding of their binding interactions is important for the development of a validated inhibitor screen. A detailed *in silico* analysis of the amino acid residues that play a role in the binding of receptor DevR to both kinase DevS and the target DNA was undertaken. A reasonable approximation of the DevS structure was produced using homologous protein structures. *In silico* docking of DevS to DevR merely produced a set of probable candidate structures, since more than one conformation with similar docked energies was observed. The decision on which one is the more correct form can only be estimated by crystallization of this complex. Therefore, the functional expression and purification of the Dev TCS components were pursued. Denaturing HIS™-select nickel affinity gel purification in the form of matrix-assisted refolding led to the production of functional Dev TCS proteins. To understand the binding of DevR to DNA consensus sequences, as well as the nature of these interactions, a model was built of the full length DevR dimer binding to DNA consensus sequences. Based on this model, single mutations were made to DevR *in vitro* and their effects assessed in order to validate the model built. During Electrophoretic Mobility Shift Assay (EMSA) analysis, it was found that K179I and N183L mutants prevented the binding of DevR to the DNA consensus sequences. If DevR and DevS binding are to be used in a drug development program, it is essential to have the protocols to accurately measure their interaction, in addition to developing a fundamental understanding of how their interactions occur. The binding affinity of DevR to both DevS and the truncated soluble fragment of DevS (DevS201) were explored, using the BIAcore instrument, an SPR-based biosensor. For sufficiently strong binding between a histidine kinase and a response regulator, the KD needs to be in the nM range. The KD was calculated to be 255 nM for DevS201 and 184 nM for DevS. Therefore it can be concluded that DevS201 binds DevR strongly enough to be used in future studies, and that the BIAcore could be used to screen small-molecule inhibitors of DevR-DevS interactions.

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.

Novel Linear Diamine Disubstituted Polycyclic'Cage' Derivatives as Potential Antimycobacterials

As a part of an ongoing project to develop highly potent antituberculosis therapeutics, a series of novel polycyclic 'cage' tetra-amines were synthesized and screened for in-vitro antituberculosis activities against the H37Rv strain of tuberculosis. Three disubstituted polycyclic moieties, namely pentacyclodecane, pentacycloundecane, and ricyclodecane, were used in this study. Compounds 5 and 7 showed similar activity to SQ109 at a MIC of 1 lM while compounds 4, 6 and 8 displayed MIC activity at 1 < MIC<10 lM against H37Rv strain of tuberculosis. Compounds 5, 7 and SQ109 were selected for further screening against, multi-drug resistant, (R1097) and extensively drug resistant, (X149) strains of tuberculosis. Compound 5 showed anti-TB activity of <1 lM against extensively drug resistant strain (X149) and a MIC = 1 lM against multi-drug resistant strain (R1097), while compound 7 and SQ109 showed excellent anti-TB activity against both drug-resistant strains at a MIC <1 lM. This study demonstrates the first reported analysis of pentacyclo-undecane as a potential therapeutic agent.

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.

Eliminating *M.bovis* from raw milk with Kefir fermentation.

M.bovis may contaminate raw milk which is used in rural areas in most African countries and fermented into traditional beverages. This study was initiated in collaboration with the department of Food Sciences of Stellenbosch University in order to establish a fermentation method that would simultaneously eliminate *M.bovis* from the raw milk. Kefir is a type of fermented milk in which Kefir grains are used as a starter. Due to lactic acid bacteria (LAB) and other fermentative microorganism count of the Kefir grains, the fermentation process and the reported ability to inhibit other pathogenic and spoilage bacteria takes place at household temperatures (25^{o}C). Fermented and fermenting Kefir beverages were used as a representative of fermented milk in this study. Currently, there is a lack of reported information on the prevalence of *M. bovis* in milk and milk products, as well as studies on the effect of milk fermentation on the survival of *M. bovis*. This is in part, due to the lack of recognized quantitative culture and molecular methods for *M. bovis* detectionfrom dairy matrices. In order to establish an efficient method for the isolation and quantification of viable *M.bovis* from milk and fermented milks, two chemical decontamination protocols, namely: N-acetyl-Lcysteine-NaOH (NALC-NaOH) and 0.75% HPC (hexadecylpyrimidinium chloride for 5 h), as well as sample treatments using the two antibiotics, Erythromycin and PANTA^{TM} were evaluated. The addition of PANTA^{TM} (Becton Dickinson), a lyophilized antimicrobial preparation into the 7H10 culture media, was found to be the most effective method for enumeration of *M.bovis*. The HPC method showed itself to be an economic alternative for decontamination of fermented milk samples, however, the inclusion of a chemical treatment prior to culturing, drastically affected the viability of *M.bovis*. This method was shown to be capable of suppressing organisms associated with raw end fermented milk with minimum effect on the mycobacteria. These two methods were used for all further studies. The effectiveness of each extraction method was also evaluated by PCR, using primers targeting a 580bp IS6110 fragment (Vitale et al., 1998). This insertion sequence is specific for MTBC. The PCR method showed promising features for detection of *M.bovis* from farm milk in Africa. However the method did not show repeatability. This might be due to the fact of the target element can be scarce (1 to 6 copies present) in *M.bovis* strains. The classical human M. tuberculosis presents many copies of IS6110 fragment.

The results of this study indicated that milk fermentation has potential method to prevent milk-born tuberculosis, however, the ability of Kefir to inhibit *M.bovis* depended strongly on the initial load of Mycobacterium in milk. As the exact amount of *M.bovis* present in infected cows' milk is not known, we considered a high bacterial load in order to clearly demonstrate anti-microbial activity. The implications of these results on public health depend on the technology of each local product. The microbiological safety of fermented milk increases with an increase in the time of fermentation towards 60 h.

Drugs designed against mycothiol and ergothioneine 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 and inhibitors against enzymes of the mycothiol pathway is based on the premise that mycothiol is unique to mycobacteria, and is thus 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. Nikkomycin exhibited no activity in the extracellular assay.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 drug was, however, cytotoxic for macrophages, since they became permeabilized or underwent lysis. This occurred also with lower concentrations, eg 1µg/ml. In 2012, 21 newly designed drugs will first be subjected to a high throughput mshA enzyme screening assay, before testing for antitubercular activity.

Determining whether the designed drugs will be viable to investigate further as potential new antitubercular lead compounds is impeded by the unknown contribution of ergothioneine which is another thiol compound present in mycobacteria. In 2011 we initiated a major study to understand the compensatory role of mycothiol and ergothioneine in the survival of mycobacteria.

Thus far, we have knocked out a gene coding for EgtD (an enzyme involved in ergothioneine biosynthesis) from the wild type (mc^{2}155) and mycothiol deficient mutant (Δ*mshA*) strains. We discovered that EgtD is imperative

for biosynthesis since the mutants were found to be deficient in ergothioneine. In stress assays we found that the ergothioneine deficient single mutant (Δ*egtD)* and mycothiol deficient single mutant (Δ*mshA)* were slightly sensitive to oxidative stress conditions generated by cumene hydroperoxide, compared to the wild type, while the double mutant (Δ*mshA/egtD)* was significantly affected. This suggests a co-operative antioxidative role of ergothioneine and mycothiol in mycobacteria. In addition to that, as opposed to previous reports on mycothiol, we could detect extracellular ergothioneine and we have a preliminary proof that extracellular ergothioneine results from an excretion/secretion system. The MIC (minimum inhibitory concentration) of first-line anti-TB drugs against the Δ*mshA* was not changed by knocking out Δ*egtD,* except that their Ethionamide MIC was higher, suggesting an ergothioneine contribution in the activation of this drug in mycobacteria.
We still have to evaluate the role and association of mycothiol and ergothioneine in the survival of mycobacteria in nitrosative stress conditions, and in macrophages. We also aim to validate the finding that ergothioneine is actively secreted.

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 anti tuberculosis 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. A recent survey conducted by the CDC suggested that more frequent inspections are on the cards. Only few centres globally can currently fulfil those requirements. 2011 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 were evaluating combinations in EBA studies (TMC207, PZA, PA-824, and Moxifloxacin) and have completed the clinical evaluation of SQ-109 as well as PNU-100480. 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 (Study 29). 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.
We have during this year collaborated with the Global Alliance for TB Drug Development (GATB, 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. We have submitted a draft to the ACTG Aids/tuberculosis to collaborate in a study A5312, “Early Bactericidal activity of high-dose isoniazid among adult patients with different genetic variants of INH-Resistant Tuberculosis.
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. The TBTC is expanding its Study 29 to Study 29X to incorporate the use of the GeneXpert to evaluate the data generated by it as an effective biomarker of response to treatment. This will complement ongoing investigation within the TBTC of the relationship between sputum AFB smear, solid and liquid media results and time to detection on liquid media during TB therapy. GATB will continue both Phase IIA and IIB work with novel drug combinations. 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.
The laboratory comprises of two components, a clinical trial side and a research side. Capacity of the laboratory is the current bottleneck for further expansion of activities, and its lack of accreditation is 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 with ongoing commitment. We see this as a weak point in the centre at present. Active attention is being given to get the laboratory accredited. Visits and documentation were already conducted by and submitted to a South African accreditation body.

Prof Diacon and his research group are also involved in several scientific projects and collaborations. Detection of M.tb using the GeneXpert PCR machine was investigated in several types of body fluids (blood, cerebrospinal and pleural fluids, gastric aspirates and stools) from adults and children suspected to be infected with M.tb and M.tb meningitis. The study of pulmonary infections with M.tb was investigated by analysis of exhaled breath samples. During an EBA study testing a new antibiotic, PNU-100480, drug containing whole blood samples were collected from patients and incubated with H37Rv and the bacteria retrieved by blood lysis.

Changes of growth caused by the drug present in blood were determined by liquid culture. This whole blood assay for the determination of drug activity in blood was novel to our centre. Two students (MSc and PhD) are conducting their studies testing the new drug SQ109. Several technicians from the CCTR laboratory were trained to use the GeneXpert to analyse sputum samples as part of the drug trial screening or follow-up procedure.

Veterinary Tuberculosis

Diagnostics

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. Ongoing epidemics of bovine TB (BTB) are currently experienced in the Kruger National Park (KNP) and Hluhluwe-iMfolozi National Park (HiP). In 2010 we validated the modification of a human interferon-gamma release assay (IGRA), the QuantiFERON-TB Gold assay, for use in African buffaloes (*Syncerus caffer*). A description of this modification (mQFT) has been published (Parsons et al 2011) and further evaluation of its use in the field was done in 2011. This subsequent work has established that the mQFT is significantly more sensitive than the tuberculin skin test (TST), the BTB test traditionally used in HiP. Additionally, use of the mQFT reduces, by two days, the length of time that animals must be kept captive, and significantly reduces the intensity and costs of animal handling. These factors reduce the risks of severe trauma to both animals and handlers. In 2012, an evaluation of the use of the mQFT as the primary BTB test in the HiP will be undertaken.

As result of the BTB epidemic in buffaloes, other species are exposed to this disease, resulting in severe disease in some and concerns about their ecology (most notably lions). In 2011 we confirmed the utility of a commercial feline interferon-gamma (IFN-γ) ELISA kit to detect the IFN-γ of lions. This preliminary finding validates the use of this ELISA kit in a potential modification of the QFT for use in lions. In 2012, this potential will be further explored by testing of lions in the KNP.

Rhinoceroses are another species of concern with regards to BTB. It is believed that in the wild this species rarely develops clinical TB, however, numerous rhinoceroses are annually translocated from both the KNP and HiP to locations which are BTB-free, with the risk of the introduction of BTB to these areas. As such, a BTB test for rhinoceroses is urgently needed. In 2011, we validated the use of bovine IFN-γ ELISA kit for the detection of rhinoceros IFN-γ in preparation for the development of a BTB test. In 2012, a controlled rhinoceros BTB infection study in the KNP will provide an opportunity to potentially validate the use of the mQFT in this species.

A recurrence of *M. tuberculosis* infections in the primate colonies of the MRC was also investigated by our division. As done previously, use of the QFT assay contributed to the sensitive detection of TB cases in baboons and rhesus macaques.

The development of IGRA tests for animal species is dependant of the availability of appropriate reagents (in particular antibodies which are specific for the host IFN-γ). To overcome this limitation so that the assay can be adapted for use in exotic species we investigated the use of the QFT in a modified gene expression assay (GEA). Buffaloes from the HiP were used as model hosts, and IFN-γ gene expression was measured as a test outcome. In this pilot study, we showed that the GEA had a sensitivity of 80% and a specificity of 95% to detect BTB-positive buffaloes. In 2012, this assay will be further refined and validated for use in other species.

Ongoing projects included the support of local State Veterinary control of BTB in dairy cattle herds in the Western Cape. The laboratory provided diagnostic and bacteriological support to Dr S. Davey of the Malmesbury State Veterinary office.

Whole genome and disease susceptibility work

Sequence data previously generated in collaboration with the University of Liverpool identified approximately 6.5 million novel SNPs in the African buffalo genome, of which nearly two million were within gene regions. Bta4 (*Bos taurus*) gene annotation was added to all SNPs identified within whole gene regions. Inspection of the SNPs identified allowed for the selection of SNPs in particular genes or pathways of interest for investigation in BTB susceptibility in African buffalo. Selected SNPs were subject to validation and fluorescent genotyping in approximately 900 African buffalo samples. This sample set included both TB skin test positive and negative individuals. Association testing was subsequently performed on the validated SNPs using the genotype data and various analysis parameters. An additional epidemiological dataset collected over a seven year period was collated and analysed to identify the prevalence and incidence changes in bovine TB in African buffalo in Hluhluwe iMfolozi Park due to the test and cull BTB programme. This work forms part of a PhD thesis and as a series of future publications.

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 Prof G van der Spuy. Amongst the various services provided by the server platforms are three 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 two years on a remote server and currently comprises 1.9 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.

A recent development is the acquisition of a dedicated high-end bioinformatics server for the analysis of large and complex datasets generated by high-throughput technologies such as whole-genome sequencing and expression micro-array chips. The presence of this machine in our centre is facilitating the development of in-house bioinformatics capacity with respect to both staff and students.