



FACULTY OF HEALTH SCIENCES UCT

Department of Clinical Laboratory Sciences
Department of Human Biology
(incorporating members of the IIDMM)



4th POSTGRADUATE RESEARCH DAY
7 September 2011

**Lecture Theatre, Learning Centre
Anatomy Building**

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Welcome from the Organizing Committee

We would like to welcome the students, staff, and representatives from some of our sponsors to the 4th Postgraduate Research Day that incorporates the Departments of Human Biology, Clinical Laboratory Sciences and the Institute of Infectious Disease and Molecular Medicine. The School is engaged in a wide spectrum of basic and applied research activities, from the level of the gene via the cell and organ to the whole body.

The purpose of this Research Day is two fold, firstly, to give postgraduate students and postdoctoral fellows of the School an opportunity to present their research work, since communication of research is an important element of research activity and secondly, to expose student researchers to the diverse research conducted in the Faculty of Health Sciences in order to create interest in the research of their fellow students and hopefully to promote collaboration between researchers of different disciplines. We hope the formal conference setting will be a useful learning experience and provide good preparation for developing researchers for future presentations at conferences.

We thank the Divisional/Departmental Heads and representatives for their cooperation and participation. We also thank the various companies and organizations listed on the previous page for their generous support. These contributions allow us to offer a number of prizes for the best oral and poster presentations. Special thanks to the Judges for being willing to spend their time evaluating the presentations and making the awarding of prizes as fair as possible!

The Organizing Committee

Dr. Eliya Madikane
Dr. Karen Shires
Dr. Kishor Bugarith
Prof. Peter Meissner
Dr. Roisin Kelly-Laubscher
Dr. Virna Leaner
Ms. Debbie Victor

PROGRAM

08h30 Registration

08h45 Opening and welcome
Prof. Peter Meissner

09h00 – 10h30 SESSION 1

Chair: Prof. Peter Meissner

09h00 **Colin Anthony**
The role of glycosylation in the expression, stability and crystallization of the N-domain of Angiotensin Converting Enzyme. **(A1)**

09h15 **Aretha Cooper**
A tumour suppressor role for the TBX3 transcription factor in fibroblasts. **(A2)**

09h30 **Lindie du Plessis**
MRS study of the cerebellar deep nuclei in children with fetal alcohol spectrum disorder. **(A3)**

09h45 **AD Einhorn**
The role of cytokine producing B-effector cells in murine susceptibility to *leishmania major*. **(A4)**

10h00 **Krishnamoorthy Gopinath**
Identification of genes required for vitamin B₁₂ transport in *Mycobacterium tuberculosis*. **(A5)**

10h15 – 11h00 BREAK / POSTER SESSION (P1-27T)

11h00 – 12h30 SESSION 2

Chair: Dr. Eliya Madikane

11h00 CE Haumann

Investigation of an atypical protoporphyrin family in South Africa. **(A6)**

11h15 Nina Holderness

PAK3 is an AP-1 regulated gene required for cJun/AP-1 induced cellular transformation. **(A7)**

11h30 MJ Jansen van Rensburg

The molecular characterization of methicillin-resistant *Staphylococcus aureus* from hospitals in Cape Town. **(A8)**

11h45 Agano Kiravu

Immune memory to an HIV vaccine candidate in a non-human primate model. **(A9)**

12h00 Amod Kulkarni

Development of antioxidant therapy to control neuroinflammation induced by HIV proteins gp120-41 and Tat using SK-N-SH neuronal cell lines. **(A10)**

12h15 – 13h30 LUNCH / POSTER SESSION (P1-27T)

13h30 Mary-Jessica Laguette

Sequence variants within the 3'-UTR of the COL5A1 gene alters mRNA stability: Implications for musculoskeletal soft tissue injuries. **(A11)**

13h45 G Magombedze

Dynamic explanation of the occurrence of latent TB infection using latency and dormancy time course gene expression. **(A11)**

14h00 Lindi Roberts

Role of genital tract inflammatory cytokines in susceptibility to HIV-1 infection in women who became infected despite using the Caprisa004 1% Tenofovir microbial gel. **(A13)**

14h15 Natalie Nieuwenhuizen

Regulation of allergic airway disease by IL-4/IL-13 activated macrophages and dendritic cells. **(A14)**

14h30 JS Womersley

Maternal separation affects dopamine transporter function in the spontaneously hypertensive rat: an in vivo electrochemical study. **(A15)**

15h30 – 17h00 SESSION 4

Chair: Dr. Virna Leaner

15h30 Philippa Randal

Understanding host immune function during tuberculosis meningitis infection: a neural route for *mycobacterium tuberculosis* infection. **(A16)**

15h45 Sumayah Salie

Anti-tubercular studies of phenothiazine derivatives in *mycobacterium tuberculosis*. **(A17)**

16h00 Krishna Sharma

Depigmentation in melanomas increases the efficacy of hypericin-mediated photodynamic therapy. **(A18)**

16h15 TL Sterley

Effects of maternal separation on glutamate, GABA, and potassium-evoked release of hippocampal [³H] norepinephrine in a rat model of attention deficit-hyperactivity disorder. **(A19)**

16h30 Suraj Parihar

Statin-induced autophagy and phagolysosomal maturation leads to reduced growth of intracellular pathogens. **(A20)**

17h00 COCKTAIL FUNCTION AND PRIZE GIVING

POSTERS : MORNING SESSION

P1-T	Putative polymorphic binding site for hsa-mir-608 (<i>acñ rflp c>a</i>) in the 3'-UTR of <i>col5a1</i> is functional. Yoonus Abrahams ¹ , Sharon Prince ¹ , Malcolm Collins ^{2,3,4}
P2-T	The role of muscarinic acetylcholine receptor M3 in immune response to pathogens using transgenic murine models. Alykhan Vira*, Matthew Darby*, Jurgen Wess#, Frank Brombacher*, Murray E. Selkrik [¶] , William G.C. Horsnell*.
P3-T	The physiological implications of drug resistance mutations in mycobacteria Anastasia Koch ^a , V Mizrahi ^a , DF Warner ^a
P4-T	Quantifying right ventricular motion and strain using 3D cine dense MRI D.A. Auger ^a , X. Zhong ^b , F.H. Epstein ^c , and B.S. Spottiswoode ^{a,d}
P5-T	Genome-wide haplotype and population structure of indigenous Southern African populations Wayne Delport ^{1,2} , Ayton Meintjes ¹ , Cathal Seoighe ^{1,3} , Himla Soodyall ⁴ & Raj Ramesar ⁵
P6-T	The molecular epidemiology of <i>Streptococcus pyrogenes</i> pharyngitis among children in the Vanguard Community (Bonteheuwel/Langa), Cape Town, South Africa Muhamed Babu ^{1,2} , Whitelaw Andrew ¹ , Engel Mark ² and Mayosi Bongani ² ¹ Division of Medical Microbiology, ² Department of Medicine, UCT
P7-T	Transcriptional profiling of normal and transformed oesophageal epithelial cells in response to benzo[a]pyrene AJ Bick ^a , HH Otu ^{b,c} , LF Zerbinia ^a , CG Mathew ^d , MI Parker ^a
P8-T	The role of TNF AND MYD88 in experimental Tuberculosis of the central nervous system. BF Sebesho ¹ , N Hsu ¹ , P Randall ¹ , NM Francisco ¹ , N Allie ¹ , I Dambuza ¹ , R Keeton ¹ , L Kellaway ² , M Jacobs ^{1,3}
P9-T	Substrate binding to MSHB, a zinc peptidase in the mycothiol Biosynthetic pathway of <i>Mycobacterium Tuberculosis</i>. SG Broadley ^a , BT Sewell ^a
P10-T	Matrix metalloproteinase genes on chromosome 11q22 and sit-and-reach range of motion in humans. Marilize C Burger ^{1*} , James Brown ¹ , Kevin O'Connell ¹ , Martin P Schwellnus, Stuart M Raleigh ³ , Michael Posthumus ¹ , Malcolm Collins ^{2,1}
P11-T	A novel application of isothermal titration calorimetry for analysing the kinetics of angiotensin converting enzyme Christopher J. Yates ^a and Edward D. Sturrock ^a ,
P12-T	HIV-1 superinfection results in potent neutralizing antibody responses to the superinfecting variant, but does not necessarily promote neutralization breadth Daniel J. Sheward ¹ , Penny L. Moore ^{2,3} , Maphuti C. Madiga ² , Elin S. Gray ² , Roman Ntale ¹ , Zenda L. Woodman ¹ , Jinal Bhiman ^{2,3} , Florette Treurnicht ¹ , Hayley Harvey ¹ , Sengeziwe Sibeko ⁴ , Koleka Mlisana ⁴ , Salim S. Abdool Karim ⁴ , Lynn Morris ^{2,3} , and Carolyn Williamson ¹ , and the CAPRISA002 Study Team ⁴
P13-T	<i>Bordetella Trematum</i>: an emerging human pathogen? Ilonka Engelhardt ¹ , Mischka Moodley ^{1 2}
P14-T	Risk factors for obesity development in zulu women: personal and parental weight history, weight management practices and taste sensitivity (a case-control study). Herrmann F, Harbron J, Senekal M
P15-T	Initiation of allergen specific t-helper 2 immune responses in the absence of IL-4 signals CD4⁺ t cells Frank Kirstein, Natalie Nieuwenhuizen and Frank Brombacher
P16-T	Generation and analysis of large-scale data driven <i>Mycobacterium Tuberculosis</i> functional networks for drug target identification Gaston K. Mazandu ^a , Nicola J. Mulder ^a ,

P17-T	Derivatives of natural products in the treatment of oesophageal cancer Nelusha Shunmoogam-Gounden ^a , Renatte Hans ^b , Aman Mahajan ^b , Kelly Chibale ^b , Denver T. Hendricks ^a
P18-T	Validation of Hepatitis B viral load on the Roche COBAS AmpliPrep / COBAS TaqMan instrument. Marie-Paule Henshall-Howard, Stephen Korsman, Marvin Hsiao
P19-T	The WNT signalling pathway in Ewing Sarcoma / primitive Neuroectodermal Tumour: an Immunohistochemical investigation HT Wu ¹ , H Carrara ² , N Allie ¹ , D Govender ¹
P20-T	Characterisation of the Alzheimer's Amyloid-β peptide cleavage by angiotensin converting enzyme. K. Larmuth, ED Sturrock ^a ,
P21-T	The role CD4+ T cells in host protective responses against Cutaneous Leishmaniasis using genome-wide transcriptomics. Liesel Smith, Anita Schwegmann, Fadwah Booley, Adrew Einhorn, Farahnaz Ketwaroo and Frank Brombacher
P22-T	Maternal separation impedes exercise-induced phosphorylation of ERK1/2 in adult rat hippocampus. Nokuthula Makena, Kishor Bugarith and Vivienne Russell
P23-T	The role of metabolic enzyme, phosphoglucosmutase 1 in cancer development Hapiloe Maranyane ^a , Pauline Van der Watt ^a and Virna Leaner ^a ,
P24-T	Variation in measurements of recovery following a standardized exercise bout in trained and untrained individuals T. Mann and M. Lambert
P25-T	The utility of transrenal DNA PCR as a possible diagnostic tool for tuberculosis Veronica Allen, Lemese Ah Tow Edries, Widaad Zemanay, Mark Nicol
P26-T	Development of an artificial knee meniscus: preparation and properties Tiamiyu A.O and Vaughan C.
P27-T	Generating an oxidative stress model in human skin cells for antioxidant testing Ayesha Parker ^a , Elizabeth Adelakun ^a , Tukayi Kudanga ^a , Lester Davids ^b , Marilize Le Roes-Hill ^a , Stephanie Burton ^c
POSTERS : AFTERNOON SESSION	
P1-L	The role of dectin-1 in immunity to strains of <i>Candida Albicans</i> ¹ Mohlopheni J. Marakalala, ¹ S. Vicky Tsoni, ² Ann M. Kerrigan, ² Donna M. MacCullum, ² Neil A. ² Gow, ² Frank C. Odds and ^{1,2} Gordon D. Brown
P2-L	Human Papillomavirus genome sequencing and typing using Illumina next-generation sequencing. Tracy L. Meiring ^a , Ana T. Salimo ^b , Inga Hertzeroth ^b , Ed Rybicki ^{a,b} , and Anna-Lise Williamson ^{a,c}
P3-L	Early selective pressures acting on the HIV-1 subtype c transmitted/founder whole genome in five individuals with differing disease progression profiles. Melissa-Rose Abrahams ¹ , Florette Treurnicht ² , Nobubelo Ngandu ¹ , Sarah Goodier ¹ , Jinny Marais ¹ , Ruwayhida Thebus ¹ , Michael Liu ³ , Nilu Goonetilike ³ , Helba Bredell ⁴ , Ziyaad Valley-Omar ¹ , Debra de Assis Rosa ² , Mandla Mlotshwa ² , Koleka Mlisana ⁵ , Cathal Seoighe ⁶ , Salim Abdool Karim ⁵ , Andrew McMichael ³ , Clive Gray ⁷ and Carolyn Williamson ¹
P4-L	A field evaluation of a chemiresistive sensor array as a rapid, point of care diagnostic for Pulmonary Tuberculosis V. Mischka Moodley ¹ , William Royea ² , Mark Nicol ¹
P5-L	<i>Acinetobacter baumannii</i>: an evaluation of five susceptibility test methods to detect Tobramycin Resistance in an epidemiologically related cluster. V. Mischka Moodley ^{1,2} , Stephen P. Oliver ^{1,2} , Iva Shankland ² , and B. Gay Elisha ^{1,2}
P6-L	Repigmentation in Vitiligo: an immunohistochemical analysis of melanocyte migration, proliferation and differentiation M.Petersen, L.M. Davids, and S.H. Kidson.
P7-L	Characterization of the antimycobacterial effect of a <i>Pseudomonas</i>-derived activity Krupa Naran ^a , Caryn Fenner ^b , Susan T.L. Harrison ^b , Valerie Mizrahi ^a and Digby F. Warner ^a
P8-L	IL-4Rα responsive B cells are required for protection against acute Schistosomiasis and down-regulation of gut inflammation.

	H. H. Ndlovu¹, W Horsnell¹ and F. Brombacher¹
P9-L	Investigations into identifying the possible drug targets of the garlic compound ajoene Ellen Ngarande ^{a,b} , Catherine H Kaschula ^{a,b} , M Iqbal Parker ^{a,b}
P10-L	Collagen genes and exercise associated muscle cramping in ironman triathletes Kevin O'Connell ¹ , Michael Posthumus ^{1,3} , Martin P. Schweltnus ^{1,3} , and Malcolm Collins ²
P11-L	The characterisation of novel avipoxviruses for use as potential vaccine vectors Kristy Offerman ^a , Olivia Carulei ^a , Nicola Douglass ^a and Anna-Lise Williamson ^a ^a Division of Medical Virology, Department of Clinical Laboratory Sciences, IIDMM
P12-L	DNA sequence analysis of the penguinox virus genome Olivia Carulei ^a , Nicola Douglass ^a and Anna-Lise Williamson ^{a,b}
P13-L	Overexpression of KPNB1 and KPNA2 importin proteins in cancer derives from deregulated E2F activity Pauline J. van der Watt, Ellen Ngarande and Virna D. Leaner
P14-L	TBX3, a t-box transcription factor, promotes melanoma formation and invasion by regulating key cell adhesion and extracellular matrix proteins Jade Peres ^a , Shaheen Mowla ^b , and Sharon Prince ^a
P15-L	Development of reverse tetracycline inducible system to improve stability and immunogenicity of recombinant <i>m. Bovis BCG</i> expressing HIV antigens Mbele, P ¹ ., Stutz, H ¹ ., Mayat, N ¹ ., Shephard E.G ^{1,2} ., Williamson, A-L ^{1,3} . and Chapman, R ¹
P16-L	The detection and characterisation of multi-drug resistant <i>Pseudomonas aeruginosa</i> isolates from patients in Groote Schuur Hospital Rachael Jacobson ¹ , Nadia Minenza ¹ , Maanda Mudau ^{1,2,3} and Dr Colleen Bamford ¹
P17-L	Investigating the role of IL-4 receptor-alpha deficient dendritic cells during Cutaneous Leishmaniasis. Hurdayal, R ^a , Nieuwenhuizen N ^a , and Brombacher, F ^a
P18-L	Finite element modeling and strain analysis of healthy and infarcted left ventricles. R Miller ¹ , J Kortsmi ¹ , N Davies ¹ , P Zilla ¹ , T Franz ^{1,3,4}
P19-L	Identification and characterisation of a binuclear Palladacycle Complex (AJ-5) as a novel anti-cancer drug in the treatment of human breast cancer Saeb Aliwani ¹ , Selwyn Mapolie ² and Sharon Prince ¹
P20-L	Studies into the anti-metastatic activity of ajoene and related analogues in WHCO1 Oesophageal Cancer cells Vuyolwethu Siyo, Catherine Kaschula and M Iqbal Parker
P21-L	The sensitivity and specificity of serum prolidase activity as a marker for liver fibrosis in suspected liver disease John C Stanfliet ¹ , Michael Locketz ² , Peter Berman ¹ , Tahir S Pillay ^{1,3}
P22-L	Cancer testis antigens in Multiple Myeloma Andrea Stavridis ¹ , Karen Shires ¹
P23-L	Contribution of the drug transporter <i>ABCB1</i> in predicting efavirenz plasma concentration and response to antiretroviral therapy in South African patients. ¹ M. Swart, ² Y. Ren, ² P. Smith, ³ S. Takuva, ³ P. MacPhail, ¹ C. Dandara
P24-L	Human Immunodeficiency Virus (HIV) infection in partners influences Human Papillomavirus (HPV) transmission among heterosexually active couples. Zizipho Z. A. Mbulawa ¹ , Dianne J. Marais ¹ , Leigh F. Johnson ² , David Coetzee ² , Anna-Lise Williamson ^{1, 3}
P25-L	HIV-1 GAG and NEF diversity in Cameroon: evidence of a high degree of recombinant forms. Marcel TONGO ^{1,2} , Lycias ZEMBE ¹ , Andile NOFEMELA ¹ , Roman NTALE ¹ , Eitel MPOUDI-NGOLE ² , Carolyn WILLIAMSON ^{1,3} and Wendy A. BURGERS ¹
P-26-L	Potent anti-vector responses to a candidate MVA-vectored HIV vaccine have no effect on immunity to the HIV antigens Tracey L. Muller ¹ , Anna-Lise Williamson ^{1,2} , Agano Kiravu ¹ , Rubina Bunjun ¹ , Gerald K. Chege ¹ , Nicola Douglass ¹ , Gary H. Cohen ³ , Roselyn J. Eisenberg ³ , and Wendy A. Burgers ¹
P27-L	Implementation and evaluation of a bony structure suppression software tool for chest x-rays Toinette-Lee Dixon [*] , Tania Douglas [*]

ORAL PRESENTATION ABSTRACTS

A1

THE ROLE OF GLYCOSYLATION IN THE EXPRESSION, STABILITY AND CRYSTALLISATION OF THE N-DOMAIN OF ANGIOTENSIN CONVERTING ENZYME

Colin S. Anthony^a, Sylva L.U. Schwager^a, Vincent Dive^b, Hazel R. Corradi^c, K. Ravi Acharya^c and Edward D. Sturrock^a

^aInstitute of Infectious Diseases and Molecular Medicine and Division of Medical Biochemistry

^bCommissariat à l'Énergie Atomique, France

^cUniversity of Bath, UK

Angiotensin converting enzyme (ACE) is a key regulator of blood pressure as a result of its central role in the renin-angiotensin and kallikrein-kinin systems. ACE contains two domains, the N- and C-domains, both of which contain several *N*-glycosylation sites. The N-domain also plays an important role in inactivating the regulatory peptide Ac-SDKP, which has anti-fibrotic properties. Structural studies of ACE have been plagued by severe difficulties resulting from the high degree of surface glycosylation on the protein. We attempted to investigate the role of glycosylation in the N-domain and to create forms of the enzyme suitable for crystallization. The importance of the ten potential *N*-linked glycan sites was investigated using enzymatic deglycosylation, limited proteolysis and mass spectrometry. A number of glycosylation mutants were generated *via* site-directed mutagenesis, expressed in CHO cells and analysed for enzymatic activity. Although nine out of the ten potential glycan sites are glycosylated, the presence of only three C-terminal sites was sufficient for the expression of active N-domain. The minimally glycosylated variant Ndom389 was found to crystallize reproducibly, making it a prime candidate for high throughput inhibitor-enzyme crystallization trials. Indeed this variant was crystallised in the presence of an N-domain selective phosphinic inhibitor, RXP407, and the structure solved to 2.0 Å resolution. In addition, the Ndom389-RXP407 structure confirmed the active-site residues important for the domain-selectivity of RXP407, opening the way for the development of additional N-domain selective inhibitors, which may provide therapeutic options for the treatment of pulmonary fibrosis.

A2

A TUMOUR SUPPRESSOR ROLE FOR THE TBX3 TRANSCRIPTION FACTOR IN FIBROBLASTS

Aretha Cooper¹, Sharon Prince¹

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TBX3, a member of the developmentally important family of T-box transcription factors, is overexpressed in several cancers and we have shown that it plays a direct role in melanoma and breast cancer progression. We have also shown that TBX3 is overexpressed in immortalised/transformed fibroblasts which suggest an as yet unknown role for TBX3 in fibrosarcomas. To investigate this, we stably knocked down TBX3 in an immortalised fibroblast cell line (CT-1) and characterised the resulting cells for several hallmarks of cancer. We show that silencing TBX3 in CT-1 cells caused increased proliferation, reduced growth factor dependence, anchorage independence and enhanced migration ability. These results suggest a novel role for TBX3 as a tumour suppressor and we further show that TBX3 may be mediating this activity, in part, by directly activating the COL1A2 gene which encodes type I collagen. Type I collagen is an extracellular matrix protein synthesized by fibroblasts and is downregulated in high grade fibrosarcomas. Since fibroblasts are of mesenchymal origin, and the cancers where TBX3 have previously been shown to drive oncogenesis are of epithelial origin, this raises the possibility that TBX3 may function as either oncoprotein or tumour suppressor depending on cellular context. The regulation of COL1A2 by TBX3 is interesting because, as with TBX3, increased levels of COL1A2 may be linked to tumour suppression and it is the first time that TBX3 has been shown to activate a physiological target. Results from this study have serious implications for targeting TBX3 as part of an anti-cancer regimen.

A3

MRS STUDY OF THE CEREBELLAR DEEP NUCLEI IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDER

Lindie du Plessis^{1,3}, Aaron T. Hess^{1,3}, Sandra W. Jacobson²⁻⁴, Joseph L. Jacobson²⁻⁴, Christopher D. Molteno⁴, Ernesta M. Meintjes^{1,3}

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²Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine,

³Department of Human Biology, University of Cape Town, South Africa,

⁴Department of Psychiatry, University of Cape Town, South Africa

Fetal alcohol spectrum disorder (FASD) is the most widely encountered preventable form of mental retardation worldwide and is caused by maternal drinking during pregnancy. The aim of this study was to examine whether neurochemical differences in the deep cerebellar nuclei are seen in children with varying degrees of FASD compared to healthy controls.

Alterations of metabolite concentrations in the deep cerebellar nuclei of 5 children with FAS, 15 children with partial FAS (pFAS), 8 children with heavy prenatal alcohol exposure lacking FAS dysmorphology (HE), and 10 controls were studied in Cape Town, South Africa, using an EPI volumetric navigated PRESS sequence on a 3T Siemens Allegra (Erlangen, Germany) MRI scanner.

Spectra were analysed using LCModel and statistical analysis performed using PASW, version 18.

Metabolites studied included absolute N-acetyl aspartate (NAA), total choline (Cho), total creatine (Cr) and myo-inositol (ml).

Results: NAA is inversely correlated with the severity of alcohol exposure ($r = 0.36$, $p = 0.04$), and the inverse correlation of alcohol exposure with total choline falls just short of conventional levels of statistical significance ($r = 0.31$, $p = 0.064$).

Choline is a precursor to phosphatidylcholine, which is major constituent of cell membranes, so the decreased choline could indicate reduced and insufficient membrane-mediated cell signalling in the alcohol-exposed children. Reduced NAA indicates reduced neuronal integrity in the deep cerebellar nuclei of the alcohol-exposed children, which is consistent with the dose-dependent cell loss in this region reported in ethanol-exposed rats.

A4

THE ROLE OF CYTOKINE PRODUCING B-EFFECTOR CELLS IN MURINE SUSCEPTIBILITY TO LEISHMANIA MAJOR

AD Einhorn^a, M Breton^a, R Hardayal^a, N Niewenhuizen^a, F Brombacher^{a,b}

^a Division of Immunology IIDMM, ^b ICGB Cape Town

B-cells are conventionally known as the antibody producing cells of the immune system. While this is still considered to be their primary function, several recent studies have shown that B-cells are capable of producing cytokines such as IFN γ and IL4. This finding has revolutionized the way B-cells are viewed, because it suggested they might play a role in controlling cellular immune responses.

Studies in 2000 and 2005 demonstrated *in vitro* that B-cells can assume different cytokine producing profiles, namely B-effector 1 (Be1) and B-effector 2 (Be2). They showed that differentiation of naïve B-cells into these B-effector subsets is driven by IFN γ and IL4 respectively, and that their cytokine production mirrors that of their T-helper1 and T-helper2 counterparts. To date, however, few *in vivo* studies have been published that demonstrate a role for these B-effector cells *in vivo*. Our lab is one of the first to show that different B-effector profiles can lead to markedly divergent phenotypes in disease.

By knocking out the *il4ra* on B-cells in balb/c mice, and thereby preventing the generation of Be2 cells, we show that previously susceptible balb/c mice can be made resistant to Leishmania. Furthermore, analysis of T-helper responses in these mice reveals that B-cell *il4ra* knockout mice develop a Th1 phenotype compared with wildtype Th2. We conclude that cytokine producing B-effector cells play an active role in determining the nature of the T-cell response. Where inappropriate T-helper responses to disease are responsible for pathology, this research suggests the B-cell as potential target for therapy.

A5

IDENTIFICATION OF GENES REQUIRED FOR VITAMIN B₁₂ TRANSPORT IN *MYCOBACTERIUM TUBERCULOSIS*

Krishnamoorthy Gopinath, Valerie Mizrahi, and Digby F. Warner

MRC/NHLS/UCT Molecular Mycobacteriology Research Unit, DST/NRF Centre of Excellence for Biomedical TB Research, Institute of Infectious Disease and Molecular Medicine

Vitamin B₁₂ is synthesized exclusively by prokaryotes, of which only a few possess the complete machinery for *de novo* biosynthesis. Notably, these include *Mycobacterium tuberculosis* (MTB) which, as causative agent of tuberculosis (TB), continues to devastate public health systems in endemic regions. New approaches are required for TB but are critically undermined by our poor understanding of the fundamental physiology of the organism during host infection. We demonstrated previously that B₁₂-dependent enzymes function in core metabolic pathways in MTB. Subsequently, we have established that, in addition to *de novo* synthesis, the bacillus is able to utilize exogenous B₁₂ and precursors. A key question therefore arises: does MTB synthesize B₁₂ during infection or does it satisfy its B₁₂ requirements from host sources? This knowledge is crucial to predictions of pathway utilization *in vivo*, but is complicated by the absence of an obvious B₁₂ transporter in the MTB genome. To address this, we applied whole-genome random mutagenesis in an initial screen to identify genes whose disruption restored the ability of a B₁₂-sensitive strain to grow on solid medium supplemented with B₁₂ at high concentrations. The ability of these “B₁₂-resistant” mutants to sustain propionate toxicity was then assayed in a secondary screen which reduced to 84 the number of isolates predicted to contain insertions in B₁₂-transport or assimilatory genes. Notably, *Rv1819c* mutants were heavily overrepresented, a potentially significant observation given the previous characterization of this mycobacterial *bacA* homolog as an ABC-type transporter of unknown function that is essential for chronic infection *in vivo*.

A6

INVESTIGATION OF AN ATYPICAL PROTOPORPHYRIC FAMILY IN SOUTH AFRICA

Haumann CE^a, Corrigan AV^b, Sonderup M^b, Berman PA^a, Baumgarten I^a, Wu HT^c, Pillay TS^{a,d}, Meissner PN^e

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^b Department of Medicine, University of Cape Town & Groote Schuur Hospital

^c Division of Anatomical Pathology, University of Cape Town & National Health Laboratory Service, Groote Schuur Hospital

^d Department of Chemical Pathology & College of Health Sciences, University of KwaZulu-Natal

^e Division of Medical Biochemistry, University of Cape Town

Erythropoietic protoporphyria (EPP) results from a deficiency of the final enzyme in the haem pathway, ferrochelatase, which facilitates the incorporation of iron into protoporphyrin to form haem. We investigated members of a South African family who presented with photosensitivity and raised red cell protoporphyrin levels. Although a diagnosis of EPP was considered, atypical features were noted.

We reviewed clinical and biochemical data from this family and established a fluorometric ferrochelatase enzyme assay to measure ferrochelatase activity in subjects and controls.

The assay was established in our laboratory and no significant difference was noted between the ferrochelatase activity of affected subjects and controls, suggesting that the defect is located elsewhere. Whilst this study was in progress, a collaborative effort between colleagues overseas and our laboratory led to the discovery of gain of function mutations in the X chromosome erythroid-specific 5-aminolevulinic synthase 2 (ALAS2) gene, catalysing the first committed and rate limiting step in haem biosynthesis. These were disease-causing in our family and in other (European) families with similar atypical features.

Constitutive activity of ALAS2 allows protoporphyrin to be generated faster than it can be consumed by ferrochelatase, thereby mimicking a primary defect of ferrochelatase itself.

We present data from mutational analysis of ALAS2 from our family. Further, we show data depicting protoporphyrin fluctuations during iron supplementation, a potential treatment strategy in this state of increased flux of the haem biosynthetic pathway.

We conclude that the family we have investigated show evidence of a new form of porphyria termed X-linked dominant protoporphyria.

A7 PAK3 IS AN AP-1 REGULATED GENE REQUIRED FOR CJUN/AP-1 INDUCED CELLULAR TRANSFORMATION

Nina A. V. Holderness¹, Howard Donninger², Michael J. Birrer³ and Virna D. Leaner¹.

¹Division of Medical Biochemistry, Faculty of Health Sciences, University of Cape Town, Institute of Infectious Disease and Molecular Medicine, South Africa.,

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Activating Protein 1 (AP-1) is a fundamental transcription factor that plays a role in cell proliferation, differentiation and apoptosis. Little is known about the role of the diverse downstream targets of the active AP-1 complex, a dimer comprising of the Jun, Fos or ATF bZIP-domain proteins. De-regulation of AP-1 has been linked to many cancers, with its over-expression leading to the transformation of normal cells. Previous studies utilizing a doxycycline-inducible cJun/AP-1 construct identified PAK3, a serine/threonine kinase signal transduction molecule, as a potential AP-1-target involved in the AP-1 characteristic transformation. PAK3 has been implicated in a variety of pathological disorders and over-expression of other PAK-family members has been linked to certain cancers. This project aims to investigate the role of PAK3 in cJun/AP-1 induced oncogenesis. Quantitative RT-PCR and Western Blot Analysis showed elevated PAK3 expression at both the mRNA and protein level in cJun-over-expressing cells. PAK3 expression levels were also elevated in transformed human and cancer cell lines compared to normal cells. Analysis of the PAK3 promoter using luciferase-reporter assays identified a putative AP-1 binding site, at position (+52/+60), to which cJun/AP-1 binds directly both *in vitro* and *in vivo* to transcriptionally activate PAK3 expression. siRNA inhibition of the PAK3 protein showed the regression of the morphological phenotype and migratory potential associated with cJun-transformed cells. However PAK3 inhibition showed no significant effect on the proliferative response of cJun-transformed rat cells. This study suggests a potential regulation of cJun/AP-1 on PAK3 as well as a role for PAK3 in AP-1 induced transformation; specifically that associated with cell morphology and migration.

A8 THE MOLECULAR CHARACTERISATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM HOSPITALS IN CAPE TOWN

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Comprehensive molecular epidemiological data are prerequisite for establishing control over methicillin-resistant *Staphylococcus aureus* (MRSA); however, there is currently a paucity of molecular epidemiological data available on MRSA from South Africa. A molecular characterisation of one hundred MRSA isolates collected between January 2007 and December 2008 from patients in five hospitals in Cape Town was carried out in this study.

The majority (92 %) of the MRSA segregated into six pulsed-field gel electrophoresis clusters. Each cluster contained isolates from at least two hospitals, suggesting intra- and inter-hospital transmission in Cape Town hospitals. A combination of SCC*mec* typing, multilocus sequence typing and *spa* typing was used to further characterise the MRSA. The six PFGE clusters and five sporadic isolates corresponded to four clones, while the remaining three MRSA were consistent with sporadic clones. Three of the predominant clones corresponded to frequently described pandemic clones: ST239-MRSA-III, ST36-MRSA-II and ST5-MRSA-I. ST239-MRSA-III (4%) and ST36-MRSA-II (12%) were minor clones, while ST5-MRSA-I was the second-most prevalent clone identified in this study, accounting for 37 % of the isolates.

The dominant clone identified in this study was the infrequently described multidrug-resistant ST612-MRSA-IVd, which was detected in all five hospitals. This clone appears to be endemic to South Africa, but has otherwise only been reported infrequently in Australia and the United Kingdom. The identification of the same uncommon rifampicin resistance genotype in ST612-MRSA-IV from Cape Town and isolates previously described in South Africa and Australia, suggests that ST612-MRSA-IV has undergone clonal expansion in local hospitals.

A9 IMMUNE MEMORY TO AN HIV VACCINE CANDIDATE IN A NON-HUMAN PRIMATE MODEL

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BACKGROUND: A good vaccine candidate should generate immunological memory. In this study, we investigated the ability of a candidate HIV vaccine to induce and maintain immunological memory in non-human primates.

METHODS: Rhesus macaques were given an HIV-1 subtype C DNA prime and MVA boost regimen and memory responses were evaluated at several time points. Previously described memory markers in non-human primates (CD28, CD95 and CCR7) were included in a multi-colour flow cytometry panel to delineate central memory (CD28⁺ CD95⁺), lymph-node homing central memory (CD28⁺ CD95⁺, CCR7⁺), and effector memory populations (CD28⁻ CD95⁻). The panel included markers to detect IFN- γ , TNF- α and IL-2 responses to HIV immunogens.

RESULTS: HIV-specific CD4⁺ cells were significantly skewed towards a central memory phenotype with >95% of total memory cytokine response expressing a central memory phenotype compared to only <5% expressing an effector memory phenotype at the time points evaluated. Whilst HIV-specific CD8⁺ cells showed a similar preference of central memory over effector memory phenotypes, compared to CD4s, they were relatively more distributed between the two subsets (median 65% vs. 35% of total memory, respectively). The inclusion of the lymph node homing marker CCR7 confirmed that the majority of memory responses were 'true' central memory. Our results demonstrate that this HIV-1 subtype C candidate vaccine is capable of inducing an early CD4 central memory pool and this pool of cells can be maintained following several boosting regimens.

A10 DEVELOPMENT OF ANTIOXIDANT THERAPY TO CONTROL NEUROINFLAMMATION INDUCED BY HIV PROTEINS GP120-41 AND TAT USING SK-N-SH NEURONAL CELL LINES

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Background. Human immunodeficiency virus (HIV) associated dementia (HAD) is impacting significantly on society in Africa. HIV gains entry into the brain immediately after infection and well before the symptoms of AIDS develop. Immediately after gaining entry into the brain, viral proteins such as gp120 and Tat are known to generate oxidative stress and increase the level of pro-inflammatory mediators in the brains of infected patients. It is therefore pertinent to consider the development of an effective antioxidant therapy to control the neurological complications associated with HIV.

Methods. The antioxidant capacities of compounds of natural origin (CNOs) extracted from Indian medicinal plants was determined using a standard Oxygen Radical Absorbance Capacity assay based on fluorescein (ORAC-FI assay). The CNOs were then tested for their effectiveness in reducing gp120-41 and Tat generated reactive oxygen species (ROS) in SK-N-SH cell lines. For this, the intracellular ROS levels were quantitated using a fluorescent probe 5 (6)-carboxy-2,7-dichlorofluorescein diacetate (CDFH-DA).

Results. On the basis of their antioxidant capacities measured using ORAC-FI assay, the CNOs were classified as weak, moderate and strong. Some CNOs reduced the ROS level induced by both Tat and gp120-41 in SK-N-SH cell lines, whereas others were found to be effective against only one of the pathogenic proteins.

Conclusion. These encouraging *in-vitro* preliminary findings suggest that use of CNOs may lead to the development of an effective antioxidant therapy for use in the prevention, progression, and possibly treatment of neurological complications associated with oxidative stress generated by HIV proteins.

A11
SEQUENCE VARIANTS WITHIN THE 3'-UTR OF THE COL5A1 GENE ALTERS MRNA STABILITY: IMPLICATIONS FOR MUSCULOSKELETAL SOFT TISSUE INJURIES

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COL5A1 encodes the $\alpha 1$ chain of type V collagen, a minor fibrillar collagen that regulates fibrillogenesis. A variant within the 3'-UTR of *COL5A1* is associated with chronic Achilles tendinopathy (AT) and other exercise-related phenotypes but its functional significance is unknown. The aim of this study was to identify functional differences between the *COL5A1* 3'-UTR from patients with AT and asymptomatic controls. To this end we have used a reporter assay in which the *COL5A1* 3'-UTR from AT patients and controls were cloned downstream of the firefly luciferase gene and the activity measured as an indication of mRNA stability. When the cloned *COL5A1* 3'-UTRs were sequenced, two major forms named C- and T-alleles were predominantly identified in the controls and the AT subjects respectively. The luciferase activity of the C-alleles was significantly lower than the T-alleles ($69.0 \pm 22.0\%$ (N=24) vs $90.6 \pm 13.7\%$ (N=30), $p < 0.001$) which suggests an overall increase in mRNA stability for the T-allele. Using deletion constructs we have found additional elements which regulate *COL5A1* mRNA stability. These results have important implications for our understanding of the molecular basis of musculoskeletal soft tissue injuries and other exercise-related phenotypes.

A12
DYNAMIC EXPLANATION OF THE OCCURRENCE OF LATENT TB INFECTION USING LATENCY AND DORMANCY TIME COURSE GENE EXPRESSION

G Magomedze and N Mulder

Computational Biology & IIDMM

The majority of individuals infected with *Mycobacterium tuberculosis* (Mtb) bacilli develop latent infection. Mtb becomes dormant and phenotypically drug resistant when it encounters multiple stresses within the host, and expresses a set of genes known as the dormancy regulon in vivo. These genes are expressed in vitro in response to nitric oxide (NO), hypoxia (oxygen deprivation), and nutrient starvation. The occurrence and reactivation of latent tuberculosis (TB) is not clearly understood. The ability of the pathogen to enter and exit from different states is associated with its ability to cause persistent infection. During infection it is not known whether the organism is in a persistent slow replicating state or a dormant non-replicating state, with the latter ultimately causing a latent infection with the potential to reactivate to active disease. We collected gene expression data for Mtb bacilli under different stress conditions that simulate latency or dormancy. Time course experiments were selected and differentially expressed gene profiles were determined at each time point. A mathematical model was then developed to show the dynamics of Mtb latency based on the profile of differentially expressed genes. Analysis of the time course data shows the dynamics of latency occurrence in vitro and the mathematical model reveals all possible scenarios of Mtb latency development with respect to the different conditions that may be experienced by the immune response in vivo. The mathematical model provides a suggestion of how Mtb latency occurs based on observed gene expression changes in in-vitro latency models.

A13
ROLE OF GENITAL TRACT INFLAMMATORY CYTOKINES IN SUSCEPTIBILITY TO HIV-1 INFECTION IN WOMEN WHO BECAME INFECTED DESPITE USING THE CAPRISA004 1% TENOFOVIR MICROBICIDE GEL

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Over the past 20 years, vaginal microbicide gels have been explored as a strategy to prevent sexual HIV-1 transmission to women. However, until recently, none of the candidate microbicides tested was found to protect against HIV-1 infection in clinical trials. Tenofovir (TFV) gel is the first microbicide that was shown in 2010 to significantly reduce male-to-female sexual transmission of HIV-1 by 39%. As previous studies have suggested that genital inflammation may increase the risk of HIV-1 acquisition, we investigated this as a possible mechanism for breakthrough infections in women who became HIV-1-infected despite using TFV gel. HIV-uninfected South African women (n=889) were enrolled in the CAPRISA004 trial and randomized to use either placebo or TFV gel. Luminex was used to measure the concentrations of 12 cytokines in pre-infection CVL samples from 62 women who later became HIV-1-infected and 106 women who remained uninfected. It was found that elevated cervicovaginal concentrations of IL-1 α , IL-1 β , IL-6, IL-8, IP-10, MCP-1, MIP-1 α , MIP-1 β , GM-CSF and IL-10 were associated with increased risk of HIV-1 infection, irrespective of gel use. The level of genital inflammation in individual women was similar at 2 separate time-points assessed in this study (8-104 weeks apart). These findings suggest that elevated levels of genital inflammation, which are sustained over time, may facilitate breakthrough HIV-1 infections, even in women using TFV gel. Increasing the efficacy of the TFV microbicide gel will either require better management of factors associated with inflammation or augmentation of the next generation microbicides with agents to control inflammation.

A14
REGULATION OF ALLERGIC AIRWAY DISEASE BY IL-4/IL-13 ACTIVATED MACROPHAGES AND DENDRITIC CELLS

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While IL-4R α and its ligands IL-4 and IL-13 play an important role in mouse models of allergic airway disease, the effects of IL-4 and IL-13 signaling through specific cell types are unclear. The generation of mice with cell specific impairment of IL-4R α on macrophages/neutrophils (LysM^{cre}IL-4R α ^{-/lox}) and dendritic cells/alveolar macrophages (CD11c^{cre}IL-4R α ^{-/lox}) has enabled us to investigate the effect of IL-4/IL-13 signaling on macrophage and dendritic cells in allergic airway disease.

IL-4R α ^{-/lox}, IL-4R α ^{-/-}, CD11c^{cre}IL-4R α ^{-/lox} and LysM^{Cre}IL-4R α ^{-/lox} mice were sensitized and challenged with ovalbumin. OVA-challenged IL-4R α ^{-/lox} mice had increased airway resistance and elastance, lung inflammation and mucus hypersecretion compared to PBS controls. In IL-4R α ^{-/-} mice, airway resistance and elastance, mucus hypersecretion, Th2 responses and eosinophil infiltration was decreased. However, in both LysM^{cre}IL-4R α ^{-/lox} mice and CD11c^{cre}IL-4R α ^{-/lox} mice, airway resistance and elastance were increased. This indicates that IL-4/IL-13 activated macrophages may play a role in downregulating allergic airway disease. Furthermore, lung CD4⁺ T cells from CD11c^{cre}IL-4R α ^{-/lox} mice but not LysM^{cre}IL-4R α ^{-/lox} mice had significantly increased production of the Th2 effector cytokines IL-13 and IL-5, as well as significantly increased lung eosinophils and neutrophils, suggesting an additional role for IL-4/IL-13 activated dendritic cells in downregulating Th2 responses and granulocyte infiltration into the lungs. In summary, we find a role for IL-4/IL-13 signaling through both macrophages and dendritic cells in regulating allergic airway disease in mice.

A15

MATERNAL SEPARATION AFFECTS DOPAMINE TRANSPORTER FUNCTION IN THE SPONTANEOUSLY HYPERTENSIVE RAT: AN IN VIVO ELECTROCHEMICAL STUDY

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The spontaneously hypertensive rat (SHR) is a well-characterised model of attention-deficit/hyperactivity disorder (ADHD), a developmental disorder characterised by inattention, impulsivity and hyperactivity. In comparison to its control strain, the Wistar Kyoto rat (WKY), the SHR exhibits dopamine dysregulation, one of the hypothesised causes of ADHD. Maternal separation is a model for chronic mild developmental stress that similarly affects the dopaminergic system.

The current study employed the use of behavioural tests for anxiety and locomotion with subsequent high-speed *in vivo* chronoamperometry in SHR and WKY rats exposed to either maternal separation or standard rearing. Chronoamperometry allows for the measurement of changes in striatal dopamine uptake in response to pressure ejection of a known amount of dopamine to provide a measure of dopamine transporter (DAT) function and was determined before and after the peripheral injection of cocaine, a DAT inhibitor.

Behavioural testing revealed increased locomotion in SHR and increased anxiety in maternally separated rats, both results consistent with models for ADHD and developmental stress respectively. Chronoamperometric data revealed significant effects of separation stress in SHR with a decreased rate constant, k_{-1} , and consequent increase in the total uptake time, T100, of ejected dopamine. Addition of cocaine increased the T80 clearance time for dopamine uptake in SHR compared to WKY. Taken together, these results suggest that chronic mild developmental stress impaired striatal DAT function in an animal model of ADHD, the SHR. Furthermore, challenge with cocaine illustrated reduced dopamine uptake efficiency from the striatal extracellular fluid in SHR compared to control rats.

A16

UNDERSTANDING HOST IMMUNE FUNCTION DURING TUBERCULOSIS MENINGITIS INFECTION: A NEURAL ROUTE FOR MYCOBACTERIUM TUBERCULOSIS INFECTION

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Tuberculosis (TB) is a prevalent infection within South Africa. Pulmonary infection is the primary infection but approximately 10% of disseminated forms of TB have Central Nervous System (CNS) involvement. CNS TB is difficult to diagnose and treat. The clinical features are non-specific and bacteriology is insensitive. A delay in the institution of treatment will in all certainty increase mortality and neurological morbidity.

Finding ways to diagnose CNS TB early is an area which needs attention. In order to achieve this it was imperative to gain a better immunological understanding through characterising CNS TB infection using an *in vitro*, *ex vivo* and *in vivo* CNS TB models.

The *in vitro* model involved the purchasing of neuronal cell lines HT22 and Neuro2A and microglia cell line BV2. The *ex vivo* model involved the production of primary neuronal and microglia cultures from the hippocampus and cortex of 17 day old embryonic C₅₇Bl/6 mice, respectively. The *in vivo* model focused on intra-cranial (ic) infection of mice with laboratory strain of *Mycobacterium tuberculosis* (MTB), H37Rv.

A novel finding was discovered during the initial establishment of the *in vitro* and *ex vivo* CNS TB model, MTB associated and was internalised by neurons. This internalisation was comparable to what is seen in known phagocytic cells, namely primary microglia and BV2. Visualisation of neurons actually showing uptake of MTB introduces a new dimension in studying the characteristics of CNS TB infection. Such neuronal responses may account for the neurological morbidity seen in patients suffering from CNS TB.

A17

ANTI-TUBERCULAR STUDIES OF PHENOTHIAZINE DERIVATIVES IN *MYCOBACTERIUM TUBERCULOSIS* (MTB)

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Phenothiazines are a class of psychotropic drugs known to show synergistic interactions with anti-TB drugs. At mycobacteriocidal doses however, the drugs produce undesirable side effects due to their neuroleptic nature. Thus, novel modifications would possibly render them more efficacious and with no or tolerable side effects. Our aim in the study was to screen modified phenothiazines, evaluating for antituberculosis activity.

In this study, the modified phenothiazines being tested all have the same core structure, but differ in one particular side chain. To test the direct killing effect of modified phenothiazines on MTB, we used microplate-based methods. *Mycobacterium tuberculosis* (MTB) with green fluorescence protein (H37Rv-GFP) was treated with the compounds being tested. The survival of the MTB was measured based on the relative fluorescence units was measured at various time points. The screening results showed the dosage dependant bactericidal effects of the compounds. The synergistic effect of the compounds with isoniazid (INH) was also investigated. The results demonstrated that the synergistic interaction between the compounds and INH could increase the antituberculosis activity. Furthermore, the cytotoxicity of the compounds was evaluated in macrophages in vitro. The treated macrophages were still viable after 5 days of incubation with the compound which was comparable with the treatment of INH.

In summary, we demonstrate the effect of the various side chains on the ability of the compounds to kill MTB. The synergistic effects with INH translate to lower dosing requirements of the compound and the potential to combat multidrug resistant TB.

A18

DEPIGMENTATION IN MELANOMAS INCREASES THE EFFICACY OF HYPERICIN-MEDIATED PHOTODYNAMIC THERAPY

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Melanoma is the main cause of death in skin cancers. Despite early detection, resection and post-operative therapy, melanoma remains resistant to treatment and investigations into other forms of adjuvant therapy such as photodynamic therapy (PDT) are therefore prudent. This study proposes that depigmentation in melanotic melanoma cells increases their susceptibility to PDT.

Two human melanoma cell lines were used in this study. Kojic acid (KA), a tyrosinase-specific inhibitor, was shown to inhibit melanin synthesis after 3-day exposure. Cells were then treated with hypericin (HYP) – PDT and cell viability and intracellular reactive oxygen species (ROS), measured. Apoptotic cell death was assessed by measuring caspase 3/7 activity, 4h after activation.

PDT on KA treated cells resulted in a 3.82 fold increase of ROS production which correlated to 11% increase in cell death compared to untreated controls. Moreover, cells allowed to regain their pigment failed to return to normal even after 72h. Using a DPPH* assay, the results confirmed the scavenging properties of melanin (IC₅₀18.30 µg/ml) proving that this pigment may be one of the reasons for melanoma chemoresistance. No significant difference was observed in the caspase 3/7 activity in KA treated and non-KA treated melanoma after 3µM HYP activation in comparison to UV-only treated control pigmented melanoma cells. This suggests that the HYP-PDT treatment induced a caspase-independent cell death mechanism.

Overall this study shows that pigment plays an important role in the efficacy of adjunctive PDT treatment and its removal enhances cell death susceptibility in melanomas.

A19

EFFECTS OF MATERNAL SEPARATION ON GLUTAMATE, GABA, AND POTASSIUM-EVOKED RELEASE OF HIPPOCAMPAL [³H]NOREPINEPHRINE IN A RAT MODEL OF ATTENTION DEFICIT-HYPERACTIVITY DISORDER

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The risk of developing of a psychiatric disorder, such as attention-deficit hyperactivity disorder (ADHD) or depression, involves complex interplay between genetic predisposition and environment, and is increased by continued exposure to stressful events, particularly when experienced early in life. The present study aimed to investigate effects of early life trauma on glutamate, GABA and potassium-evoked release of norepinephrine (NE, a neurotransmitter involved in the stress-response) in the hippocampus of prepubescent spontaneously-hypertensive rats (SHR, an animal model for ADHD) and Wistar-Kyoto rats (WKY, the control strain for SHR). Hippocampal slices were incubated with radio-actively labelled NE ([³H]NE), allowing uptake of [³H]NE into vesicles containing endogenous NE stores. Using an *in vitro* superfusion technique the slices were exposed to buffers containing glutamate (250μM), GABA (100μM) or potassium (25mM), thereby stimulating [³H]NE release. Maternal separation increased potassium-stimulated release of NE in SHR females, while a similar trend was seen in SHR males, suggesting elevated vesicular NE stores, or diminished negative autoreceptor feedback, in response to early life trauma. Antagonising GABA_A receptors using bicuculline (100μM) increased glutamate-stimulated NE release in both SHR female groups, but only in maternally separated SHR males, suggesting GABA_A receptor regulation of NE release is potentiated by maternal separation in SHR males. The presence of bicuculline removed the sex effects present in glutamate and GABA stimulations in WKY, suggesting that these sex effects were largely due to differential GABA_A receptor functioning. Our results support the hypothesis that neurochemical effects of early life trauma are dependent on genetic predisposition.

A20

STATIN-INDUCED AUTOPHAGY AND PHAGOLYSOSOMAL MATURATION LEADS TO REDUCED GROWTH OF INTRACELLULAR PATHOGENS

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Abstract

Cholesterol has been shown to play an important role in the pathogenesis and persistence of intracellular pathogens. Here, we modulate host cholesterol biosynthesis pathway using statins, which are pharmacological inhibitors of HMG-CoA reductase enzyme. We investigated the role of statins in inducing host protective responses against intracellular pathogens. We report reduced growth of *Listeria monocytogenes* (LM) and *Mycobacterium tuberculosis* (Mtb) in murine macrophages. We show prominent immunomodulatory activity induced by statins, mainly increased phagolysosomal maturation and autophagy resulting in decreased bacterial growth in macrophages. Subsequently, statin-treated mice showed decrease in bacterial loads, accompanied by reduced histopathology during listeriosis and tuberculosis. Furthermore, we found reduced growth of Mtb in peripheral blood mononuclear cells (PBMC) isolated from patients with familial hypercholesterolemia on statin therapy when compared to healthy subjects. Together, our results show that statins induce host protective responses in macrophages and increases resistance to intracellular pathogens.

POSTER PRESENTATION ABSTRACTS

MORNING SESSION:

P1-T

PUTATIVE POLYMORPHIC BINDING SITE FOR Hsa-miR-608 (*Acil* RFLP C>A) IN THE 3'-UTR OF *COL5A1* IS FUNCTIONAL.

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COL5A1 encodes the rate-limiting pro- α chain of type V collagen, a quantitatively minor fibrillar collagen that regulates fibrillogenesis and fibril diameter. A variant of the *COL5A1* 3'-UTR has been associated with Achilles tendinopathy (AT) and other soft tissue injuries but the functional significance is yet to be identified. To elucidate the mechanisms involved, we used a reporter assay in which the *COL5A1* 3'-UTR from AT patients and controls were cloned downstream of the firefly luciferase gene and the resulting activity was used as an indication of mRNA stability. When the 3'-UTRs were sequenced, two major allelic forms were identified. The more stable TEN allelic form (A at the *Acil* RFLP) was over-represented in AT patients while the CON allelic form was over-represented in controls. These two constructs were co-transfected with 1; 10; 50 or 100 pmol of Hsa-miR-608 miRNA mimic or scrambled siRNA. The results indicate that the miRNA binding site is functional in both CON (1 pmol n=12; p<0.001) and TEN (1 pmol n=9; p<0.001) forms however, the TEN allelic form shows greater repression at all tested concentrations with $36.4 \pm 15.6\%$ reduction in luciferase activity as compared to the CON allelic form with $13.7 \pm 7.6\%$ (n=9, p<0.003). This study indicates that this polymorphic miRNA binding site as well as elements within the 3'UTR may be contributing to the tendinopathic phenotype.

P2-T

THE ROLE OF MUSCARINIC ACETYLCHOLINE RECEPTOR M3 IN IMMUNE RESPONSE TO PATHOGENS USING TRANSGENIC MURINE MODELS.

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Muscarinic receptors are G protein coupled receptors (GPCRs) that bind to acetylcholine, the principal neurotransmitter, to initiate an intracellular signalling cascade. There are 5 subtypes of muscarinic receptors (i.e M1-M5), first identified in the central nervous system (CNS) where they mediate nerve impulse transmission across neuronal and neuromuscular synapses. The M3 muscarinic receptors in particular are expressed on smooth muscle cells, salivary glands as well as on immune cells such as T cells, macrophages and dendritic cells. However, the function of M3 receptors on immune cells is largely unknown.

In our study, we aim to elucidate the role of M3 receptors on immune cells, using a genetically modified mouse strain lacking the M3 receptor (*M3R*^{-/-}), after infection with the bacterial pathogen *Salmonella Typhimurium*. Preliminary findings show delayed bacterial clearance in the *M3R*^{-/-} mice with a 100 fold higher bacterial burden at day 27 post-infection. Higher IL-10 production from restimulated *M3R*^{-/-} splenocytes was observed during early stages of infection together with increased IFN- γ production (10 fold) during the latter stages of infection which may explain the observed delay in bacterial clearance in *M3R*^{-/-} mice compared to wildtype mice.

Future studies will examine in detail the specific immune cell types affected by *M3R* deletion. Results from this study will provide a novel insight into the role of M3 receptors in the regulation of immunity. M3 receptor specific drugs are already available on the market and therefore understanding its immunological role may allow for adaptation of these drugs to treat infectious diseases.

P3-T

THE PHYSIOLOGICAL IMPLICATIONS OF DRUG RESISTANCE MUTATIONS IN MYCOBACTERIA

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Resistance to the frontline anti-tuberculosis (TB) drug, rifampicin (RIF), maps primarily to mutations in *rpoB*. Numerous studies have assessed the impact of RIF^R-associated mutations on selected fitness indicators in *Mycobacterium tuberculosis* (Mtb). In contrast, the specific consequences of RIF^R for mycobacterial physiology remain poorly understood. In *Escherichia coli*, adaptation to growth in minimal media is a result of clinically-relevant *rpoB* mutations, while in *Bacillus subtilis* these mutations give the organism the ability to utilise a variety of unusual metabolites. These observations suggest the potential for *rpoB* mutations to impact multiple functions independent of their RIF^R-associated properties. Moreover, they support the idea that *rpoB* mutations might confer a benefit in certain environments and so might be maintained in the human population in the absence of antibiotic treatment. We have generated a panel of mutants containing RIF^R-associated *rpoB* genotypes in the non-pathogenic mycobacterium, *M. smegmatis* (Msm). Mutants were constructed without RIF selection to enable assessment of the impact of single *rpoB* mutations on fitness in the absence of possible second-site mutations. Competition experiments revealed a slight fitness defect for mutants containing an S531L mutation. However, even when grown in axenic culture, mutants had decreased “culturability” on RIF-containing media. These data suggest that using RIF to distinguish mutant and wild-type strains may bias competition assays, and that *rpoB* mutants might have higher fitness than previously thought. Present work is directed at generating similar mutants in Mtb, and applying a collection of assays to probe the physiology of these strains.

P4-T

QUANTIFYING RIGHT VENTRICULAR MOTION AND STRAIN USING 3D CINE DENSE MRI

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Imaging studies of myocardial mechanics are typically limited to the left ventricle (LV) as the right ventricle (RV) is notoriously difficult to image because of its thin wall, asymmetric geometry and unruly motion. However, the RV plays a critical role in the cardiovascular system and it is thus important to have a good understanding of its kinematics and function. Regional deformation, or strain, is a useful clinical indicator of myocardial function and viability. Previous studies have demonstrated myocardial strain to be useful for mapping infarcts and regions of mechanical dyssynchrony.

The purpose of this study is to introduce techniques to quantify detailed RV motion and strain in healthy volunteers. This is achieved using three dimensional cine displacement encoding with stimulated echoes (DENSE) magnetic resonance imaging (MRI). DENSE is an MRI technique developed to quantify myocardial motion and strain at a high spatial and temporal resolution. Myocardial tissue displacement is directly encoded into the MRI image phase, which allows for the direct extraction of motion data at a pixel resolution. Tissue tracking techniques and surface strain methods based on 3D DENSE data are introduced, and used to resolve midwall myocardial strain as a function of time for regional cardiac segments in five normal volunteers.

Results compare favorably with previous myocardial tagging and DENSE studies. Peak strain values are shown to vary between -0.1 and -0.25. This work presents 3D motion tracking and strain quantification of the RV at a previously unattainable spatial resolution.

P5-T

GENOME-WIDE HAPLOTYPE AND POPULATION STRUCTURE OF INDIGENOUS SOUTHERN AFRICAN POPULATIONS

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Recent technological advances in high-throughput genotyping have facilitated an improved understanding of genomic structure, particularly evident in results from the International HapMap Consortium and Human Genome Diversity Project. These studies, and others, confirm that Africans have higher levels of haplotypic and genotypic diversity than non-Africans, consistent with an African origin for modern humans. However, southern Africans, including the Khoisan, who are thought to represent an ancestral human lineage, are under represented in these studies, with seven or fewer samples. Here we report the genotypes of approximately 906,000 SNPs, obtained using the Affymetrix Genome-Wide Human SNP Array 6.0 platform, from representatives of five indigenous southern African populations, including 25 Khoisan individuals and four southern African Bantu-speaking populations with comparable sample sizes. Our analyses of haplotype structure, linkage disequilibrium, recombination, copy number variation and genome-wide population structure highlight the unique position of Khoisan relative to both African and non-African populations. Furthermore, we identify an as yet unreported divergence between southwestern and southeastern Bantu-speaking populations, consistent with hypotheses of the expansion of Bantu-speaking people. Our results support an early divergence of proto-Khoisan from other African populations, yet also provide evidence of a proportion of Khoisan ancestry among southern African Bantu-speaking populations. The data and results presented here provide an important resource for subsequent genome-wide research on southern African and other African populations.

P6-T

THE MOLECULAR EPIDEMIOLOGY OF *STREPTOCOCCUS PPYOGENES* PHARYNGITIS AMONG CHILDREN IN THE VANGUARD COMMUNITY (BONTEHEUWEL/LANGA), CAPE TOWN, SOUTH AFRICA

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Background

Acute rheumatic fever (ARF) is one of the non-infectious complications of pharyngitis due to *Streptococcus pyogenes* (Group A Streptococcus or GAS). Acute rheumatic fever can lead to rheumatic heart disease (RHD), which is a huge health care burden in many developing countries. Prevention of ARF, and thus RHD can be accomplished by timely treatment of streptococcal pharyngitis, or by provision of prophylaxis to patients who already had ARF. However, implementing these interventions is challenging. The alternative approach would be to develop a vaccine against GAS; however the fact that over 100 serotypes of the organism exist complicates this approach. Current vaccine candidates aim to cover the commonest serotypes causing pharyngitis.

Emm typing is a molecular typing method that has been widely used to study the prevalence of *emm* types of GAS and aid to predict the potential coverage of a 26-valent GAS vaccine which is currently under development. This study was designed to determine the distribution of *emm*-types from children with pharyngitis and thus to estimate the coverage of the proposed 26-valent GAS vaccine in Cape Town, South Africa.

Methods

Throat swabs were taken from school-age learners with pharyngitis attending the Vanguard Community Clinic. Isolates of GAS from these swabs were stored, and *emm*-typing was performed using PCR amplification of the M-protein gene followed by DNA purification and direct sequencing. The sequence data is then compared to an online database maintained by the Centers for Disease Control and Prevention, CDC.

Results

A total of 146 isolates have been typed. Amongst the 128 isolates, a total of 31 different *emm* types were represented, with *emm* 48 (12%) and *emm* 12 (9%) being the dominant *emm* types.

Discussion

This study has shown diversity in the *emm* types from our GAS isolates. The 26-valent GAS vaccine is currently estimated to cover 43% of the *emm* types, and this vaccine will provide less coverage in South Africa as compared to high income countries. It is our opinion that more studies on the distribution of *emm* types are crucial to inform the design of a vaccine that will prove to be effective in prevention of rheumatic heart disease in the developing world.

P7-T

TRANSCRIPTIONAL PROFILING OF NORMAL AND TRANSFORMED OESOPHAGEAL EPITHELIAL CELLS IN RESPONSE TO BENZO[A]PYRENE

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Tobacco smoking is a major risk factor for developing oesophageal squamous cell carcinoma (OSCC). Benzo[a]pyrene (BaP), a tobacco smoke procarcinogen, is metabolically activated into the carcinogenic benzo[a]pyrene diol-epoxide (BPDE) by the CYP1 family of cytochrome P450 enzymes. BaP is also a ligand for the aryl hydrocarbon receptor (AhR) which activates CYP1 gene transcription. Transcriptional profiling of the effects of BaP has been studied in several cell lines, but not in oesophageal cells.

Transcriptional profiling of the effects of 48h 10 μ M BaP treatment on global gene expression in a normal oesophageal epithelial cell line (EPC-2) and an OSCC cell line (WHCO1) was carried out using an Illumina microarray and analysis with Ingenuity Pathways Analysis (IPA) software. Microarray fold change was validated by quantitative real-time RT-PCR on selected genes.

In WHCO1 cells, xenobiotic metabolism and oxidative stress signaling were differentially regulated, whereas in EPC-2 cells, cell cycle regulation, the DNA damage response and DNA repair pathways were differentially regulated. AhR signaling and the inflammatory response were more significant in WHCO1 than EPC-2 cells. Meta-analysis of published BaP microarray data showed common gene expression in response to BaP in multiple cell types.

These results suggest that WHCO1 cells are more efficient at BaP metabolic activation and subsequent formation of DNA-damaging BPDE and reactive oxygen species (ROS), while EPC-2 cells protect against transformation by regulating the cell cycle and repairing BPDE-damaged DNA. By characterizing the complex transcriptional responses to BaP we gain understanding of the molecular mechanisms behind BaP-associated cancer initiation and progression.

P8-T

THE ROLE OF TNF AND MYD88 IN EXPERIMENTAL TUBERCULOSIS OF THE CENTRAL NERVOUS SYSTEM.

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Tuberculosis (TB) remains the cause of mortality worldwide and can involve any organ system of the body. Infection in the central nervous system (CNS) is the most severe form of tuberculosis and a serious public health problem in developing countries. In the CNS tubercle bacilli can cause tuberculous meningitis, abscess, tuberculoma in brain and spinal cord. In pulmonary TB, Tumor Necrosis Factor (TNF) plays a critical role in immune responses to *M. tuberculosis* in a Myeloid differentiation factor 88 (MyD88)-dependent manner. TNF Knock-out (KO) and MyD88KO mice showed comparable susceptibility to pulmonary TB. We therefore investigated the roles of TNF and MyD88 in protective immunity against CNS TB.

C57BL/6 (Wild-type), TNFKO and MyD88KO mice were infected with *M. tuberculosis* H37Rv via intracerebral inoculation.

TNFKO mice succumbed to *M. tuberculosis* infection by day 21. However, MyD88KO mice survived the infection three fold longer. TNFKO and MyD88KO strains had 100 fold increases in bacilli burden in the brain when compared to WT at day 21. However MyD88 had comparable Th1 cytokines comparable to WT while TNFKO mice showed increased IL-1, IL-6 and IFN- γ .

These results suggest a crucial role of TNF and MyD88 in CNS TB and a MyD88-independent mechanism in MyD88KO mice which may have conferred prolonged survival of MyD88KO mice.

P9-T

SUBSTRATE BINDING TO MSHB, A ZINC PEPTIDASE IN THE MYCOTHIOIOL BIOSYNTHETIC PATHWAY OF *Mycobacterium tuberculosis*.

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New drugs are needed to combat the ever increasing incidence of drug resistant tuberculosis. Eukaryotes and gram negative bacteria make use of a low molecular weight thiol containing peptide, glutathione (GSH) in their defense against toxins and oxidative stress (Forman, Zhang & Rinna, 2009). In mycobacteria a carbohydrate based thiol, mycothiol (MSH) is used instead. This results in the detoxification of the two frontline antitubercular antibiotics, rifampicin and isoniazid.

MshB catalyses the third step in the mycothiol biosynthetic pathway which involves the deacetylation of GlcNAc-Ins, via cleavage of a peptide bond, to GlcN-Ins (Newton, Av-gay & Fahey 2000). MshB shares homology with another Zinc peptidase which is found in the mycothiol recycling pathway, MCA. Both enzymes cleave the same peptide bond, they have overlapping enzyme activity and their products feed into the same step of the mycothiol synthetic pathway (Steffek et al., 2003, Newton et al., 2006).

MshB was crystallized in the presence of the inhibitor VU5 (Gammon et al., 2010). X-ray diffraction data enabled the structure of the co-crystal to be determined. Interpretation of the map identified the ligands bound in the active site as glycerol and acetate rather than VU5. The glycerol was hydrogen bonded to residues Arg 68, Asp 95 and His 144. The acetate was co-ordinated to the catalytic zinc. All the glycerol atoms can be mapped onto the corresponding atoms of the glucopyranoside ring of MSH strongly suggesting that the hydrogen bonds are important in positioning the natural substrate. This has been previously suggested by Fan et al., 2009.

P10-T

MATRIX METALLOPROTEINASE GENES ON CHROMOSOME 11q22 AND SIT-AND-REACH RANGE OF MOTION IN HUMANS

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Introduction A heritability study has demonstrated that human range of motion (ROM) has a substantial genetic component. Furthermore, the *COL5A1* Bst¹UI RFLP has been identified as the first gene variant associated with human ROM. Other musculoskeletal soft tissue coding and/or signalling genes may also associate with ROM measurements. A haplotype containing the *MMP1*, *MMP10*, *MMP3* and *MMP12* genes have been associated with risk of anterior cruciate ligament rupture. The aim of this study was to investigate the association between these four MMP gene variants and sit-and-reach (SR) ROM in physically active individuals.

Methods 292 Caucasian individuals were included in this study. All participants were genotyped, using a Taqman assay, for the *MMP1* 1G/2G rs1799750, *MMP10* C/T rs486055, *MMP3* G/A rs679620, and *MMP12* A/G rs 2276109 variants. A sit-and-reach (SR) ROM test was performed on all participants. Significance was accepted $P < 0.05$.

Results There were no significant genotype associations between any of the gene (*MMP1*, $P=0.377$; *MMP3*, $P=0.701$; *MMP10*, $P=0.182$; *MMP12*, $P=0.213$) variants and SR ROM. It was however interesting that there was a tendency ($P=0.104$) for individuals with the rare *MMP12* GG genotype (329 ± 60 mm, $N=7$) to have increased SR ROM measurements when compared to those with an A allele (262 ± 109 mm, $N=285$).

Conclusion There was no evidence that the *MMP1*, *MMP3*, *MMP10* and *MMP12* genes associate with ROM. However, the rare *MMP12* GG genotype may associate with increased ROM. Due to the rarity of this genotype, a larger sample size is required to test this association.

P11-T

A NOVEL APPLICATION OF ISOTHERMAL TITRATION CALORIMETRY FOR ANALYSING THE KINETICS OF ANGIOTENSIN CONVERTING ENZYME

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Angiotensin-converting enzyme (ACE) is a zinc-metalloprotease with dipeptidyl carboxypeptidase activity that primarily cleaves the vasoactive peptides angiotensin-I (Ang 1) and bradykinin, as well as a number of other physiologically relevant peptides *in vitro*. However, there is no simple, efficient method for obtaining Michaelis-Menten rate constants for these physiological substrates, limiting effective *in vitro* study of these reactions.

Isothermal Titration Calorimetry (ITC) is a powerful thermodynamic technique widely used in the study of protein–ligand and protein–protein interactions. Recently however, ITC has been increasingly applied to the study of enzyme reaction rates. Two distinct methods are used extensively and are based on the proportionality between the rate of a reaction and the heat absorbed (endothermic) or released (exothermic).

These two methods were evaluated using ACE and a synthetic substrate (HHL) and a physiological substrate AngI. Both methods were shown to be limited and severely affected by both substrate inhibition (HHL) and product inhibition (AngI). This necessitated the development of a unique approach where the single injection (SI) method was adapted to produce a Progress Curve, which was analysed using a recently described variation of the Michaelis-Menten equation. The assay described is extremely robust and circumvents the limitations of the two more prevalent methods when applied to ACE kinetics. Whilst developed for the study of ACE, it nevertheless represents a broadly applicable approach to enzyme kinetics that has a number of advantages over current ITC-based approaches.

P12-T

HIV-1 SUPERINFECTION RESULTS IN POTENT NEUTRALIZING ANTIBODY RESPONSES TO THE SUPERINFECTING VARIANT, BUT DOES NOT NECESSARILY PROMOTE NEUTRALIZATION BREADTH

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A better understanding of how neutralizing antibody responses develop during natural infection and the factors that augment them would be invaluable in the design of improved immunogens and immunization protocols. HIV dual infection (infection by >1 distinct HIV strains) provides a unique opportunity to evaluate whether polyvalent immunogens and prime-boost strategies are likely to enhance the breadth and potency of vaccine-induced responses against HIV.

Ten dual-infected participants were identified from the CAPRISA002 cohort, 3 of whom were superinfected within the first year of infection. Neutralization breadth at 3 years post-infection was compared to 16 singly-infected participants using a panel of 44 heterologous viruses, including subtypes A, B, and C. Autologous plasma neutralizing titers in 3 superinfected, and 3 co-infected individuals were assessed longitudinally using representative clones from multiple timepoints in the pseudovirus-based TZM-bl assay.

There was no association between co-infection, superinfection, or diversity in early infection and the development of greater neutralization breadth. Furthermore, titers to the primary-infecting variants were not boosted following superinfection. However, 2 of the 3 superinfected participants developed elevated titers against the superinfecting virus, with ID₅₀ values exceeding 1:20,000 and 1:40,000 respectively. None of the 3 co-infected participants generated similarly elevated titers, suggesting that sequential infection was a driving factor.

The lack of an association between dual-infection and breadth highlights the fact that polyvalent immunogens may not necessarily improve the breadth of elicited humoral responses. However, the high titers generated against the superinfecting variants suggest that a prime-boost approach has the potential to increase the potency of neutralizing responses against the boosting immunogen. As existing specificities were not boosted, the mechanism is likely distinct from the anamnestic response.

P13-T

***BORDETELLA TREMATUM*: AN EMERGING HUMAN PATHOGEN?**

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We recently isolated a non-fastidious, non-lactose fermenting, gram-negative bacillus on routine culture media. This organism was isolated from a biopsy of the middle ear of a 13 year old, HIV-negative female with chronic suppurative otitis media. The Vitek2 microbiology system identified this organism as *Bordetella trematum* with 99% confidence. This result was then confirmed using 16S rRNA sequence analysis.

B. trematum was first proposed as a novel species in 1996. This organism has been isolated from ear infections and wounds in humans, but the pathogenic significance remains unknown. To date only 10 cases of human infection/colonisation have been described in the literature and according to our knowledge, this is the first reported case in South Africa. *B. trematum* may be an emerging pathogen and further studies are required to determine its exact role in human disease.

The Vitek2 is an automated system used by clinical diagnostic laboratories for the identification of a variety of bacteria based on their biochemical reactions. Recently *B. trematum* has been added to its database. However, misidentification is a major problem with low reactive organisms such as *B. trematum*. Awareness needs to be created within the routine diagnostic microbiology laboratories in order to improve chances of correct identification so that this organism's true significance as a potential pathogen may be more accurately established.

P14-T

RISK FACTORS FOR OBESITY DEVELOPMENT IN ZULU WOMEN: PERSONAL AND PARENTAL WEIGHT HISTORY, WEIGHT MANAGEMENT PRACTICES AND TASTE SENSITIVITY (A CASE-CONTROL STUDY).

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Background: Obesity is a significant health problem in South Africa. Intervention should be based on appropriate formative assessment of target populations. For these purposes associations between weight status, personal and parental weight history, weight management practices and 6-n-propylthiouracil (PROP) taste sensitivity in Zulu female adults were investigated in a case control study.

Methods: A convenience sample of 94 overweight/obese Zulu women (BMI>27kg/m²) who entered a weight-loss programme and 86 normal weight controls (BMI<25kg/m²) completed the PROP taste test and a self-administered questionnaire developed for this study. Group comparisons were conducted using Chi-square tests followed by multivariate regression analysis to determine odds ratios, adjusting for age, marital-status and education.

Results: Zulu women were more likely to be obese as adults if they had been overweight as children (OR 20.1), as adolescents (OR 17.4) and as young adults (OR 95.2); if they perceived their mother as having been overweight during their childhood (OR 2.56), were dissatisfied with their weight (OR 27.5) and/or feared weight gain (OR 19.8). While weight loss attempts were significantly more prevalent in cases (85%) than controls (28%), 28% of controls wanted to gain weight. PROP taster-status was not associated with weight status after controlling for eating behaviours.

Conclusion: Risks for obesity development in Zulu women include having a personal history of overweight, having an overweight mother in childhood and fearing weight gain. Indicators of both acculturation to a slim western body weight/shape norm and upholding cultural norms in terms of acceptance of a larger body size, are evident.

P15-T

INITIATION OF ALLERGEN SPECIFIC T-HELPER 2 IMMUNE RESPONSES IN THE ABSENCE OF IL-4 SIGNALS CD4⁺ T CELLS

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Background

Allergies are characterized by an inappropriate immune response to environmental antigens. T-helper2 (Th2) cells are associated with allergies and are critical for disease development. *In vitro* studies demonstrated that IL-4 signalling on CD4⁺T cells leads to the development of Th2 cells, suggesting a crucial role for IL-4R α expression on Th cells.

Aims and Objectives

Although the initiation of Th2 responses *in vitro* is understood in great detail, less is known about Th2 cell induction *in vivo* as it occurs in the absence of IL-4. This study investigates *in vivo* the contribution of IL-4R α expressing T-helper cells to disease pathology.

Methods

The role of T cell IL-4R α in the development of Th2 responses is investigated using Lck^{cre}IL4R α ^{-/lox} mice, which have a deletion of IL-4R α on CD4⁺T cells. Mice are immunized with ovalbumin and symptoms of allergic asthma are induced by intranasal instillation of ovalbumin. Lung function, airway inflammation, cytokine and antibody responses were analysed.

Results

Ovalbumin instillation induced airway hyperresponsiveness, goblet cell hyperplasia and infiltration of the airways by eosinophils independently of IL-4R α on CD4⁺T cells. In agreement, CD4⁺T cells in Lck^{cre}IL4R α ^{-/lox} mice produced IL-13 and IL-5. IgE levels in Lck^{cre}IL4R α ^{-/lox} mice were reduced and correlated with reduced production of IL-4. Furthermore, Lck^{cre}IL4R α ^{-/lox} present elevated numbers of Th17 cells which correlates with a neutrophil infiltration of the airways.

Conclusion

These findings suggest that IL-4 signals in CD4⁺ T cells are not necessary for the initial development of a Th2 response and the induction of allergic symptoms; but prevent a Th17 response to allergens.

P16-T

GENERATION AND ANALYSIS OF LARGE-SCALE DATA DRIVEN *MYCOBACTERIUM TUBERCULOSIS* FUNCTIONAL NETWORKS FOR DRUG TARGET IDENTIFICATION

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Technological developments in large-scale biological experiments, coupled with bioinformatics tools, have opened the doors to computational approaches for the global analysis of whole genomes. This has provided the opportunity to look at genes within their context in the cell. The integration of vast amounts of data generated by these technologies provides a strategy for identifying potential drug targets within microbial pathogens, the causative agents of infectious diseases. As proteins are druggable targets, functional interaction networks between proteins are used to identify proteins essential to the survival, growth and virulence of these microbial pathogens. Here we have integrated functional genomics data to generate functional interaction networks between *Mycobacterium tuberculosis* proteins, and carried out computational analyses to dissect the functional interaction network produced for identifying drug targets using network topological properties.

We elucidated the interplay between *Mycobacterium tuberculosis* proteins, and identified proteins that may be essential for intracellular life on the basis of the network topological properties. This enhances our understanding of cell functioning and organism development and facilitates the determination of potentially important proteins that may be suitable drug targets. This study has provided the opportunity to expand the range of potential drug targets and to move towards optimal target-based strategies.

P17-T

DERIVATIVES OF NATURAL PRODUCTS IN THE TREATMENT OF OESOPHAGEAL CANCER

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Artemisinin derivatives are effective antimalarial agents that were recently found to have potent anticancer activity in a variety of cancer cell lines. The effects of these compounds in oesophageal cancer cells have not been elucidated. The first generation derivatives, artesunate (ART) and dihydroartemisinin (DHA), as well as novel artemisinin derivatives synthesized by the Chemistry Department of UCT were included in this study, which aimed to investigate the potential of artemisinin derivatives as chemotherapeutic agents and to determine the mechanism of action in oesophageal cancer cells. The artemisinin derivatives, ART and DHA, were shown to be effective against oesophageal cancer cells and the novel UCT derivatives were more potent than ART and DHA in both oesophageal cancer and other cancer cells, as shown by the MTT assay. The artemisinins were selective to cancer cells as they had significantly decreased activity against the normal cells treated. ART and DHA induced higher levels of apoptosis compared to the UCT derivatives which induced autophagy, determined by the PARP cleavage assay using Western blot analysis and Beclin-1 assay using qRT-PCR, respectively. The UCT derivatives induced a G1 cell cycle arrest whereas ART and DHA induced a G2-M block of the cell cycle. The results of this project indicate that the artemisinin derivatives are active against oesophageal cancer cells and that the novel UCT artemisinin derivatives display more potent activity against the cancer cells tested in comparison to the first generation derivatives ART and DHA. The novel UCT artemisinin derivatives may have potential as cancer chemotherapeutic agents.

P18-T

VALIDATION OF HEPATITIS B VIRAL LOAD ON THE ROCHE COBAS AMPLIPREP / COBAS TAQMAN INSTRUMENT.

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Background:

Currently the NHLS Groote Schuur Hospital Virology Laboratory performs HBV viral load testing on the Roche Light Cycler instrument using the QIAgen Artus HBV LC PCR Kit. The price of the QIAgen HBV Kit has increased by 30%. The COBAS AmpliPrep/COBAS TaqMan instrument (CAP/CTM) was installed in the laboratory as part of the Early Infant Diagnosis of HIV programme. The CAP/CTM HBV assay is more sensitive than the QIAgen assay and will be more cost effective.

Objective: To validate results of Hepatitis B (HBV) viral load on CAP/CTM for routine testing HBV viral loads received in the Virology Laboratory.

Method:

The validation was performed using past and current samples.

1) Same day analyses were done with both methods obviating freezing/thawing. 2) Analyses performed on CAP/CTM on stored thawed samples. 3) Dilutions of sample and effect of various diluents were tested with both assays. 4) Four known HBV positive patients with a LDL result on the Light Cycler were analysed on the CAP/CTM

Results:

The two kits compare well. The CAP/CTM tends to read slightly higher. Only two samples crossed the threshold of 4 log affecting treatment decision. In the low range, the CAP/CTM assay was more sensitive. The tested dilution matrices made no difference on the CAP/CTM. Results from serial dilutions of samples did multiply to the original result.

Conclusion:

The CAP/CTM HBV viral load test compares with the QIAgen Artus HBV LC PCR, and is more sensitive.

P19-T

THE WNT SIGNALLING PATHWAY IN EWING SARCOMA / PRIMITIVE NEUROECTODERMAL TUMOUR: AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Aim: To examine the immunohistochemical expression of the WNT pathway components in Ewing sarcoma (ES) / primitive neuroectodermal tumour (PNET)

Method and results: Twenty five cases originally diagnosed with ES/PNET were retrieved from the archives and stained with antibodies against WNT1, WNT5A, DVL1, GSK3 β , β -catenin, MYC, cyclin D1, E-cadherin (extracellular domain) and E-cadherin (cytoplasmic domain). Of the 25 cases analysed, 23 cases were confirmed as ES/PNET on review. Of the 23 cases, WNT1 was positive in 7 cases (30%), WNT5A in 12 cases (52%), DVL1 in 11 cases (48%), MYC in 11 cases (48%), cyclin D1 in 20 cases (87%), and nuclear localisation of the cytoplasmic domain of E-cadherin in 11 cases (48%). Nuclear β -catenin localisation was seen in 2 cases (8%). Membranous E-cadherin staining was absent in all cases. Except for an association of age with DVL1 and nuclear E-cadherin expression there was no statistically significant difference in the mean scores of the antibodies when segregated by clinicopathological parameters.

Conclusion: WNT pathway components are expressed in a subset of ES/PNET, but seldom involve β -catenin nuclear localisation. Cyclin D1 was frequently expressed. It may contribute to the proliferative potential of ES/PNET and may lead to the development of new therapeutic agents. Lack of membranous E-cadherin is postulated as a contributory factor in the micrometastatic behaviour of ES/PNET. The significance of nuclear E-cadherin expression remains to be elucidated

P20-T

CHARACTERISATION OF THE ALZHEIMER'S AMYLOID-B PEPTIDE CLEAVAGE BY ANGIOTENSIN CONVERTING ENZYME.

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Angiotensin-converting enzyme (ACE), present in the brain renin-angiotensin system, is critical in the regulation of blood pressure. Hypertension is a major risk factor for Alzheimer's disease and ACE is implicated in the breakdown of the Alzheimer's-associated amyloid beta (A β) peptide where it cleaves the A β *in vitro*. This study aims to investigate the kinetic differences between the catalytic domains and the A β substrate; towards a clearer understanding of the link between hypertension and Alzheimer's.

Wild type ACE and mutant constructs of ACE were expressed and purified. The kinetic constants for the cleavage of different A β peptides were obtained from HPLC and fluorogenic assays. The C-domain cleaved the A β peptide, although the rate was very slow. In fluorescence based assays, N-domain demonstrates faster hydrolysis of A β when compared to both somatic ACE (sACE) and the C-domain, although sACE has a 4 fold higher binding affinity. C-domain active site S₂'substitution mutants hydrolysed A β at a faster rate than the N-domain yet the sACEN-domain active site knockout had sACE equivalent kinetics. The peptide cleavage sites, determined by MS/MS, vary depending on the length of the peptide.

The individual domains clearly have different rates of hydrolysis of the A β . Although it appears that together, as sACE, there is an increase in substrate binding, which leads to greater catalytic efficiency and in the sACENKO positive co-operativity occurred. By replacing active site residues in the C-domain with corresponding N-domain residues there is a restitution of hydrolysis to the C-domain.

P21-T

THE ROLE CD4+ T CELLS IN HOST PROTECTIVE RESPONSES AGAINST CUTANEOUS LEISHMANIASIS USING GENOME-WIDE TRANSCRIPTOMICS

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IL4 & IL13 signal via IL4R α and play a role in the outcome of leishmaniasis. Protective immunity against *L. major* is dependent on a T_H1 immune response present in the healer strain C57BL6, whereas Th2 responses are detrimental as predominant in the non-healer strain BALB/c. BALB/c mice with a global deletion of IL4R α show increased resistance during acute leishmaniasis, despite the presence of an IL-4R α -independent TH2 response and developed non-healing lesions during chronic infection. In contrast, in the absence of IL4R α in T cells only, BALB/c mice becomes a healer strain due to a dominant Th1 immune response,

The aim was to uncover candidate genes and pathways from CD4⁺ T cells, which might be involved in resistance to cutaneous leishmaniasis using a genome-wide transcriptomics approach by microarray. Global IL-4R α KO, T cells specific IL-4R α KO and control mice were infected with *L. major* and 3 weeks later activated CD4⁺ T cells (CD4⁺CD44^{med-high}CD62L^{low}) and regulatory CD4⁺ T cells (CD4⁺CD25⁺) were isolated from the draining lymph nodes by FACS sorting (99% purity), RNA isolated, linear amplified and reverse transcribed to cDNA. Affymetrix and Illumina microarray was performed from 3 independent experiments. 58 differentially expressed genes from activated CD4⁺ T cells and CD⁺ Tregs between healer and non-healer strains were involved in cell-to-cell signaling, infection mechanism, inflammatory disease and response. Of interest were 4 genes (Rnf130, H2T10, Ctse, Gpb1), possibly involved in the IFN-g regulated T-helper 1 differentiation.

P22-T

MATERNAL SEPARATION IMPEDES EXERCISE-INDUCED PHOSPHORYLATION OF ERK1/2 IN ADULT RAT HIPPOCAMPUS

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Maternal separation (MS), an animal model of early life stress, interferes with the critical period of post-natal brain development, and allows for study of behavioural and neurochemical effects of early life stress. The long-term depression caused by MS is associated with impairment of memory and learning. Brain derived neurotrophic factor (BDNF) mediates exercise-induced neurogenesis and synaptic plasticity. BDNF stimulates the MAP kinase/extracellular signal-regulated kinase1/2 (Erk1/2) cascade, the role of which has been implicated in anxiety and fear conditioning. In this experiment, the effects of MS and voluntary exercise were studied to see whether exercise could reverse the MS-induced deficit in memory and phospho-Erk1/2 levels.

Sprague-Dawley rat pups were maternally separated from post-natal day 2 (P2) to P14 for 3 hours per day and allowed voluntary exercise from P50 to P70 in running-wheel cages. From P75-81, four groups of rats (MS-non-runners; MS-runners; non-separated- (NS-) non-runners; NS-runners;) were assessed behaviourally for anxiety in the open field and elevated plus maze; and for various object recognition and location memory. Later, rats were killed and hippocampal synaptophysin and phospho-Erk1/2 measured using Western blot analysis.

Exercise reduced the MS-induced anxiety-like behaviours. MS rats had better recall for location and temporal ordering of objects. Non-stressed exercised rats had increased levels of synaptophysin and phospho-Erk1/2 but phospho-Erk1/2 was unchanged in MS-runners. MS antagonised the exercise-induced increase in Erk1/2. A possible mechanism for this would be the upregulation of MKP-1 which dephosphorylates phospho-Erk1/2, which would be consistent with the association of elevated MKP-1 and long-term depression.

P23-T

THE ROLE OF METABOLIC ENZYME, PHOSPHOGLUCOMUTASE 1 IN CANCER DEVELOPMENT

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Cancer cells undergo metabolism that is significantly altered compared to normal cells. This metabolic disruption can be an adaptive response to the tumour microenvironment or consequent to aberrant signalling due to oncogenic activity. Much still needs to be understood about specific key players that drive and maintain cancer development under conditions that would seemingly appear unfavourable. This project aims to contribute to answering questions that pertain to cancer cell metabolism and biology particularly looking into the function of Phosphoglucomutase 1 (PGM1), a metabolic enzyme identified in microarray analysis to be upregulated in cervical cancer patient tissue compared to normal cervical tissue. PGM1 reversibly catalyses the first step in glycogen synthesis and the last in glycogen breakdown involving the conversion of glucose-1-phosphate into glucose-6-phosphate, an intermediate for glycolysis and the pentose phosphate pathway. The upregulation of PGM1 in cancer was confirmed using qRT-PCR, Western blot analysis and immunofluorescence. To determine the requirement of PGM1 to cancer cells, PGM1 expression was inhibited using siRNA and effects on cell proliferation, glycogen content and NADPH levels under normal culture conditions and decreased glucose levels was investigated. Our investigations indicate that inhibition of PGM1 expression in Caski and HepG2 cancer cells results in a slight reduction in cancer cell proliferation under conditions of glucose depletion. A decrease in glycogen content and increase in NADPH levels was observed when PGM1 expression was inhibited. Taken together our results suggest that PGM1 may confer a growth advantage to cancer cells under conditions of nutrient deficiency, while substrate depletion maintains low NADPH levels.

P24-T

VARIATION IN MEASUREMENTS OF RECOVERY FOLLOWING A STANDARDIZED EXERCISE BOUT IN TRAINED AND UNTRAINED INDIVIDUALS

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Introduction

The return to resting metabolism following a bout of exercise is more rapid in physically trained individuals than in untrained individuals. However, there has been no consensus on how recovery should be quantified or compared. Therefore the aim of this study was to investigate metabolic recovery, or excess post-exercise oxygen consumption (EPOC), as a means of interpreting the acute, internal response to an exercise bout. Specifically, this study will determine which of the 9 EPOC-related outcomes under investigation should be used as a marker of exercise response.

Methods

Healthy, untrained individuals (UT) (n = 11), moderately-trained runners (MT) (n = 13) and well-trained runners (WT) (n = 12) performed a standardized protocol consisting of baseline metabolic measurements, a 3km treadmill run at 70% of VO_2 max and 1 hour of controlled, resting recovery. Nine different EPOC measurements, relating to the magnitude, duration and rate of the post-exercise recovery response were calculated using graphing software.

Results

Measures relating to EPOC duration and EPOC rate were able to distinguish between trained and untrained individuals (UT vs. MT, $p=0.003-0.002$)(UT vs. WT, $p=0.0001-0.0006$) whereas measures of EPOC magnitude were not. EPOC measures showed only weak to moderate correlations with performance, the highest correlation being performance and EPOC rate ($r=0.80$), followed by performance and EPOC half-life and normalized EPOC half-life ($r= -0.66$).

Conclusion

Of the 9 measurements of recovery, EPOC rate was the most consistently promising measurement, strongly differentiating between trained and untrained participants, showing a high degree of variation and also showing the best relationship with VO_2 max and performance.

P25-T

THE UTILITY OF TRANSRENAL DNA PCR AS A POSSIBLE DIAGNOSTIC TOOL FOR TUBERCULOSIS

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Childhood tuberculosis remains a diagnostic challenge. There is a need for non-invasive specimen collection methods for the detection of *M. tuberculosis*. Detection of transrenal DNA (Tr-DNA) in urine has recently been described as a promising tool for diagnosis of TB. The purpose of this study was to optimise *M. tuberculosis* Tr-DNA isolation procedures prior to testing of samples from children with suspected TB. Initial experiments comparing three different DNA isolation procedures: Promega Wizard® Genomic DNA purification kit; Invitrogen Dynabeads® DNA Direct™ Universal kit and the Norgen urine DNA isolation kit (Norgen Biotek Corporation) showed the Norgen isolation procedure to be most efficient in isolating *M. tuberculosis*. Urine specimens from a healthy individual were spiked with sheared *M. tuberculosis* DNA in duplicate with a range of DNA concentrations. Specimens were either processed immediately or frozen and processed the following day using the Norgen Biotek kit according to the manufacturer's instructions. The efficiency of isolating sheared *M. tuberculosis* DNA was thereafter tested by a highly sensitive in-house real-time PCR assay.

The Norgen urine DNA isolation kit yielded large amounts of total DNA. The in-house real-time PCR assay allowed low levels of sheared *M. tuberculosis* DNA to be detected in the presence of background DNA. The yield of total DNA isolated from frozen urine specimens.

P26-T

DEVELOPMENT OF AN ARTIFICIAL KNEE MENISCUS: PREPARATION AND PROPERTIES

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The meniscus is a C-shaped fibrocartilaginous structure in the knee, between the femur and the tibia. Meniscal damage can occur through football knee injuries, accident, repeated small injuries to the cartilage, or degeneration of the meniscus cartilage in older people. Meniscus cartilage does not heal very well once it is torn due to lack of blood supply. The aim of this study was to design novel meniscus prosthesis that has comparable mechanical properties to the native meniscus such that it will serve to eliminate or reduce articular cartilage degeneration.

Medical grade silicone elastomer reinforced with standard monofilament nylon fishing lines were fabricated into composites using compression moulding technique. Samples of both fibre and non-fibre reinforced polymeric samples were tested under compression using Zwick material testing machine at a crosshead speed of 5 mm/min. Stress-strain plots were recorded and viscoelastic behaviour was observed.

Our results showed a modulus of elasticity of 41.9 ± 0.23 MPa for fibre reinforced polymers while 100% polymeric samples have a higher value of 77.1 ± 0.48 MPa. However, the stress-strain curves showed a definite elastic behaviour in fibre reinforced samples similar to the natural meniscus tissue compared to those samples without fibres. It is essential for a functional meniscus to be able to protect the articular cartilage and distribute load in the knee joint. The characteristics of the developed fibre reinforced composite showed a similar viscoelastic and compressive properties to that of the natural meniscus.

P27-T

GENERATING AN OXIDATIVE STRESS MODEL IN HUMAN SKIN CELLS FOR ANTIOXIDANT TESTING

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The balance of reactive oxygen species (ROS) for physiologically relevant functioning of the cell is maintained by endogenous antioxidants and antioxidative enzymes. High levels of ROS cause oxidative damage to cellular proteins, DNA and lipids. All of these may contribute to aging, progression of cardiovascular disease and cancer development. Therefore, the addition of exogenous antioxidants could be helpful in the prevention of ROS-mediated damage elicited by UV. The study aimed to establish a model to test antioxidant efficacy in human skin cells. Human keratinocyte and fibroblast cell lines, representing cells of the epidermis and dermis respectively, were exposed to different UVA doses (5, 10 and 20 J/cm²). Cells were tested for cell viability, ROS production and DNA damage by XTT, flow cytometry and agarose gel electrophoresis, respectively. The results showed that the 2 cell types respond differently to UV. In keratinocytes, the response was immediate in terms of mitochondrial activity, increased ROS production; and 8 hours after UV, DNA damage was observed. In fibroblasts, the metabolic response to UV was slower and less ROS was produced. In conclusion, a partial model has been established for the assessment of the protective effects of antioxidants. Therefore in this model, keratinocyte cell viability and DNA damage will be tested 4 and 8 hrs after UV irradiation, respectively, while fibroblast cell viability will be evaluated after 24 hrs.

AFTERNOON SESSION:

P1-L

THE ROLE OF DECTIN-1 IN IMMUNITY TO STRAINS OF *CANDIDA ALBICANS*

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Fungal infections are an emerging problem resulting from modern medical interventions, immunosuppression and acquired immunodeficiency. The innate recognition of fungal pathogens is a crucial first step in the induction of protective anti-fungal immunity, and Dectin-1 is one of the key receptors involved in this process. This pattern recognition receptor possesses an extracellular carbohydrate recognition domain, which recognises beta-glucans, and a cytoplasmic signalling motif which can induce cellular activation. In response to several fungal pathogens, including *Candida albicans*, Dectin-1 mediates various cellular responses, including DC maturation, the respiratory burst, phagocytosis, endocytosis and the induction of numerous cytokines and chemokines. Work from our laboratory has demonstrated that this receptor plays an essential role in immunity to *C. albicans in vivo*. However, another study has shown that Dectin-1 was not required for protection against this pathogen. We set out to clarify this controversy by examining the role of this receptor in various strains of *C. albicans* in different mouse backgrounds. We show here that the involvement of Dectin-1 in anti-fungal immunity is critically dependent on the fungal strain, and that the role of this receptor is not influenced by the mouse genetic background. Our results further demonstrate that the protection of mice against the fungal infections stems from the ability of Dectin-1 to promote cytokine production and fungal clearance. These results provide critical new insights into the role of Dectin-1 in anti-fungal immunity and have relevance for our understanding of the role of this receptor in control of *Candida* infections in humans.

P2-L

HUMAN PAPILLOMAVIRUS GENOME SEQUENCING AND TYPING USING ILLUMINA NEXT-GENERATION SEQUENCING

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Human papillomavirus (HPV) infection is the highest in Africa, where concurrent HIV infection is additionally high. HIV positive women are infected with multiple, and often rare, HPV types. Most commercial HPV DNA detection kits are PCR-based and target types commonly circulating in the developed world. There is limited information on HPV types in Africa not detected by commercial kits.

The aim of this project was to evaluate the applicability of Illumina sequencing in detecting and genotyping multiple co-infecting HPV types in comparison to a commercial kit. Total DNA was extracted from a cervical specimen from a South African HIV positive woman with 12 HPV types identified by Roche linear array (LA). Circular HPV DNA was enriched using rolling circle amplification and sequencing performed with the Illumina Genome Analyser II. Reads were *de novo* assembled and mapped to reference HPV sequences. A total of 16 HPV types (16, 30, 35, 39, 40, 45, 53, 55, 56, 70, 74, 81, 86, 90) were identified. Types 30, 35, 56, 74, 86 and 90 were not detected by the commercial LA kit. Type-specific PCR amplification of HPV types 30, 74 and 86 revealed a prevalence of 14.6%, 12.8% and 4.6%, respectively, in a cohort of 109 HIV positive South African women.

Next-generation sequencing allows for rapid, accurate and unbiased detection of circulating HPV types without prior sequence information. As the costs decrease this technology will become more widely used and will aid the development of regionally specific diagnostic kits.

P3-L

EARLY SELECTIVE PRESSURES ACTING ON THE HIV-1 SUBTYPE C TRANSMITTED/FOUNDER WHOLE GENOME IN FIVE INDIVIDUALS WITH DIFFERING DISEASE PROGRESSION PROFILES

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Immune pressures acting on transmitted HIV-1 affect virus diversification and potentially disease course. Characterizing these pressures is essential to vaccine development for the globally dominant subtype C virus. We aimed to map changes across the virus genome in 5 women from the CAPRISA002 acute infection cohort, Durban, in the first 6 months of infection.

We generated whole (n=118) and half (n=7) genome sequences at screening/enrolment, 3 and 6 months post-infection. Sites under positive selection and peptides undergoing change due to putative reversion, CD8+ T lymphocyte (CTL) or antibody pressure were identified. Autologous peptides were tested in interferon-γ ELISPOT assays.

A total of 59 peptide regions were identified as under putative immune pressure (containing 51/57 positively selected sites). Fifteen epitopes (24%) were recognized by T cells of which 3 have not been previously described; 7 epitopes spanned known class I HLA-associated CTL epitopes but with no matching ELISPOT responses; 20 were identified as under putative antibody pressure; 9 as under putative reversion and 8 were unclassified. CTL pressure was most frequent in Nef. The earliest observed CTL escape mutation was at two weeks post-infection. Shuffling/toggling of mutations was observed in 81% of CTL epitopes. We observed changes in Env hypervariable loops prior to autologous neutralizing antibody detection. HIV rapidly escapes immune pressure, with escape most frequently detected within 5 weeks of infection, the earliest of which was detected at 2 weeks post infection. CTL pressure accounted for the majority of changes however we found no association between escape and disease progression.

P4-L

A FIELD EVALUATION OF A CHEMIREACTIVE SENSOR ARRAY AS A RAPID, POINT OF CARE DIAGNOSTIC FOR PULMONARY TUBERCULOSIS

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BACKGROUND

Chemiresistive sensor arrays are promising point-of-care diagnostics which detect volatile organic compounds (VOCs) liberated by the metabolic activities of bacteria and oxidative stress of infection. Sensors discriminate the signature of a causative organism using pattern recognition software. We conducted a study evaluating the use of such a sensor for the detection of *M. tb* directly from the breaths of patients suspected of having pulmonary TB.

METHOD

We compared the sensitivity and specificity of the sensor (NextDimension Technology, California) to that of TB microscopy, and culture or GeneXpert. TB suspects were recruited at PHC facilities in the Paarl area. Forty smear positive, culture/ Xpert positives, 28 smear negative, culture/ Xpert positive, 48 smear negative, culture/ Xpert negative TB suspects and 50 healthy volunteers were recruited over a 6-month period. Forty-two percent of the study population was HIV-positive. The test, performed in triplicate, consisted of a patient exhaling into a bag, which was then attached to the sensor and analysed in real-time.

RESULTS

Linear discriminant analysis revealed the sensitivity and specificity of the sensor were as follows: 100%, 92% (all culture positives vs. healthy volunteers), 70%, 83% (smear positive, culture positive vs. culture

negative), 36%, 70% (smear negative, culture positive vs. culture negative). The sensor was able to accurately discriminate between TB patients and healthy volunteers, but less effectively between TB patients and patients with other causes for their lower respiratory tract symptoms.

CONCLUSION

This device shows promise as a rapid, point of care diagnostic for pulmonary tuberculosis.

P5-L

ACINETOBACTER BAUMANNII: AN EVALUATION OF FIVE SUSCEPTIBILITY TEST METHODS TO DETECT TOBRAMYCIN RESISTANCE IN AN EPIDEMIOLOGICALLY RELATED CLUSTER.

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BACKGROUND

Acinetobacter baumannii is a major pathogen causing nosocomial infections, particularly in critically ill patients. This organism has acquired the propensity to rapidly develop resistance to most antibiotics. At several hospitals within Cape Town, tobramycin and colistin remain frequently the only therapeutic options. The Vitek2 automated susceptibility testing (AST) is used in the clinical laboratory to determine selected susceptibility profiles. The suspicion of a possible AST-related technical error when testing for susceptibility to tobramycin in *A. baumannii* precipitated this study.

METHODOLOGY

Forty *A. baumannii* strains isolated from clinical specimens (June-December 2006) which exhibited MICs close to the tobramycin breakpoints were included in this prospective study. AST was compared to disk diffusion, Epsilometer test and agar dilution using broth microdilution (BMD) as the reference method. Additionally, PCR was performed to detect the *aac(3)-II'* gene which encodes an aminoglycoside modifying enzyme with activity against tobramycin.

RESULTS

The tobramycin susceptibility results revealed errors in 25/39 isolates (10 very major and 15 minor errors) when AST was compared to BMD ($p < 0.001$), 12/39 (1 very major and 11 minor errors) when Etest was compared to BMD, and 15 errors (3 very major and 12 minor errors) when disk diffusion was compared to BMD. Additionally, the tobramycin resistance gene, *aac(3)-II'*, was detected in 21/25 of the discrepant isolates, confirming the resistant phenotype detected by the reference method. Molecular typing showed that these isolates were genetically related.

CONCLUSION

Clinical laboratories using the Vitek2 system for routine use should consider an alternative susceptibility testing method to determine susceptibility to tobramycin.

P6-L

REPIGMENTATION IN VITILIGO: AN IMMUNOHISTOCHEMICAL ANALYSIS OF MELANOCYTE MIGRATION, PROLIFERATION, AND DIFFERENTIATION

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Vitiligo is an acquired, hypopigmentary disorder that affects 1-2% of the global population. The etiopathogenesis of vitiligo is complex and several theories exist in an attempt to explain the lack of melanocytes. Following psoralens-UVA therapy however, perifollicular repigmentation is often seen, which suggests that melanocyte precursor cells found in the putative stem cell niche in the hair follicle bulge region are able to ensure repopulation of basal melanocytes. BrdU, a thymidine analogue, is a commonly used marker of cellular proliferation *in vitro*. Immunodetection of the incorporated BrdU necessitates the unmasking of antigenic epitopes by a harsh DNA denaturation step, precluding its use for double-labelling. Double-labelling is necessary in this instance to demonstrate both proliferating cells and melanoblasts or melanocytes in the basal layer of the epidermis. The main objective of this study was therefore to investigate this theory by uncovering the location of melanoblasts/melanocytes in the niche area, the hair follicle and the basal layer of the epidermis with an overall aim to contribute to better repigmentation in vitiligo patients. BrdU-treated, PFA-fixed punch biopsies of normal, vitiliginous, and repigmenting skin samples from vitiligo patients were processed to paraffin wax. Double-labelling of skin sections was achieved by replacing DNA denaturation with heat-mediated antigen retrieval. Using an optimised double-labelling immunofluorescent method, proliferating cells (BrdU-positive), and melanocytes (MART-1 positive) were simultaneously demonstrated. While increasing patient biopsy evaluations remain an option for future studies, our results presented herein suggest that the response to vitiligo therapy allows for the tailoring of treatment regimens to be more patient

P7-L

CHARACTERIZATION OF THE ANTIMYCOBACTERIAL EFFECT OF A *PSEUDOMONAS*-DERIVED ACTIVITY

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The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB), reinforces the need for the development of novel antimycobacterial compounds. Antibacterial secondary metabolites constitute a potentially rich source of anti-TB drugs. In previous work, we identified a *Pseudomonas* strain which inhibited growth of the non-pathogenic mycobacterium, *M. smegmatis* (MSM), on solid media. The active compound(s) was isolated in a crude extract and shown to inhibit growth of all Gram-positive organisms assayed, including other actinobacteria, but not the Gram-negative *E. coli* – a result which suggests a potentially Gram-restricted target range. Notably, a parallel extraction on an unrelated *Pseudomonas* isolate failed to inhibit growth of MSM, thereby confirming that the inhibitory effect is limited to our strain, designated *Pseudomonas* αMB (anti-mycobacterial). Moreover, the crude extract was shown by broth microdilution assay to inhibit growth of MTB at a concentration of 14-16 µg/ml, a value only ten-fold higher than key frontline anti-TB agents tested. These results identified the *Pseudomonas*-derived active agent(s) as a compelling candidate for further investigation as a potential lead compound. Therefore, subsequent work has focused on the need to scale up production of the inhibitory compound in liquid cultures through the use of fermentation technology. Although preliminary data indicate that differential growth dynamics are associated with different carbon sources, the link between carbon utilization and production of the inhibitory compound(s) is yet to be established. This and other variables will be investigated in developing an optimised protocol for the production and isolation of the active compound(s).

P8-L

IL-4R α RESPONSIVE B CELLS ARE REQUIRED FOR PROTECTION AGAINST ACUTE SCHISTOSOMIASIS AND DOWN-REGULATION OF GUT INFLAMMATION.

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IL-4R α dependent TH2 immunity is essential for host control of and survival from *S. mansoni* infection. B cells are known to play important roles in controlling *S. mansoni* infection but whether an ability to respond to TH2 stimuli impacts on this is unknown. To address this we used a novel mouse model lacking IL-4R α expression on B cells (MB-1^{cre}IL-4R α ^{-lox}) to identify possible roles for B cell IL-4R α responsiveness in controlling host immunity to acute Schistosomiasis.

We found B cell-specific IL-4R α -deficient mice had heightened susceptibility to *S. mansoni* infection compared to immune-competent control mice. Increased susceptibility was related to increases in granuloma size, liver fibrosis and gut inflammation. Related to this were reduced Th2 cytokines responses in B cell-specific IL-4R α -deficient mice. The critical role of TH2 mediated host control and survival from acute *S. mansoni* infection is the generation of a pathology controlling alternative macrophage (AM) response. Analysis of liver granuloma associated macrophages in B cell-specific IL-4R α -deficient mice showed impaired induction of AM marker YM-1 and increased classical macrophage associated iNOS production. This demonstrates that IL-4R α responsive B cells are required for optimal induction of alternatively activated macrophages in the liver.

These data demonstrates that B cells driven TH2 immunity plays a critical role in co-ordinating the induction of the immunological responses against acute Schistosomiasis that are essential for host survival.

P9-L

INVESTIGATIONS INTO IDENTIFYING THE POSSIBLE DRUG TARGETS OF THE GARLIC COMPOUND AJOENE

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Crushed garlic contains many organosulphur compounds (OSC) which share a similar structure to contain a sulphide or polysulphide group flanked by allyl groups. Ajoene is one of these OSC which is reported to be an active growth inhibitor of cancer cells. The hypothesized mechanism of its action is forming mixed disulphides with cysteine residues in proteins thereby affecting protein function leading to cell death. The specific drug targets of OSC at present are unknown, although it has been reported to form mixed disulphides with β -Tubulin and glutathione reductase in in vitro systems. We have synthesised a fluorescein labelled ajoene analogue, FOX and aim to use this analogue to identify proteins which may bind to ajoene in oesophageal cancer (WHCO1) and breast cancer (MDA) cell lines.

Cells were treated with FOX and the resulting cell lysate analysed by protein dialysis, TLC and western blot. The results showed strong affinity between FOX and the lysate supporting the hypothesis that the ajoene maybe bound to some proteins in the lysate. Further stringent proteomic analysis involving isolating potential drug targets of ajoene, such as β -Tubulin, from FOX-treated cell lysate by immunoprecipitation, subjecting them to non-denaturing SDS-page and MALDI-TOF mass spectrometry is required. We also report on confocal microscopy of live and fixed FOX treated MDA cells in an attempt to identify fluorescently labelled proteins, cell structures and the localisation of FOX in the cells.

Preliminary conclusions show that fluorescein-labelled ajoene is strongly associated with the lysate from FOX-treated MDA and WHCO1 cancer cells supporting the hypothesis that FOX is possibly bound to protein targets within the cell.

P10-L

COLLAGEN GENES AND EXERCISE ASSOCIATED MUSCLE CRAMPING IN IRONMAN TRIATHLETES

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Introduction

Exercise Associated Muscle Cramps (EAMC) is a common condition affecting participants of endurance events such as ultra-marathons and triathlons. Despite its high prevalence, little is known about the etiology of EAMC. There is evidence that tissue damage may play a role in the etiology of EAMC. It has also been speculated that genetic predisposition may also play a role in the etiology. We propose variants within the *COL5A1*, *COL3A1* and *COL6A1* genes may modulate risk of developing EAMC, since these genes are known to be associated with injury of soft-tissue, including tendons and ligaments.

Methods

Two hundred and ninety eight participants with self-reported history of EAMC within the last 12 months prior to an Ironman Triathlon were included as cases in this study (hEAMC group). One hundred and fifty nine triathletes with no self-reported history of previous (lifelong) EAMC were included as controls (CON group). All triathletes were genotyped for the *COL5A1* rs12722 (C/T), the *COL3A1* rs1800255 (G/A) and *COL6A1* rs35796750 polymorphisms.

Results

The *COL5A1* CC genotype was significantly over-represented ($p=0.041$) among the CON (24.0%) when compared to hEAMC group (15.9%). This association was strengthened when only older (>35 years) individuals were analyzed ($p=0.006$). No significant genotype differences were found for the *COL3A1* ($p=0.731$) and *COL6A1* ($p=0.986$) genotypes between the hEAMC and CON groups.

Conclusion

This study identified, for the first time, the *COL5A1* gene as a potential marker for a past history of EAMC. These effects may be mediated through the effects that type V collagen exerts on collagen fiber diameter and strength in the endo- and perimysium.

P11-L

THE CHARACTERISATION OF NOVEL AVIPOXVIRUSES FOR USE AS POTENTIAL VACCINE VECTORS

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Avipoxviruses are excellent candidates for vaccine vectors due to their safety profile and host range restriction. Several novel South African avipoxvirus isolates, infecting birds of the Columbidae family (pigeons and doves), have been characterised. These isolates were grown on chorioallantoic membranes of embryonated hen eggs and resulted in variable pock morphology with severe thickening of the membrane tissue. Differences in avipoxvirus growth on these membranes were investigated. Two conserved poxvirus genes corresponding to Vaccinia Virus (VACV) P4b (*fpv167*, VACV A3L) and G8R (*fpv126*, VLTF-1) were amplified and sequenced. These genes were analysed and Neighbour-Joining phylogenetic trees were constructed from MUSCLE nucleotide and amino acid alignments of published poxvirus sequences at these loci. Based on these two genes, the South African avipoxvirus isolates in this study were all found to group in either clade A, subclade A2 or A3. Avipoxviruses have been seen to naturally infect 232 out of 9000 species of bird. To date, members of the Avipoxvirus genus have been assigned species names according to the bird species which they infect. There are 10 defined and 3 tentative species of avipoxvirus. It has been shown that not all poxviruses originating from the *Columbidae* family (pigeons and doves) group together in the same phylogenetic clade. Moreover, the same species of bird can be infected by different poxviruses. This supports the notion that avipoxvirus evolution is independent of the evolutionary taxonomy of the host and suggests an alteration of the existing avipoxvirus nomenclature and taxonomy.

P12-L

DNA SEQUENCE ANALYSIS OF THE PENGUINPOX VIRUS GENOME

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Penguinpox virus (PEPV) is a novel, avian poxvirus isolated from African Penguins in the Western Cape. Infection with PEPV results in mild cutaneous lesions and very low mortality rates. PEPV progresses through early stages of replication in mammalian cells but no infectious progeny virus is produced. The Avipoxviruses are divided into three clades (A, B and C) and 6 subclades (A1-A4 and B1 and B2). Viruses belonging to clade A are Fowlpox (FWPV) like viruses, clade B Canarypox (CNPV) like viruses and clade C, Parrotpox like viruses. Multiple sequence alignment and phylogenetic analysis of three genes, VLTf-1, P4b and H3L showed that PEPV belongs to clade A, subclade A2 and is most closely related to isolates from Turkey and Ostrich. FWPV and CNPV are the only avian poxviruses to have their genomes completely sequenced.

The genome of PEPV was sequenced using the Roche 454 GS-FLX system. Genome sequence analysis has resulted in the discovery of 260 open reading frames (ORFs) with 240 conserved between FWPV and PEPV and 20 showing no significant homology to previously described sequences. Gene synteny between PEPV and FWPV is generally conserved though there are several insertions/deletions and rearrangements in the PEPV genome relative to FWPV.

P13-L

OVEREXPRESSION OF KPN β 1 AND KPN α 2 IMPORTIN PROTEINS IN CANCER DERIVES FROM DEREGULATED E2F ACTIVITY

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Karyopherin β 1 (Kpn β 1) and Karyopherin α 2 (Kpn α 2) are nuclear transport proteins that work in concert to transport cargo proteins into the nucleus. We previously identified increased expression of Kpn β 1 and Kpn α 2 in cervical tumours compared to normal epithelium and in transformed cells compared to their normal counterparts. This study aims at identifying the transcription regulatory mechanisms associated with high Kpn β 1 and Kpn α 2 levels in cancer cells. Kpn β 1 (-2013 to +100) and Kpn α 2 (-1900 to +69) promoter fragments were separately cloned into the reporter vector, pGL3-basic, and luciferase assays revealed both as significantly more active in cancer and transformed cells compared to normal, correlating with endogenous protein levels. A series of deletion constructs identified the promoter regions responsible for the differential activity, and a number of highly conserved E2F binding sites were identified within these regions. Mutation analysis confirmed the functionality of several E2F sites, and siRNA inhibition of Dp1, the co-activator necessary for E2F function, resulted in decreased Kpn β 1 and Kpn α 2 protein levels. E2F/Dp1 binding to the Kpn β 1 and Kpn α 2 promoters was detected by ChIP analysis. E2F activity is known to be increased in cervical cancer cells due to the inhibition of its repressor, Rb, by HPV E7. E7 was thus inhibited using siRNA, and decreased activities of both promoters observed, supporting the crucial role of E2F in maintaining high Kpn β 1 and Kpn α 2 levels in cervical cancer cells. This study is a first to report Kpn β 1 and Kpn α 2 promoter regulation in cancer cells and to correlate their promoter activities with deregulated E2F.

P14-L

TBX3, a t-box transcription factor, PROMOTES melanoma formation and invasion BY REGULATING KEY cell adhesion and EXTRACELLULAR MATRIX PROTEINS

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Melanoma is a highly aggressive cancer and its incidence is increasing faster than most other cancers. While it only constitutes 4 percent of all dermatological cancers, it is responsible for 80 percent of deaths caused by skin cancer. Malignant melanoma occurs as a result of melanocytes progressing through a series of steps which are well defined phenotypically but which remain poorly understood at a molecular level. Interestingly, the T-box transcription factor, TBX3, has been implicated in melanoma progression but its precise role is yet to be elucidated.

This study provides compelling evidence that TBX3 plays an important role in the vertical growth phase of melanoma progression which is characterised by cells acquiring the ability to proliferate indefinitely, to cross the basement membrane, invade the dermis and form tumours. Importantly, we show, using a shRNA approach that TBX3 is specifically involved in reducing cell adhesion and enhancing cell migration of melanoma cells in anchorage independent and cell motility assays. Furthermore, using an *in vivo* nude mouse animal model we demonstrate that TBX3 is required for melanoma formation and metastasis. Finally, we have data from quantitative real time PCR and luciferase assays to suggest that the molecular mechanism underlying TBX3's involvement in melanoma progression is through its ability to repress several cell adhesion and extracellular matrix molecules including E-cadherin, and Fibronectin

P15-L

DEVELOPMENT OF REVERSE TETRACYCLINE INDUCIBLE SYSTEM TO IMPROVE STABILITY AND IMMUNOGENICITY OF RECOMBINANT *M. BOVIS* BCG EXPRESSING HIV ANTIGENS

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Live recombinant *Mycobacterium bovis* BCG (rBCG) expressing HIV-1 antigens have shown promise as HIV vaccine vectors, however, have not been used in clinical trials due to poor viral antigen expression and genetic instability. We aim to overcome these problems by down-regulating HIV-antigen expression during preparation of rBCG stocks and up-regulating it prior to vaccination. Our design is based on constitutively expressing the DNA binding regulatory protein revTetR, such that in the presence of anhydrotetracycline (ATc), revTetR-ATc complex binds *tet* operator sites placed within the promoter, causing shut-down of antigen-expression during *in vitro* growth. Thus rBCG stocks expressing HIV antigens can be expanded in the presence of ATc and vaccine remain stable.

A series of revTetR regulated expression systems were constructed containing different combinations of operator sequences and two different revTetRs. These systems were assessed in mycobacteria with and without ATc using GFP as reporter antigen. Stable systems were chosen and *gfp* gene replaced with HIV-1 *rt* gene.

Stable vectors showed repression of HIV-RT expression in the presence of ATc which was reversed upon its removal. Vaccine stocks of this rBCG were prepared and evaluated for immunogenicity. BALB/c mice were primed with rBCG-RT intraperitoneally and boosted with a multi-gene HIV-1 subtype C recombinant MVA vaccine (SAAVI MVA-C) via intramuscular route. This combination regimen elicited antigen-specific intracellular IFN- γ activity.

We showed that an rBCG vaccine expressing HIV-RT could be down-regulated *in vitro* and up-regulated *in vivo* to improved vector stability, overcome the challenges of low antigen expression and induce strong immune responses.

P16-L

THE DETECTION AND CHARACTERISATION OF MULTI-DRUG RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATES FROM PATIENTS IN GROOTE SCHUUR HOSPITAL

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Pseudomonas aeruginosa is a Gram-Negative rod capable of causing severe, even fatal infections particularly in immunocompromised patients. During the latter part of 2010 an outbreak of multi-resistant *P. aeruginosa* occurred among patients' in the Haematology ward at Groote Schuur Hospital. Molecular typing using Pulsed-field gel electrophoresis (PFGE) confirmed that all isolates belonged to a single clonal type. The aim of this study was to investigate an alternative, more rapid typing method as well as exploring the genetic background of our local *P. aeruginosa* populations and characterize the carbapenem resistance mechanisms.

Variable Number Tandem Repeats (VNTR) based on the amplification of 9 alleles is an alternative more rapid typing technique to PFGE. Multi-Locus Sequencing Typing (MLST) will be used to contextualize the genetic background of these isolates by amplification and sequencing of 7 housekeeping genes and comparing the results obtained to previously published data. PCR investigations to determine mechanisms of resistance are being carried out to screen for the presence or absence of β -lactamase genes, *bla*_{SPM-1}, *bla*_{KPC}, *bla*_{IMP} and *bla*_{VIM}.

Preliminary results show good correlation between the PFGE and the VNTR profiles obtained for these strains and thus far none of these isolates harbor the *bla*_{SPM-1} gene. Further results are pending. This work therefore suggests that VNTR offers a more rapid epidemiological typing tool than PFGE in an outbreak situation.

P17-L

INVESTIGATING THE ROLE OF IL-4 RECEPTOR-ALPHA DEFICIENT DENDRITIC CELLS DURING CUTANEOUS LEISHMANIASIS

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Leishmania major-susceptible BALB/c mice develop an interleukin (IL)-4-driven T helper type 2 (T_{H2}) response with increased production of IL-4 and IL-13. IL-4 and IL-13 signal through a common IL-4 receptor-alpha chain (IL-4R α). Global abrogation of IL-4R α is not sufficient to confer complete resistance in BALB/c mice, however CD4⁺ T cell-specific IL-4R α -deficient mice are resistant to *L. major*. This indicates a protective role of IL-4R α signaling on non-CD4⁺ T cells during infection. Previous studies have shown that an initial burst of IL-4 following *L. major* infection instructs dendritic cells (DCs) to produce IL-12 leading to protective T_{H1} responses. The present study aims to confirm a possible role for IL-4 and/or IL-13 signaling in DC mediated T_{H1} differentiation. Dendritic cell-specific IL-4R α -deficient BALB/c mice (CD11c^{cre}IL-4R α ^{-/-lox}) were generated by gene targeting and site-specific recombination using the cre/loxP system under control of the CD11c promoter. Knock-out and control mice were injected sub-cutaneously with 2 x 10⁶ *L. major* LV39 (MRHO/SV/59/P) promastigote parasites into the left-hind footpad. Disease progression was monitored weekly by measuring footpad swelling. Mice were analyzed for parasite burden, cytokine and antibody production and lymphocyte populations at eight weeks post-infection. In the absence of IL-4R α -responsive DCs, IL-12 and hence protective T_{H1} responses were reduced. This was accompanied by upregulated T_{H2} cytokine and antibody responses. Furthermore, CD11c^{cre}IL-4R α ^{-/-lox} mice presented with increased footpad swelling and increased parasite burden in footpads and associated draining lymph-nodes. These results demonstrate that IL-4 and/or IL-13 signalling on DCs is critical for early protection against cutaneous leishmaniasis.

P18-L

FINITE ELEMENT MODELING AND STRAIN ANALYSIS OF HEALTHY AND INFARCTED LEFT VENTRICLES

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Currently, cardiovascular diseases account for approximately one third of all deaths in the world. More than 33% of these deaths are related to ischemic heart disease, involving a myocardial infarction (MI). Computational biomechanics can offer considerable benefits to the study of infarcted myocardium and novel therapies.

In this study, MRI images of a healthy heart were segmented and hexahedral meshes of the left ventricle (LV) at end-diastolic and end-systolic time points were created from the contours. Simulations of diastolic filling and systolic contraction were run using a transversely isotropic strain energy function and a model for active tension. An MI was applied to the anterior apical region of the LV encompassing approximately 15% of the LV wall. The infarct was modelled at acute and fibrotic phases of post-MI remodelling by altering the constitutive and active stress models. In the initial phase of this research, a comparison was done of LV diastolic inflation between healthy and two infarcted models.

The end-diastolic pressure volume relationship showed a greater end diastolic volume for the acute ischaemic case and a diminished EDV for the fibrotic infarct. The acute MI model resulted in a 33% increase in average fibre strain across the ischaemic region whereas the average fibre strain decreased by 78% in the fibrotic model. The fibrotic infarct also significantly reduced strain values in the border zone region.

The FE models provide meaningful strain data, corresponding well with data from literature, which can be used for further research into the mechanical effects of an anterior-apical infarct.

P19-L

Identification and characterisation of a binuclear Palladacycle Complex (AJ-5) as a novel anti-cancer drug in the treatment of human breast cancer

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Despite considerable research efforts, cancer continues to represent one of the most serious health problems worldwide. Many tumours develop resistance mechanisms to current therapeutic drugs and their severe side effects underscore the urgent need to develop improved antitumor agents. For many years, most tumours have been treated with platinum (Pt) drugs, the most common being cisplatin. Recently, many other metals have been tested for anti-cancer properties and palladium complexes are emerging to be especially interesting because although structurally similar to Pt(II), they have been shown to be more effective. Here we tested the anti-tumour properties of a novel binuclear palladacycle complex (AJ-5), containing palladium, in a human breast cancer cell line (MCF-7) and normal breast epithelial cells (MCF-12A). The results obtained show that AJ-5 is more effective in inhibiting the proliferation of MCF-7 cells with an IC₅₀ of 0.07 µM compared with 0.1 µM in MCF-12A cells. Flow cytometry analyses of MCF-7 cells show that AJ-5 induces a G1 arrest and apoptosis (sub-G1 peak). The pro-apoptotic effect of AJ-5 was confirmed by Annexin V-FITC/Propidium Iodide double-staining and an increase in the levels of PARP and PARP cleavage. We show, using an antibody to phosphorylated H2AX that the anti-tumour function of AJ-5 is achieved through inducing DNA damage which correlates with an increase in p53 and p21 levels. Taken together, these results suggest that AJ-5 may serve as an effective drug in the treatment of human breast cancer and reveal a potential mechanism for the anti-cancer effect of AJ-5.

P20-L

STUDIES INTO THE ANTI-METASTATIC ACTIVITY OF AJOENE AND RELATED ANALOGUES IN WHCO1 OESOPHAGEAL CANCER CELLS

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Oesophageal cancer is the seventh leading cause of cancer related death worldwide, with the main cause being tumour metastasis. Metastatic cancer seldom responds to clinically available cancer therapy. Natural compounds and their synthetically derived analogues are the current forerunners of drug development for improvement of cancer therapy. Ajoene is one of the major organosulfur compounds found in garlic (*Allium sativum* L) and is reported to inhibit the metastasis of B16/BL6 melanoma cells in C57BL/6 mice (Taylor et al., 2006). In the current study we aim to test whether ajoene and ajoene analogues will inhibit tumour metastasis, by investigating the *in vitro* effect of ajoene and ajoene analogues on WHCO1 cancer cell migration and invasion. Migrations is being tested with wound-closure assay, while invasion is tested with matrigel-invasion assay; these findings are confirmed with western blotting which will be used to determine the expression analysis of E-cadherins, which are transmembrane proteins that play an important role in cells to cell adhesion. The preliminary findings show that the Z-isomer of the ajoene analogues have a significant inhibitory effect on cell migration, with no significant effect being observed with the E- analogues. Thus our findings suggest that ajoene analogues inhibit migration and invasion of WHCO1 cancer cells and that this effect is exclusively related to the Z- stereochemistry of the compound. This implies that specific protein-drug interactions are likely required for the anti-metastatic activity of ajoene.

P21-L

THE SENSITIVITY AND SPECIFICITY OF SERUM PROLIDASE ACTIVITY AS A MARKER FOR LIVER FIBROSIS IN SUSPECTED LIVER DISEASE

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Background Liver dysfunction is common and often unrecognised. Liver biopsy is the gold standard in the assessment of liver fibrosis, but has disadvantages. Many viable options have been investigated. We assessed the diagnostic accuracy of serum prolidase enzyme activity (SPEA) in predicting the presence and degree of liver fibrosis, as compared with liver biopsy.

Methods We undertook a prospective case control study. 38 outpatients without apparent liver illness and 20 patients with liver pathology scheduled to undergo liver biopsy had their SPEA levels measured. We evaluated a SPEA assay for linearity, imprecision, stability of the measurand and the effect of haemolysis. Data from the control group were used to establish a reference interval for SPEA

Results The SPEA assay is linear and has reasonable imprecision. It is affected by haemolysis and freeze-thaw cycles should be avoided. A reference interval of 22 - 504 IU/l was obtained. Patients undergoing liver biopsy had higher SPEA levels (361 (268) IU/L [median (interquartile range)]) compared with controls (169 (160) (p < 0.001)). A SPEA cut-off value of 200 IU/l yielded a sensitivity of 89%, specificity of 59%, an odds ratio of 11.5, negative predictive value of 92% and a positive predictive value of 50%.

Conclusions Higher SPEA levels in patients undergoing liver biopsies compared with controls may reflect the presence of liver fibrosis. SPEA levels could not be used to stage the degree of fibrosis. The utility of SPEA as a biomarker of liver injury should be investigated further.

P22-L

CANCER TESTIS ANTIGENS IN MULTIPLE MYELOMA

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Cancer testis antigens (CTA's) are a group of highly immunogenic proteins that show restricted testis germ-line tissue expression, as well as aberrant expression in a large variety of cancers. The study of the expression profiles of these genes in disease not only offers insight into disease pathogenesis, but their expression has also been linked to other prognostic factors in some cancers, indicating more advanced disease. We proposed to investigate the simultaneous expression of a novel set of 11 CTA's in newly diagnosed, untreated Multiple Myeloma patients and try to establish a link to known markers of risk stratification such as DNA ploidy, tumor proliferation index, chromosome 13 deletions, chromosome 14 translocations, lytic lesions, renal damage or other cellular markers. The aim being to investigate the use of a simple CTA panel as a cost-effective prognostic tool in MM. In this first component of the study. RNA was extracted from BM and used for RT-PCR analysis of the following CTA genes simultaneously: BAGE, NY-ESO-1, SpanXb, PAGE, LAGE, PRAME, SSX-2, MAGE-A3, MAGE-C1, CPT-11 and SCP-1. MM patients at various stages of disease were analysed using this multi-parameter approach. Multiple CTA's were expressed in these patients, with at least 1 CTA being expressed in every patient. The most commonly expressed CTA's were Mage-A3 and MAGE-C1, indicating a putative role of these proteins in the pathogenesis of the disease. Several patients expressed >5 CTA's and we are now collating all the other prognostic data to try to establish a possible link to these key indicators.

P23-L

CONTRIBUTION OF THE DRUG TRANSPORTER *ABCB1* IN PREDICTING EFAVIRENZ PLASMA CONCENTRATION AND RESPONSE TO ANTIRETROVIRAL THERAPY IN SOUTH AFRICAN PATIENTS.

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Introduction: Efavirenz is used in first-line ARV therapy of HIV-infected patients, and is metabolised into three major metabolites: 8-hydroxy-efavirenz, 7-hydroxy- efavirenz and N-glucuronide- efavirenz. The *ABCB1* gene encodes P-glycoprotein, an ATP-dependent drug efflux pump, which is responsible for drug transport across extra-and intra-cellular membranes. The genetic variability in the *ABCB1* gene may contribute to variability in efavirenz plasma concentrations; resulting in variable suppression of the HIV-virus as well as exhibition of CNS side-effects in some patients. The aim is to evaluate the contribution of polymorphisms in the *ABCB1* gene in HIV-patients receiving HAART treatment for at least 6 months.

Methods: Patients were recruited from Themba Lethu Clinic in Johannesburg. SNaPshot was used to genotype known SNPs in approximately 300 healthy, black, South Africans as well as 300 South African patients receiving ARV therapy. Efavirenz drug plasma concentration levels were measured using LC-MS/MS.

Results: We report on the frequencies of SNPs in this gene in the South African population and their correlation, alone or in combination, with efavirenz plasma concentration, viral load, CD4-cell count and CNS adverse events. Preliminary results show no LD between the *ABCB1* alleles, which is in contrast with other populations. Data analysis is in progress and will be complete by the time of the meeting.

Conclusion: The contribution of all SNPs in the pharmogenetically relevant genes involved in efavirenz transport, compared to the general reporting of single SNPs, is likely to give a good prediction of the contribution of pharmacogenetics.

P24-L

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN PARTNERS INFLUENCES HUMAN PAPILLOMAVIRUS (HPV) TRANSMISSION AMONG HETEROSEXUALLY ACTIVE COUPLES.

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Background: Human papillomavirus (HPV) infection is associated with genital cancers in both women and men. Human immunodeficiency virus (HIV)-seropositive women progress to cervical cancer approximately ten years earlier than HIV-seronegative women.

Objectives: This study investigated factors influencing genital HPV transmission.

Methods: Participants were black, heterosexually active couples aged 19 to 65 years that were either both HIV-seropositive or HIV-seronegative or HIV-discordant. There were 486 couples at the baseline visit, 285 at the 6-month visit, 209 at the 12-month visit, 152 at the 18-month visit and 65 at the 24-month visit. Cervical and penile HPV types were determined by Roche Linear Array HPV genotyping assay. HPV transmission was defined as the acquisition of an HPV type that was detected in their partner at the previous visit.

Results: The female to male HPV transmission rate (transmission events per 1000 person-months) was 28.0, while the male to female HPV transmission rate was 11.7. Male to female transmission of LR-HPV types occurred at a higher rate (15.8) than male to female transmission of HR-HPV types (6.0). In contrast, female to male transmission of HR-HPV types and LR-types was similarly frequent (29.2 and 26.4 respectively). HIV-positive women were found to be at higher risk of HPV acquisition from their male partners compared to HIV-negative women (RR: 2.31, 95% CI: 1.08-4.92, P=0.03). HIV-positive men with a CD4 count <350/mL had a higher risk of HPV acquisition from their female partners compared to HIV-positive men with CD4 counts ≥350/mL (RR: 3.17, 95% CI: 1.05-9.55, P=0.04). In women, the risk of HPV infection from their male partners was significantly associated with young age at sexual debut (P=0.03).

Conclusion: HIV infection and a low CD4 count increases the rate of HPV acquisition from sexual partners, indicating a high risk of HPV associated cancers in both HIV-positive women and men.

P25-L

HIV-1 GAG AND NEF DIVERSITY IN CAMEROON: EVIDENCE OF A HIGH DEGREE OF RECOMBINANT FORMS.

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Unlike South Africa, which has a monoclade epidemic of HIV-1 subtype C, Cameroon, in west central Africa, has an extraordinary degree of HIV diversity. This diversity presents a major challenge to the development of an effective HIV vaccine. There is thus a continuing need to closely monitor the emergence of new HIV variants in the country.

In an effort to characterize HIV genetic diversity in Cameroon, 54 plasma samples from HIV-infected blood donors were analysed. Full length HIV gag and nef sequences were generated and phylogenetic analyses were performed, including the assessment of intragene recombination.

All gag and nef sequences clustered within HIV-1 group M. As has been described previously from Cameroon, circulating recombinant form CRF02_AG predominated the clade distribution, infecting 49% of the participants, followed by subtype G (11%), subtypes D and CRF37_cpx (4% each), and subtypes A1, F2, CRF01_AE and CRF36_cpx (2% each). Remarkably, 20% of the sequences were unique intergene recombinants, with the majority involving CRF02_AG in one gene, and the remainder involving CRF09_cpx, CRF11_cpx and CRF22_cpx. Four samples (8%) showed evidence of unique intragene recombination in gag, and one sample did not cluster with any known clade.

Although Cameroon has a low prevalence of HIV (5.6%), the HIV epidemic is highly diverse, and constantly evolving. Our results indicate that HIV-1 CRF02_AG recombinant predominates the HIV epidemic in Cameroon. However, with the circulation of several HIV-1 variants within the population, the emergence of second generation recombinants with unknown diagnostic and clinical consequences is a concern.

P26-L

POTENT ANTI-VECTOR RESPONSES TO A CANDIDATE MVA-VECTORED HIV VACCINE HAVE NO EFFECT ON IMMUNITY TO THE HIV ANTIGENS

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We characterized the humoral and cellular immune responses to MVA from a candidate MVA-vectored HIV-1 subtype C vaccine (SAAVI MVA-C) in non-human primates, and examined the effect these had on the response to the HIV immunogens after successive vaccinations.

Low titer neutralizing antibodies to MVA were detected using a MVA-GFP neutralization assay, consistent with low titer binding antibody responses to vaccinia virus envelope proteins A33 and B5R detected by ELISA. In contrast, high magnitude IFN- γ ELISPOT responses to MVA were induced in all (8/8) animals. These rose dramatically from a median of 497 SFU/10⁶ PBMC after the first MVA vaccination, to 4455 SFU/10⁶ PBMC after the third MVA, given over a year later. Responses to the HIV immunogens showed similar magnitudes and kinetics of boosting, reaching a median of 4459 SFU/10⁶ PBMC after the third MVA vaccination. MVA-specific CD8+ responses were predominately single function IFN- γ producing T-cells, while lower magnitude CD4+ T-cell responses largely produced TNF- α . Greater polyfunctionality to the HIV immunogens was evident, which may have been the result of earlier recombinant DNA prime vaccinations. Phenotypically there were no differences in memory responses to HIV and MVA, with antigen-specific CD4+ T-cells being of the central memory phenotype, and CD8+ T-cells showing a more balanced central and effector memory profile.

This anti-vector immunity did not dampen the generation or boosting of potent, polyfunctional CD4+ and CD8+ responses to the HIV inserts. These data support further development and testing of this candidate MVA vaccine.

P27-L

IMPLEMENTATION AND EVALUATION OF A BONY STRUCTURE SUPPRESSION SOFTWARE TOOL FOR CHEST X-RAYS

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Pulmonary tuberculosis (TB) is responsible for a large number of deaths in South Africa. The diagnosis is often performed using chest X-rays, and the reliability of interpretation depends on the experience of the radiologist. Computer aided diagnosis (CAD) may be used to increase the accuracy of diagnosis. Overlapping structures in chest X-rays hinder computer-aided analysis of the lung texture to detect abnormalities - this project aims at determining whether the performance of TB CAD tools may be improved by the suppression of bony structures in the chest region.

The project implements a rib suppression tool and analyses its effects on an existing texture-based classification algorithm developed by a previous UCT student. The dataset used here consists of 150 paediatric images.

Rib suppression is divided into two stages: segmentation and rib subtraction. In segmentation, the ribs overlapping the lungs are automatically identified in the image. The segmentation is performed using two separate methods: active shape models and pixel classification. This segmentation is then used to derive a model of the ribs which is subtracted from the original image. The rib models are built using existing methods that make use of principle component analysis.

The active shape model segmentation, when implemented as a semi-automated process, returns an overlap of 0.7, between manually and algorithmically segmented ribs. Textural-analysis on rib-suppressed images showed an improvement of 0.01 in the area under the ROC curve over textural analysis of the original images. Given the already high performance of the original CAD tool without rib suppression, the small improvement may not warrant its inclusion in the algorithm.