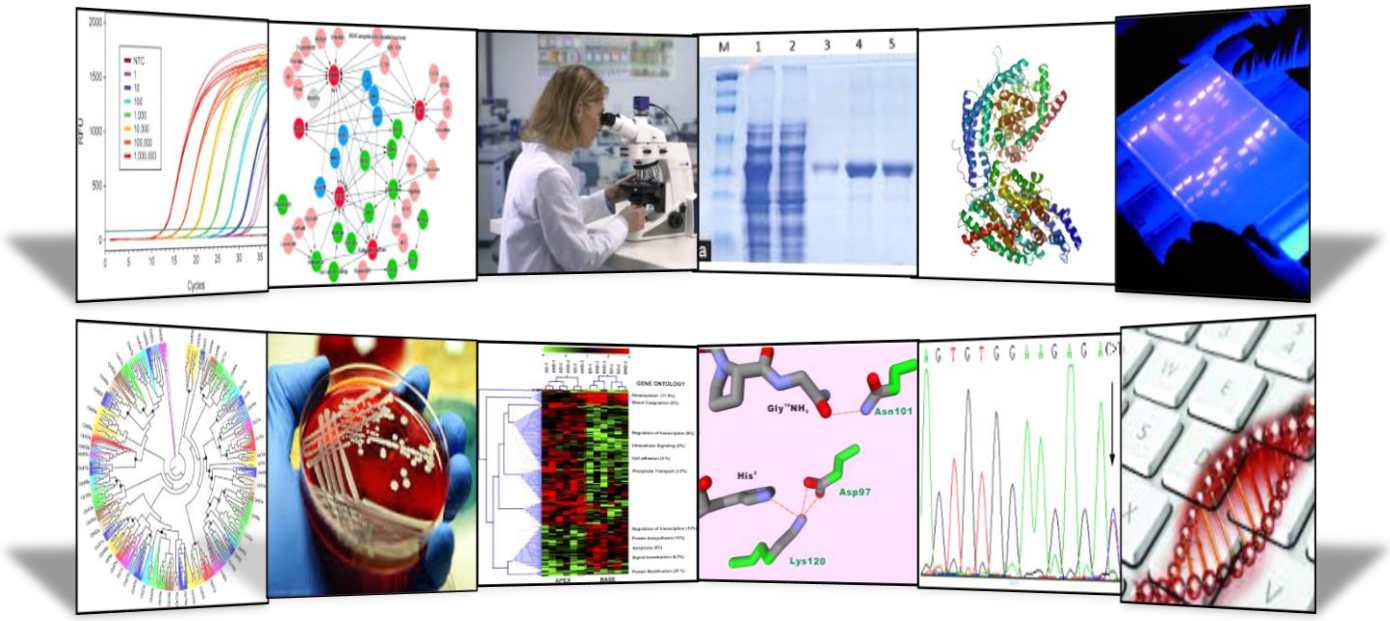
FACULTY OF HEALTH SCIENCES

DEPARTMENT OF INTEGRATIVE BIOMEDICAL SCIENCES



FIRST RESEARCH DAY

1 September 2016

Wolfson Lecture Theatre
Wolfson Pavilion, IDM

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Welcome from the Organizing Committee

We would like to welcome the students, post-doctoral fellows and staff of the Department of Integrative Biomedical Sciences and representatives from some of our sponsors to the first Research Day of the Department of Integrative Biomedical Sciences.

The purpose of the Departmental Research Day is several fold, firstly, to provide a sense of identity and belonging to the staff and students of the newly formed Department. Secondly to expose the students and staff of the Department to the diverse research conducted in the newly formed Department in order to create interest in the research of their fellow colleagues and hopefully to promote further collaboration between researchers of the Department. Thirdly, to provide the postgraduate students and postdoctoral fellows of the Department an opportunity to present their research work, since communication of research is an important element of the research activity. 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 Departmental staff and students for their cooperation and participation. We also thank the various companies and organizations listed on the previous page for their generous support. Their contributions allow us to offer prizes for the three best oral and poster presentations.

Special thanks to Jene Ward for her excellent administrative and logistic support she has provided to the Organizing Committee of the Research Day. Moreover, we thank Jene for her initiative and willingness in organizing the Research Day.

Lastly, we would like to thank 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
Prof Arieh Katz
Dr. Nelson Da Cruz Soares
Dr. Jeremy Woodward
Dr. Pauline van der Watt
Dr. Alecia Naidu

PROGRAM

09h30 Registration

10h00 Opening and welcome
Prof Arieh Katz

10h15 – 11h30 SESSION 1 Chair: Dr Nelson Da Cruz Soares

10h15 Lauren Arendse
Dual C-Domain Selective ACE and Neprilysin Inhibitors for the Treatment of Hypertension and Cardiovascular Disease. **(O1)**

10h30 Melissa Blumenthal
The impact of EPHA2 polymorphism on KSHV infectivity and/or KS prevalence among HIV/AIDS patients in South Africa. **(O2)**

10h45 Shaun Garnett
Generating a Proteomic Profile of Neurogenesis, Through a Quantitative Comparison of Neuroepithelial and Radial Glial Like Stem Cells. **(O3)**

11h00 Jon Ambler
Gengraph Toolkit, Simple Generation and Manipulation of Graph Genomes. **(O4)**

11h15 Shivan Chetty
The use of Humanized Cytolytic Fusion Proteins (hCFPs) for Targeted Immunotherapy. **(O5)**

11h30 – 12h15 COFFEE BREAK / POSTER SESSION (P1-P22)

12h15 – 13h15 SESSION 2 Chair: Dr Pauline van der Watt

12h15 **Aderonke Ajayi-Smith**
Novel Small Molecule Inhibitor of KPNB1 Induces Apoptosis and Interferes with Nuclear Import in Cancer Cells. **(O6)**

12h30 **Jeremy Burgess**
Three-Dimensional Reconstruction of Helical Assemblies formed by Mycothiol S-Conjugate Amidase of *Mycobacterium Smegmatis*. **(O7)**

12h45 **Afolake Arowolo**
An Investigation into the Subcellular Localization of FAM111B. **(O8)**

13h00 **Richard Burman**
Benzodiazephines and Epileptic Seizures. **(O9)**

13h15 – 14h15 LUNCH / POSTER SESSION (P1-P22)

14h15 – 15h30 SESSION 3 Chair: Dr Alecia Naidu

14h15 **Kehilwe Nakedi**
Identification of in vivo substrates of Mycobacterium bovis BCG Ser/Thr protein kinase PknG. **(O10)**

14h30 **Chacha Issarow**
Impact of the Vaccines on Population Tuberculosis Control Using Age-Structured Model. **(O11)**

14h45 **Clemens Hermann**
Comprehensive Characterisation of the Cell Wall Proteome of Mycobacterium Smegmatis using Gel-Free Mass Spectrometry. **(O12)**

15h00 **Wessel Moolman**
Homo- and Heterodimerisation of Angiotensin-Converting Enzyme. **(O13)**

15h15 **Eden Padayachee**
Photoimmunotheranostic Agents for Triple-Negative Breast Cancer (TNBC) Diagnosis and Therapy that can be activated on demand. **(O14)**

15h30 – 15h45 BREAK / POSTER SESSION (P1-P22)

15h45 – 16h45 SESSION 4 Chair: Dr Jeremy Woodward

15h45 **Zac MacDonald**
Targeted Proteomics: Time to Dust off the Hypothesis **(O15)**

16h00 **Tamara Stelma**
KPNB1-Mediated Nuclear Import is required for Inflammatory Cytokine Expression, Invasion and Survival of Cancer Cells. **(O16)**

16h15 **Andani Mulelu**
Factors Involved in the Oligomerisation of the Cyanide Dihydratase from *Bacillus Pumilus C1.* **(O17)**

16h30 **Janique Peyper**
The Neutrophil Proteome: Insights into TB-IRIS. **(O18)**

17h00 COCKTAIL FUNCTION AND PRIZE GIVING

POSTERS :

P1	**ARTEMISININ DERIVATIVE ACTIVATES AUTOPHAGY IN CANCER CELL LINES** **U.C. Andong** and D. T Hendricks.
P2	**THE INFLUENCE OF ANGIOTENSIN CONVERTING ENZYME MUTATIONS ON THE KINETICS AND DYNAMICS OF N-DOMAIN SELECTIVE INHIBITION** **L. Lubbe**, B.T. Sewell and E.D. Sturrock
P3	**INVESTIGATION OF Ac-SDKP LEVELS AND ACE AND POP ACTIVITIES IN THE PATHOPHYSIOLOGY OF CONSTRICTIVE TUBERCULOUS PERICARDITIS** **Ramasamy V**, Ntsekhe M and Sturrock ED
P4	**UNDERSTANDING THE ROLE OF MMPs IN TB CAVITATION** **Palesa Seele**, Nelson Soares, Jonathan Blackburn and Edward Sturrock
P5	**VIMENTIN MODULATES INFECTIOUS INTERNALISATION OF HPV16 PSEUDOVIRIONS** **Lisa Graham**, Melissa Blumenthal, Martina Bergant Marusic, Georgia Schafer, Arieh Katz
P6	**HIGH-THROUGHPUT DETERMINATION OF *MYCOBACTERIUM SMEGMATIS* PROTEIN COMPLEX STRUCTURES** **Angela M Kirykowicz** and Jeremy D Woodward
P7	**DYNAMICS OF PROTEOME AND PHOSPHOPROTEOME DURING RESPONSE OF *MYCOBACTERIUM SMEGMATIS* TO VITAMIN C** **Claudia Albeldas**, Naadir Ganief, Kehilwe C Nakedi, Shaun Garnett, Andrew Nel, Jonathan M Blackburn and Nelson C Soares
P8	**SUB-MIC LEVELS OF NITRIC OXIDE AND HYDROGEN PEROXIDE *IN MYCOBACTERIUM SMEGMATIS*: A PROTEOMIC TIME COURSE INVESTIGATION** **Naadir Ganief,** Jessica Sjoureman, Nelson Da Cruz Soares, and Jonathan M. Blackburn
P9	**TIME COURSE PROTEOMIC ANALYSIS ON EFFECT OF EXPOSURE TO SUB-LETHAL RIFAMPICIN CONCENTRATION ON MYCOBACTERIUM SMEGMATIS** **Alexander D. Giddey,** Elise de Kock, Kehilwe C. Nakedi, Shaun Garnett, Andrew JM Nel, Nelson C Soares and Jonathan M Blackburn
P10	**IDENTIFYING NOVEL CANCER ANTIGENS USING IMMUNOPROTEOMICS** **Muneerah Smith**, and Jonathan M. Blackburn
P11	**THE ORGANOID MODEL FOR PRECISION MEDICINE IN SOUTH AFRICA** **Maria Gagliardi**, Geoffrey Bartholomeusz, Stefan Barth, Musa Mhlanga.
P12	**INVESTIGATING THE POTENTIAL OF NUCLEAR TRANSPORT PROTEINS AS CANCER BIOMARKERS BY EXAMINING CANCER CELL SECRETOME AND EXOSOMES** **Andrew Wishart**, Pauline van der Watt and Virna D. Leaner
P13	**INVESTIGATING SMALL MOLECULES AGAINST NUCLEAR TRANSPORT AS ANTI-CANCER AGENTS** **Nonkululeko Mkwanazi,** and Virna. D. Leaner
P14	**INVESTIGATING THE SPECIFICITY OF THE SMALL MOLECULE INHIBITOR, INI-43, FOR KPNβ1** **Sarah Carden**, P. van der Watt, A. Verrico, P. Lavia and V. Leaner
P15	**INVESTIGATION INTO THE EFFECTS OF AN HIV-1 SUBTYPE C TRANSMISSION MOTIF ON ENVELOPE EXPRESSION, PROCESSING AND VIRAL ENTRY.** **Riley Traviss,** and Zenda Woodman
P16	**INVESTIGATION INTO THE ROLE OF THE HIV-1 SUBTYPE B SIGNAL PEPTIDE MOTIF ON SUBTYPE C TRANSMITTED FOUNDER ENVELOPE FUNCTION** **Bahiah Meyer,** and Zenda Woodman
P17	**PHOTOIMMUNOTHERANOSTIC AGENTS FOR TRIPLE-NEGATIVE BREAST CANCER (TNBC) DIAGNOSIS AND THERAPY THAT CAN BE ACTIVATED ON DEMAND.** **Eden Padayachee**, Mateen Wagiet**,** and Stefan Barth
P18	**SELECTIVE ELIMINATION OF CD64-POSITIVE DYSREGULATED M1-MACROPHAGES ALLOW TREATMENT OF CHRONIC INFLAMMATORY DISEASES.** **Olusiji Akinrinmade,** Eden Padayachee, Shivan Chetty, and Stefan Barth
P19	**SYNTHETIC PEPTIDE ADAPTORS IMPROVING TRANSLOCATION AND CYTOSOLIC ACCUMULATION OF HUMAN CFPs** **Sandra Jordaan**, Shivan Chetty, and Stefan Barth
P20	**SELECTIVE ELIMINATION OF KEY IMMUNE EFFECTOR CELLS IN ASTHMA PATHOLOGY BY RECOMBINANT IMMUNOTOXINS** **Adebukola Daramola**, S. Chetty, Frank. Kirstein, Frank. Brombacher, and S.Barth.
P21	**THE FUNCTIONAL ROLES OF HPV11E6 AND HPV18E6 IN INITIATING CELLULAR TRANSFORMATION** **Lamech M. Mwapagha** and M. Iqbal Parker
P22	**BisPMB INDUCED CYTOTOXICITY AGAINST OESOPHAGEAL CELLS LINES IS MEDIATED BY CHOP AND ERK1/2** **Vuyolwethu Siyo,** Catherine H. Kaschula and M. Iqbal Parker

ORAL PRESENTATION ABSTRACTS

O1

DUAL C-DOMAIN SELECTIVE ACE AND NEPRILYSIN INHIBITORS FOR THE TREATMENT OF HYPERTENSION AND CARDIOVASCULAR DISEASE

Lauren B. Arendse,[1,2] Kelly Chibale,[2]¶ and Edward D. Sturrock[1]
[1]Division of Chemical and Systems Biology, Department of Integrative Biomedical Sciences, Faculty of Health Sciences[1], and [2]Department of Chemistry, Faculty of Science, University of Cape Town.

The simultaneous modulation of several neurohumoral mediators in key interconnected blood pressure regulatory pathways has been an attractive approach to improve the efficacy of treatment for hypertension and cardiovascular disease. The dual ACE/neprilysin (NEP) inhibitor omapatrilat that simultaneously blocks the ACE-mediated formation of the vasoconstrictor angiotensin II (Ang II) and the NEP-mediated degradation of vasodilator natriuretic peptides showed superior efficacy in hypertension, but its progress in clinical trials was halted by bradykinin-mediated adverse effects.

ACE consists of two domains: the C-domain is primarily responsible for the formation of Ang II, while both the N- and C-domain breakdown bradykinin. In an ex vivo study in human blood plasma a combination of the selective NEP inhibitor sacubitrilat and our C-domain selective ACE inhibitor Lis-W resulted in a unique and favourable peptide-metabolism profile, with efficient suppression of Ang II production and metabolism of bradykinin. This peptide-metabolism profile is distinct from the profile observed for omapatrilat and provides support for the development of dual C-domain selective ACE/NEP inhibitors.

In this study we aim to design, synthesise and test a new series of omapatrilat derivatives that are selective for the C-domain. X-ray crystal structures of NEP and the ACE N- and C-domains in complex with various inhibitors together with extensive mutagenesis studies have shed light on interactions important for domain selectivity, providing a good foundation for structure-based drug design. *In vitro* fluorogenic inhibition assays using recombinant ACE and NEP enzymes produced in mammalian cell expression systems will enable the rapid screening of new inhibitors.

O2

THE IMPACT OF EPHA2 POLYMORPHISM ON KSHV INFECTIVITY AND/OR KS PREVALENCE AMONG HIV/AIDS PATIENTS IN SOUTH AFRICA

Melissa Blumenthal[1], Sumir Panji[2], Graeme Meintjes[3,4], Zainab Mohamed[5], Marc Mendelson[4], Denise Whitby[6], Arieh Katz[1] and Georgia Schäfer[1]
[1]Division of Medical Biochemistry and Structural Biology, [2]Division of Computational Biology, Department of Integrative Biomedical Sciences, [3]Institute for Infectious Disease and Molecular Medicine, [4]Department of Medicine; [5]Division of Radiation Oncology, Department of Radiation Medicine; Faculty of Health Sciences, University of Cape Town. [6]Viral Oncology Section, AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research.

Kaposi's Sarcoma (KS) is the most common AIDS-related malignancy worldwide and is of particular significance in sub-Saharan Africa where, due to the HIV epidemic, KS is associated with significant morbidity and mortality. The oncogenic Kaposi's Sarcoma-associated herpes virus (KSHV) is the etiological agent of KS. The uptake mechanism of KSHV into endothelial cells has recently been elucidated and highlights EPHA2 as a specific host receptor for the virus. Despite this key function, little is known about the consequences of polymorphism in the *EPHA2* gene. A cohort of South African HIV positive patients with or without KS was recruited. KSHV serostatus was assessed by ELISA to latent and lytic KSHV antigens. All patients in our cohort presenting with KS were shown to be KSHV seropositive by ELISA, and further, our data indicate a 44.76% prevalence of KSHV seropositivity among the HIV positive South African cohort. Genomic DNA was isolated from blood leukocytes and the EPHA2 coding region consisting of 17 exons was amplified by PCR and subsequently sequenced. The data have identified a number of previously reported single nucleotide variants in the EPHA2 gene as well as a number of novel variants that occur in the functionally important protein tyrosine kinase domain and are significantly overrepresented in patients with KS. Despite the high burden of disease in Southern Africa, research into KS and KSHV is extremely underrepresented, highlighting the importance of this, the first study to investigate polymorphism in EPHA2 in the South African population in relation to KSHV infectivity and/or KS prevalence.

O3

GENERATING A PROTEOMIC PROFILE OF NEUROGENESIS, THROUGH A QUANTITATIVE COMPARISON OF NEUROEPITHELIAL AND RADIAL GLIAL LIKE STEM CELLS

Shaun Garnett[1]; Jignesh Tailor[3]; Austin Smith[3]; Kathryn Lilley[3]; Susan Kidson[2]; Jonathan Blackburn[1]
[1]Division of Chemical and Systems Biology, Department of Integrative Biomedical Sciences, and
[2]Department of Human Biology Faculty of Health Sciences, University of Cape Town. [3]University of Cambridge, United Kingdom.

Traditionally neural stem cells were cultured as neurospheres, a heterogenous agglomeration of neural cells at various stages of differentiation. This heterogeneity prevented accurate quantitative analysis. In 2008 Sun et al produced the first non-immortalised human foetal neural stem (NS) cell line from nine week old human foetal cortex. These cells are cultured as monolayers, have a radial glia like appearance, self renew and form all three neural cell types, neurons, astrocytes and oligodendrocytes upon differentiation. More recently human foetal neuroepithelial like (NES) stem cells have been produced from five week old human foetal hindbrain, they resemble neuroepithelial cells, with characteristic rosettes, upon differentiation they form a pure population of neurons. These homogeneous monolayer cultures enable quantitative proteomic analysis, to increase our understanding of early brain development. Three NES and two NS cells lines were available for analysis. They proliferate with stimulation from FGF and EGF, removal of these growth factors results in spontaneous differentiation. Proliferating NES and NS cells were compared using SILAC labelling. NES cells are less differentiated, expressing SOX2 and LIN28, have active cell cycle processes, DNA elongation, histone modification and miRNA mediated gene silencing. Whereas NS cells are more developmentally defined, express multiple membrane proteins, have activated focal adhesion, thereby increasing their binding and interaction with their environment. NS metabolism is more oxidative, utilises lipid metabolism, the pentose phosphate pathway and produce creatine phosphate. This work represents a detailed in vitro characterisation of non immortalised human foetal neural stem cells, it describes the regulatory, metabolic and structural changes occurring within neural stem cells in early brain development. The information herein points towards de-differentiation as a means to produce more neurogenic neural stem cells in vitro thus aiding regenerative therapies, as well as provides a wealth of information for better understanding.

O4

GENGRAPH TOOLKIT, SIMPLE GENERATION AND MANIPULATION OF GRAPH GENOMES

Jon. M. Ambler, and Nicola Mulder
Division of Computational Biology, Department of Integrative Biomedical Sciences, Faculty of Health Science University of Cape Town.

The current standard for representing genomes is as a linear consensus sequence, limited in terms of its ability to represent variation. Currently, there is a movement towards the use of genome graphs, an integrated structure able to reflect the variation amongst its constituent genomes.
We present GenGraph, a simple toolkit for generating and utilising graph genomes. The tool creates a single graph containing multiple genomes from an alignment that can either be provided or created by the tool itself. The tool also provides some useful functions utilising the graph structure including extracting relevant information.
In a graph structure, all the relevant sequence information of the original genomes as well as their structural differences are preserved. This allows the extraction of the original genome sequences from the graph, the generation of gene homology matrices or pan-transcriptomes from provided annotations, as well as the generation of pan-genomes. The use pan-genomes and pan-transcriptomes is highly advantageous when mapping NGS data, maximising the percentage of mapped reads. Visualisation of graph genomes can be done by exportation to a widely used format, viewable with common tools like Cytoscape.

As such, GenGraph provides useful tools for the generation and utilisation of graph genomes, and demonstrates their utility in the context of genomic and transcriptomic based studies

O5

THE USE OF HUMANIZED CYTOLYTIC FUSION PROTEINS (hCFPs) FOR TARGETED IMMUNOTHERAPY

Shivan Chetty, Sandra Jordaan, Neelakshi Mungra, Adebukola Daramola, Mukit al Islam, and Stefan Barth
Division of Chemical and Systems Biology, Department of Integrative Biomedical Science, Faculty of Health Science University of Cape Town.

The ability to selectively target and kill cells of interest whilst minimizing the adverse effects to healthy cells is a central focus of current biotechnology based therapeutic strategies for a variety of diseases including cancer. Traditionally, cell specific therapeutic approaches have focused on combining antibodies for cell surface receptors to biologically active drugs or bacterial toxins. These immunotoxins and antibody-drug conjugates have been shown to be reasonably effective but are often associated with some level of toxicity due to the presence of a non-human effector component. As an improvement, the newer generation of fusion proteins called targeted human cytolytic fusion proteins (hCFPs) incorporate lysis inducing human enzymes. Examples of human enzymes that have been employed as cytolytic effectors to induce apoptosis in disease-specific target cells include granzyme B (initiates proteolytic cascades), MAP *tau* (modifies microtubule formation) and angiogenin (cleaves tRNA and blocks translation). However, some challenges do exist with using human enzymes as compared to bacterial toxins. Lacking the translocation sequences of bacterial toxins, internalized hCFPs are largely trapped in the endosome, and do not efficiently get into the cytosol. Our research focuses on the use of novel enzymes for disease specific hCFP targeted approaches as well as developing strategies to overcome the mentioned challenges. By tight collaboration with Paolo Carloni (Institute for Advanced Simulation and Institute of Neuroscience and Medicine Forschungszentrum Jülich, Germany) we are using supercomputing simulations of dynamic protein interactions to identify crucial amino acids.

O6

NOVEL SMALL MOLECULE INHIBITOR OF KPNB1 INDUCES APOPTOSIS AND INTERFERES WITH NUCLEAR IMPORT IN CANCER CELLS

A.F. Ajayi-Smith and V.D. Leaner
Division of Medical Biochemistry and Structural Biology, Department of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town.

Karyopherin beta 1 (KpnB1) is a major nuclear import receptor that mediates the import of cellular cargoes into the nucleus. Recently it has been shown that KpnB1 is highly expressed in several malignant cancers such as ovarian, cervical, neck, and lung cancers. We investigated the role of KpnB1 as a potential therapeutic target in oesophageal and cervical cancers using siRNA silencing. *In* vitro effects of KpnB1 siRNA silencing include inhibition of proliferation and cell death via apoptosis. The aim of this study is to identify novel small molecule inhibitors of KpnB1and determine their anti-cancer activity. *In silico* screening was used to identify novel small molecule inhibitors of KpnB1 and Compound 60 *(9-[1-methyl-3-piperidinyl)methoxy]-4-[6-methyl-2-pyridinyl)methyl]-7-(5-methyl-2-thienyl)-2,3,4,5-tetrahydro-1,4-benzoxazepine)* was futher investigated as it was able to inhibit the proliferation of oesophageal and cervical cancer cells and it interfered with nuclear import of KpnB1 and its cargoes such as NFAT and NFkB. Compound 60 induced a G2/M cell cycle arrest and resulted in cell death via apoptosis. Inhibition of KpnB1 using Compound 60 represents a potential therapeutic approach for cancers of different tissue origin by promoting growth inhibition and apoptosis.

O7

THREE-DIMENSIONAL RECONSTRUCTION OF HELICAL ASSEMBLIES FORMED BY MYCOTHIOL S-CONJUGATE AMIDASE OF *MYCOBACTERIUM SMEGMATIS*

J.G.Burgess, B.W.Weber, J.D. Woodward and B.T.Sewell
Division of Medical Biochemistry and Structural Biology, Department of Integrative Biomedical Sciences, University of Cape Town.

Mycothiol, a low molecular weight thiol produced in actinomycetes, is a major intracellular antioxidant, playing a role similar to that of glutathione in eukaryotes. Mycothiol forms conjugates with various electrophilic toxins and antibiotics, which are cleaved by Mycothiol S-conjugate amidase (Mca) before being excreted from the cell and the GlcN-Ins is recycled to the mycothiol synthetic pathway. Enzymes in this pathway have been considered as potential antibiotic targets but multiple issues have yet to be resolved.

Mycothiol S-conjugate amidase (Mca) of *Mycobacterium smegmatis* was produced recombinantly within *E.coli* BL21 and purified to homogeneity. We have discovered that it forms ordered helical assemblies that can be readily visualized by negative stain electron microscopy in the presence of low concentrations of ammonium sulfate.

We have produced a low resolution reconstruction of these helical assemblies in which the monomeric subunits can clearly be visualized. Helical segments were classified according to helical width, and used in separate reconstructions to refine the helical symmetry within each class. Subunits were modelled using a homology model of Mca, and likely interactions between subunits are discussed. The approach may be used with CryoEM techniques to obtain a high resolution structure of the Mca monomer.

O8

AN INVESTIGATION INTO THE SUBCELLULAR LOCALIZATION OF FAM 111B

Afolake Arowolo,[1] Dirk Lang [2], Bongani Mayosi [3] and Edward Sturrock [1]**.**
[1]Division of Chemical and Systems Biology, Department of Integrative and Biomedical Science, and [2]Division of Physiological Sciences, Department of ;Human Biology, [3]Hatter Institute for Cardiovascular Research in Africa, Department of Medicine, University of Cape Town.

Mutations in the FAM111B gene are associated hereditary fibrosing poikiloderma (HFP), a multisystemic fibrotic disease. An earlier study identified a total of three missense mutations (Y621D, R626G, and S628N) that span five families of different ethnic origin. However, the physiological role of FAM111B, wild-type and mutant forms, in the disease patients is yet to be understood. Through bioinformatics studies, we identified a putative nuclear localization signal (NLS) in the amino acid sequence of FAM 111B. Thus to provide insight into the possible role of FAM 111B in subcellular localization, we expressed EGFP-tagged FAM111B wild-type (FAM 111B WT), mutants derived from HFP patients, and truncated constructs (FAM111B- M255 and A375, up- and downstream of putative NLS sequence respectively) in HEK293 cells, and cellular expression was analysed by Confocal microscopy. FAM111B WT showed variable subcellular distribution as it localises to discrete, densely labelled, extranuclear structures of variable size, as well as diffusely marked nuclear or cytoplasmic areas. The expression patterns of truncated/patient's mutant, however, differ consistently in the subcellular distribution among groups, as well as from FAM111B WT. These data suggest a possible association of FAM111B with centrosomes or centrosome-associated structures. Further studies to confirm this interaction as well as the identity of the smaller densely labelled structures with suitable organelle markers are underway.

O9

BENZODIAZEPHINES & EPILEPTIC SEIZURES

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Status epilepticus (SE) describes a state of persistent seizures that are unrelenting, and is considered as a medical emergency. The preferred first-line management of this condition includes the use of benzodiazepines (BZPs). These agents assert their effect by acting as allosteric modulators of the γ-butyric acid (GABA) A receptor ($GABA_AR$) which forms the principle mechanism of fast synaptic inhibition in the brain. While the BZPs are often effective in terminating SE, it has been observed in both local and international cohorts that in a select number of, more commonly paediatric, patients they fail.

Previous data from animal models has demonstrated that during SE, instead of being inhibitory, GABA can in fact become excitatory. It is believed that this may be due to a disruption in the chloride gradient that has been shown to occur during periods of neuronal hyperexcitability. Furthermore, we postulate that this short-term activity- dependent disruption in the transmembrane chloride gradient and subsequent change in GABA function may have an effect on BZP states during prolonged seizure-like events (SLEs).

The experimental aim of this research is to explore the effect of benzodiazepines on normal GABA function and on SLEs using different *in vitro* animal models. Furthermore, we aim to demonstrate the transient changes in transmembrane chloride and GABA function that occur during these states and investigate the effect BZP may have on this phenomenon.

O10

IDENTIFICATION OF *IN VIVO* SUBSTRATES OF *MYCOBACTERIUM BOVIS* BCG SER/THR PROTEIN KINASE PknG.

Kehilwe C Nakedi, Bridget Calder, Mousumi Barnerjee, Alexander Giddey , Andrew Nel, Shaun Garnett, Jonathan M Blackburn and Nelson C Soares
Division of Chemical and Systems Biology, Department of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town.

Ser/Thr kinases play a critical role in bacterial physiology and pathogenesis. The challenge now lies in linking kinases to their physiological substrates, thereby elucidating their exact functions. The aim of this work is to associate protein phosphorylation in mycobacteria with important subsequent macro cellular events by identifying the physiological substrates of PknG in *Mycobacterium bovis* BCG. The study compared the phosphoproteome dynamics during the batch growth of *M. bovis* BGC versus the respective PknG knock-out mutant (ΔPknG-BCG) strains. We employed TiO_2 phosphopetide enrichment techniques combined with label free quantitative phosphoproteomics work flow on LC/MS/MS. The comprehensive analysis of label free data identified 603 phosphopeptides on 307 phosphoproteins with high confidence. 55 phosphopeptides in were differentially phosphorylated, of these, 23 phosphopeptides were phosphorylated in *M. bovis* BCG wild type only and were further validated through targeted mass spectrometry assays (PRM's). The docking studies based on a published crystal structure of PknG in complex with GarA revealed that the majority of identified p-sites presented docking scores close to that seen in previously described PknG substrates, GarA Thr 20 and ribosomal protein L13 Thr11. 6/23 phosphoproteins had even higher docking scores than GarA, suggesting that the proteins identified here are truly PknG substrates. Based on protein functional analysis of the PknG substrates identified, the study confirms that PknG play an important regulation role in mycobacterial metabolism, but also indicated its association with the machinery of protein translation and folding.

O11

IMPACT OF THE VACCINES ON POPULATION TUBERCULOSIS CONTROL USING AGE-STRUCTURED MODEL

Chacha M. Issarow[1], Robin Wood[2] and Nicola Mulder[1].
[1]Computational Biology Division, Department of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town, Cape Town,
[2]Desmond Tutu HIV Research Centre, Faculty of Health Sciences, University of Cape Town.

Despite the fact that tuberculosis (TB) vaccine has been implemented globally, TB remains by sure a public health concern that causes morbidity and mortality in the community. In this study, we design an age-structured mathematical model that incorporates vaccination for susceptible individuals (young age groups [0, 5) and [5, 15) years) to explore the impact of the vaccines on TB control. Furthermore, we examine the conditions under which a TB vaccine can be useful in a high transmission and high re-infection community. In order to explore the impact of the vaccines, the study was divided into two parts and simulated the model: (i) without vaccination (ii) with vaccination, and we observed TB notification in different age groups. In the first part (without vaccination), high active TB was detected for the age groups [0 - 5), [15 - 25), [45 - 55) and [55 - 65) years with notification rates 562, 484, 505 and 484 per 100, 000, respectively. In the second part, when the vaccine introduced in the population, active TB decreased for the age groups [0 - 5), [5 - 15), [15 – 25) years by 43.92%, 60.20% and 71.61%, respectively. The lowest active TB was detected in the age group [5 - 15) years in both cases. However, in both cases (with and without vaccination), active TB remains high for the age groups [25, 35) to >= 75 years, suggesting that these age groups are at excessively high risk of developing TB disease. The study shows that active disease progression depends on age and average duration of the waning of the vaccine effect.

O12

COMPREHENSIVE CHARACTERISATION OF THE CELL WALL PROTEOME OF MYCOBACTERIUM SMEGMATIS USING GEL-FREE MASS SPECTROMETRY

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Infection by *Mycobacterium tuberculosis* remains one of the biggest global health threats. Understanding the cell wall of mycobacteria represents one of the most important tasks to better design drugs that target mycobacteria or to identify B or T cell antigens that are suitable for vaccination strategies, but remains a challenge due to its complexity in composition. In this study, we successfully developed a gel-free approach to specifically look at cell wall proteins in *Mycobacterium smegmatis*. The cell wall was subjected to differential centrifugation, differential detergent solubilisation and phase separation to yield the genuine cell wall proteome. The protein extracts were digested by filter-assisted sample preparation for LC-MS/MS analysis on a Q Excative mass spectrometer and identified proteins subjected to a strict bioinformatics pipeline. This resulted in the unprecedented identification of 95 lipoproteins, 481 membrane proteins containing at least one transmembrane helix and 141 secreted proteins. Gene ontology enrichment showed that these proteins are involved in cell wall biogenesis, virulence, phosphorylation and transport. This list might represent the most comprehensive list of cell wall proteins in *Mycobacterium smegmatis* thus far. Our approach might provide the basis for further quantitative proteomic studies into the role of the cell wall proteome of mycobacteria in virulence or during drug exposure.

O13

HOMO- AND HETERODIMERISATION OF ANGIOTENSIN-CONVERTING ENZYME

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Clinical treatment of hypertension involves the use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs). ACE is a zinc metallopeptidase that plays a central role in the renin-angiotensin system (RAS), which is primarily responsible for blood pressure homeostasis. ACE cleaves angiotensin-I to form angiotensin-II, which acts as a vasoconstrictor *via* the angiotensin-II receptor type 1, a G-protein coupled receptor (GPCR). However, this mechanism of action is increasingly being scrutinised, since the action of ACE inhibitors cannot be fully attributed to the inhibition of ACE catalytic activity. Accumulating evidence indicates that ACE has non-catalytic functions, including acting as a receptor and inducing a signalling response. These activities are thought to facilitate the mechanism of action of ACE inhibitors due to specific protein-protein interactions that allow for functional regulation and cross-talk between the different components of the RAS. We studied the mechanism and functional relevance of ACE homo- and heterodimerisation, as well as the effects that ACE inhibitors and ARBs have on these interactions, since it could explain some of the therapeutic benefits of these compounds that cannot be directly linked to the inhibition of catalytic activity. Single- and two-domain ACE constructs, tagged with Cerulean and Venus fluorescent proteins, were used to investigate the structural requirements for dimerisation, effect of inhibitor treatment and GPCR related interactions. FRET was utilised to demonstrate testis ACE and somatic ACE homodimerisation in CHO-K1 and HEK293T cells and the specificity of these interactions were confirmed by co-transfection with unlabelled ACE constructs.

O14

SNAP-TAG TECHNOLOGY MEDIATES SITE SPECIFIC CONJUGATION OF ANTIBODY FRAGMENTS WITH SYNTHETIC SMALL MOLECULES FOR DIAGNOSIS AND THERAPY OF CANCER.

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One of the greatest challenges in chemical conjugation of small chemical agents to antibodies is efficient site-specific attachment. The SNAP-tag is providing a unique solution which might result in a versatile alternative to heterogeneous antibody drug conjugates. The SNAP-tag is an engineered human DNA repair enzyme, alkylguanine-DNA alkyltransferase, which when reacting with benzylguanine modified substrates is able to transfer a benzyl group to its active site and release free guanine in a nucleophilic reaction. By fusion of the SNAP-tag with a recombinant antibody fragment, a diagnostic and imaging tool becomes available that can be covalently conjugated to any benzyl-guanine modified substrate including e.g. fluorescent dyes or small molecule toxins in a 1:1 stoichiometry. SNAP-tag based imaging probes are not limited to diagnosis alone, but are also representing a promising tool for the generation of novel and improved immunotheranostics when using near infrared fluorophores with photosensitizer activity. These SNAP-tag based antibody fusion proteins can be easily used to generate homogeneous recombinant antibody drug conjugates, antigen specific nanoparticles delivering anti-cancer drug, as well as photoimmunotherapeutic agents. To introduce this novel technology to South Africa, we are currently starting collaborations with Professor Roger Hunter (Dept of Organic Chemistry, UCT), Professor Lester Davids (Dept. of Human Biology, UCT), Professor Nonhlanhla Khumalo (Dept of Dermatology, UCT), Professor Jill Farrant (Dept. of Molecular & Cell Biology, UCT), and Professor Musa Mhlanga (Dept. IBMS, UCT).

O15

TARGETED PROTEOMICS: TIME TO DUST OFF THE HYPOTHESIS

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With the growing availability of mass spectrometry (MS) platforms for proteomics research the need for verification and validation of the resultant discovery datasets has increased significantly. Traditionally, verification and validation of MS outputs (e.g. in the form of putative biomarkers) has been done using some form of antibody based approach (such as ELISA, Western Blotting, or bead-based immunoassays). However, these techniques can be costly, are not always amenable to multiplexing, and can present insurmountable technical difficulties. An alternative is the application of MS instrumentation to perform quantification using multiple reaction-monitoring (MRM) on triple quadrupole instrumentation or parallel reaction monitoring (PRM) on high resolution instruments. This targeted proteomics approach enables the quantification of proteins and peptides with high reproducibility, accuracy and sensitivity. In addition to validation of discovery work, MS based targeted proteomics is at the forefront of a return to a more hypothesis driven approach in biological and biomedical proteomics research. Scientists, in particular systems biologists, are applying targeted proteomics strategies to test hypotheses generated from insights gained from a range of omics studies. While discovery proteomics has provided a valuable map to “scientific gold”, targeted proteomics provides the means to extract and refine biologically significant knowledge from the ever expanding mass of information. As more and more ‘omic’ data is produced, the trend towards more focussed, targeted proteomics is likely to continue, and with it the need to access relevant hardware and expertise.

O16

KPNB1-MEDIATED NUCLEAR IMPORT IS REQUIRED FOR INFLAMMATORY CYTOKINE EXPRESSION, INVASION AND SURVIVAL OF CANCER CELLS

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Karyopherin β1 (KPNB1) is a nuclear import protein involved in the transport of transcription factors and other proteins containing a nuclear localisation sequence. Elevated KPNB1 expression has been reported in cancer and transformed cells. Transcription factors such as NFκB and AP-1 contain a NLS and have been suggested to require KPNB1 for nuclear import. These transcription factors initiate the expression of multiple cytokines and factors associated with inflammation and cancer cell biology. An inflammatory microenvironment, a hallmark of cancer, contributes to factors such as sustained proliferation, invasion and neoangiogenesis. Our study aimed to investigate the effect of inhibiting nuclear import via KPNB1 as a potential anti-cancer and anti-inflammatory approach using siRNA and the novel small molecule, inhibitor of nuclear import- 43 (INI-43). We found that inhibiting KPNB1 lead to reduced migration and invasion of cervical cancer cells while extended inhibition caused decreased proliferation and apoptosis. KPNB1 is essential for the translocation of NFκB into the nucleus as inhibition of nuclear import resulted in its cytoplasmic retention and decreased transcriptional activity of both NFkB and AP-1. DNA-binding studies confirmed a reduced binding-ability or presence of NFkB in the nuclear extract of KPNB1-inhibited cells. Consequently reduced IL-6, IL-1β, TNF-α and cJun target gene expression was observed. INI-43 was able to inhibit tumour growth in an ectopic xenograft mouse model while the effect on inflammation and cancer cell biology was further explored using immunohistochemistry. Our study provides evidence that inhibiting KPNB1 has anti-inflammatory and anti-cancer effects and shows promise as a chemotherapeutic target.

O17

FACTORS INVOLVED IN THE OLIGOMERISATION OF THE CYANIDE DIHYDRATASE FROM *BACILLUS PUMILUS C1*

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The cyanide dihydratase enzyme from *Bacillus pumilus* ($CynD_{pum}$) is a member of the nitrilase superfamily and is known to specifically catalyse the conversion of cyanide into formic acid and ammonia. This enzyme is a good candidate for bioremediation of cyanide waste but the high alkaline pH of the cyanide waste water poses a problem in that it inactivates the Wild type enzyme and therefore improvement of stability is required in order to synthesize an effective enzyme. Over the pH range of 6-8 the enzyme exists as short 18-subunit spirals which associate to form long, more stable helical fibres at pH 5.4. The reason for this pH dependent transition is not fully understood but it is hypothesized to be due to changes in the charge of histidine residues. The aim of this project is to obtain a high resolution structure of $CynD_{pum}$, relate this to its function, and investigate the role of the histidines in oligomerisation with aid of the structure. Using Cryo-electron microscopy techniques a three dimensional reconstruction structure of purified $CynD_{pum}$ was obtained at a resolution of 7Å. Using this high resolution structure we were able to identify amino acid residues involved in oligomerisation as well as the role of the histidines, with aid from additional mutagenesis studies.

O18

THE NEUTROPHIL PROTEOME: INSIGHTS INTO TB-IRIS

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Approximately 80% of global HIV-associated TB occurs in sub-Saharan Africa. High TB incidence, HIV sero-prevalence, and improved ART access increase TB-IRIS incidence during co-treatment. TB-IRIS is associated with significant morbidity and mortality, but immunopathogenesis is incompletely understood. To advance the understanding of TB-IRIS immunopathogenesis, we are comparing neutrophil proteomes of TB-IRIS cases with those of appropriate controls. ART-naïve patients being treated for HIV-associated TB (and at risk for TB-IRIS) have undergone phlebotomy prior to, and at the typical time of TB-IRIS onset after, ART initiation. One-hundred-twenty-four snap-frozen isolated neutrophils are undergoing mass spectrometry-based differential proteomic profiling in order to generate biological hypotheses for orthogonal validation. This sample size provides >95% power to detect a two-fold change in protein expression with 99% probability, and will facilitate sub-group analyses (e.g. effects of prednisone exposure). Neutrophil isolation yield (cells per sample) and purity exceed $19x10^6$ and 90% respectively. Protein extraction yield exceeds 60µg per 10^6 cells. Liquid chromatography-coupled mass spectrometry, with and without pre-fractionation, identifies over 2000 and 1500 neutrophil proteins respectively. Preliminary data (N=8) suggest that neutrophils from TB-IRIS cases (relative to controls, at the time of onset) have disturbed energy metabolism and elevated intracellular Ca^{2+} levels, and are primed to undergo necrosis. A better understanding of the immunological pathways underlying TB-IRIS pathogenesis will facilitate improved prediction, prevention, diagnosis, and management.

POSTER PRESENTATION ABSTRACTS

P1

ARTEMISININ DERIVATIVE ACTIVATES AUTOPHAGY IN CANCER CELL LINES

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Earlier evidence in our laboratory showed that a novel artemisinin derivative, EXP57EA, killed cancer cell lines (based on two cell lines) through a process that involved autophagy. In this study, we determined the effect of the novel compound on a wider panel of cancer cell lines (five cell lines). We firstly determined the mode of cell death and then investigated the signalling pathways that triggered the mode of cell death. The results showed that EXP57EA did not induced apoptosis based on the PARP cleavage assay. However, vacuoles were seen in treated cells compared to untreated cells which were suggestive of autophagy. Autophagy was then monitored by analysing the expression level of two autophagy markers Beclin1 and LC3-II by western blot. It was observed that EXP57EA treatment caused changes in the expression level of Beclin1 and LC3-II. Furthermore, EXP57EA activated autophagy based on increase flux in the presence of the lysosomal inhibitors Ammonium Chloride (NH_4Cl) and Chloroquine in WHCO1 esophageal cancer cell line. Real-Time PCR analysis showed that treatment with EXP57EA resulted in increased expression of CHOP, a marker of ER stress. This study showed that EXP57EA can be used as a potential anticancer targeted drug.

P2

THE INFLUENCE OF ANGIOTENSIN CONVERTING ENZYME MUTATIONS ON THE KINETICS AND DYNAMICS OF N-DOMAIN SELECTIVE INHIBITION

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Angiotensin-1-converting enzyme (ACE) is a zinc metalloprotease consisting of two domains with distinct inhibitor binding affinities despite their 90% active site identity. While the C-domain controls blood pressure, the N-domain is selective for cleaving the antifibrotic *N*-acetyl-Ser–Asp–Lys–Pro peptide. Inhibitors, such as 33RE, that selectively bind the N-domain thus show potential for selectively treating fibrosis. The aim of this study was to elucidate the molecular mechanism responsible for this selectivity.

ACE inhibition by 33RE was characterized using a continuous kinetic assay with fluorogenic substrate. The N-domain displayed nanomolar (K_i =11.21±0.74nM) and the C-domain micromolar (K_i =11278±410nM) inhibition. Residues predicted from the N-domain-33RE co-crystal structure to contribute to this 1000-fold selectivity were mutated to their C-domain counterparts. Although S_2 subsite mutation with hydrogen bond disruption drastically decreased affinity (K_i =2794±156nM), selectivity was only abolished upon additional substitution of all S_2' residues (K_i =10009±157nM). Interestingly, these residues do not directly interact with 33RE. All six mutants were therefore subjected to molecular dynamics simulations in the presence and absence of 33RE. Subsequent trajectory analyses highlighted the S_2' subsite's importance in hinging and closure of the ligand-bound complex through formation of a favourable subdomain interface. In line with this, protein thermal stabilization upon 33RE titration was significantly lower for mutant than wild-type, suggesting a more open ligand-bound conformation.
This study provides a molecular basis for the inter-subsite synergism responsible for 33RE's 1000-fold N-selectivity and aids the future design of inhibitors for fibrosis treatment.

P3

INVESTIGATION OF Ac-SDKP LEVELS AND ACE AND POP ACTIVITIES IN THE PATHOPHYSIOLOGY OF CONSTRICTIVE TUBERCULOUS PERICARDITIS

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Tuberculous pericarditis leads to constrictive pericarditis, a life threatening form of fibrosis, in 60% of patients despite therapy. There are currently no effective predictors and prophylactics for the condition. N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), an antifibrotic peptide, made by prolyl oligopeptidase (POP) and degraded by angiotensin converting enzyme (ACE) as well as the pro-fibrotic Galectin-3 have been detected in pericardial fluid. We investigate the role of Ac-SDKP, ACE and Galectin-3 in the pathophysiology of TB pericarditis and the *in vitro* effects of Ac-SDKP analogues and ACE inhibitors on the prevention of fibrosis. Ac-SDKP and Galectin-3 levels in TB pericardial fluid were compared to controls using specific ELISAs whilst the enzymatic activities of ACE and POP were measured by fluorigenic assays. The effects of Ac-SDKP analogues (Ac-SDψKP, Ac-SDK, Ac-DKP, SDK and DKP), and ACE inhibitors, on the prevention of fibrosis in a lung fibroblast cell line, were measured by quantitative Western Blotting for TGF-β levels and a hydroxyproline assay. HPLC analysis was used for assessing the cleavage of Ac-SDKP peptides by ACE. Levels of Ac-SDKP in participants with TB pericarditis were significantly lower ($p=0.04$) than in controls without pericardial disease and a mild upregulation in Galectin-3 levels and ACE activities could be observed in TB pericardial fluid. *In vitro* studies revealed that Ac-SDKP alone and in combination with the ACE inhibitors reversed the effect of AngII on TGF-β and collagen levels in the fibroblasts.

P4

UNDERSTANDING THE ROLE OF MMPs IN TB CAVITATION

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Cavitation is a key factor in the transmission of tuberculosis (TB) and an independent factor in therapeutic relapse rate. Matrix metalloproteinases (MMPs) are zinc-dependent enzymes whose main function is to degrade the extracellular matrix. Their activity is endogenously inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs). Upregulation of MMP-1 and MMP-9 has been documented in TB patients, while TIMP expression was either unaffected or downregulated. Apart from evidence of collagen breakdown products in the sputum of TB patients, animals which can develop cavities have well-conserved MMP-1 orthologs suggesting a key facilitator role of MMP-1 in cavitation. Time course analysis of THP-1 macrophages infected with *Mycobaterium smegmatis* (*M. smeg*), both lysate and secretome is in progress. In the preliminary data, MMP-1, MMP-8 and MMP-13 were present only in their pro-form in the infected and uninfected secretome, with slightly increased activity in the infected lysate. This was when collagen was used as a substrate. Early infection caused an increase in gelatin hydrolysis by the secreted MMP-2, while MMP-9 activity remained the same or was decreased. Preliminary data from proteomic studies showed downregulation of MMP-9 which is important in granuloma formation. These samples were prepared by FASP and analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Further optimisation of the mass spectrometry preparatory methods was done using uninfected secretome and lysate. In this case, the FASP (filter aided sample preparation) and in-solution methods were compared. So far, MMP-1, MMP-2, MMP-9, MMP-14, TIMP-1 and TIMP-2 were identified by both methods.

P5

VIMENTIN MODULATES INFECTIOUS INTERNALISATION OF HPV16 PSEUDOVIRIONS

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Human papillomavirus (HPV) is the most common viral infection of the reproductive tract, with virtually all cases of cervical cancer being attributable to infection by oncogenic HPVs. However, the exact mechanism and receptors used by HPV to infect epithelial cells is still highly debated. The current model of HPV entry suggests that the virus initially attaches to heparan sulfate proteoglycans (HSPGs) at the cell surface, followed by conformational changes and cleavage by a furin convertase which eventually leads to the transfer of the virus to an as yet unidentified high-affinity receptor. In line with this model we established an *in vitro* infection system using the HSPG-deficient cell line pgsD677 together with HPV16 pseudovirions (HPV16-PsVs) encapsidating a luciferase-encoding plasmid. While pgsD677 cells were practically non-permissive for untreated HPV16-PsVs, furin cleavage of the particles led to a substantial increase in luciferase activity. Biochemical pull-down assays followed by mass spectrometry analysis showed that furin pre-cleaved (FPC) - HPV16-PsVs specifically interacted with surface-expressed vimentin on pgsD677 cells. We further demonstrated that FPC-HPV16-PsVs co-localised with surface expressed vimentin in HaCaT and HeLa cells, while binding of incoming viral particles to soluble recombinant vimentin protein before infection led to a substantial decrease in viral uptake. Interestingly, decreasing cell surface vimentin by siRNA knockdown in HeLa cells significantly increased HPV16-PsVs infectious internalisation, while overexpression of vimentin had the opposite effect. With the identification of vimentin as an HPV binding molecule exhibiting sequestering functions, these results contribute to our understanding of the initial steps of HPV entry and may lay the basis for the design of novel antiviral drugs preventing HPV internalisation into epithelial cells.

P6

HIGH-THROUGHPUT DETERMINATION OF *MYCOBACTERIUM SMEGMATIS* PROTEIN COMPLEX STRUCTURES

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The elucidation of the structure of higher-order assemblies, though crucial to protein function, has not been a focus of Tuberculosis research. We are investigating a high-throughput search of the *Mycobacterium smegmatis* proteome to solve structures for novel soluble protein complexes (> 400 kDa); the strategy involves partial protein purification through unbiased chromatographic fractionation in conjunction with 3D reconstruction via electron microscopy (EM). Image classification of single particles in the heterogeneous sample then acts as a further “purification”. Importantly, use of semi-autonomous software, such as Appion, has made it possible to reconstruct many complexes in parallel. Use of quantitative mass spectrometry (MS) allows for the identification of protein subunits of these complexes, even if they are present at very low concentrations. Assignment of protein subunit identities to the solved complexes is achieved through various approaches: 1) by matching the mass of the subunit with the mass of the complex, 2) by correlating the frequency of the subunit peptides with the abundance of single particles on the EM grid, or 3) by using structural homologues to fit the 3D density. Pilot experiments yielded two protein complexes whose structures were solved by single-particle negative stain EM: glutamine synthetase I (700 kDa) and a novel 3.6 MDa complex. Blue and clear-native PAGE systems, in combination with grid blotting or electro-elution, is currently being tested as a more efficient high-throughput means of fractionation. In addition, high-resolution reconstructions using cryo-EM of key complexes will be completed to gain further insight into structure-function relationships.

P7

DYNAMICS OF PROTEOME AND PHOSPHOPROTEOME DURING RESPONSE OF *MYCOBACTERIUM SMEGMATIS* TO VITAMIN C

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Vitamin C has been previously found to affect the Mycobacterium in multiple ways, including increasingly susceptibility to antimicrobial drugs, inducing dormancy, and having a bactericidal effect. However, the regulatory events mediating Vitamin C related adaptations remain largely elusive. Ser/Thr/Tyr protein phosphorylation plays an important regulatory role in Mycobacterium which determines environmental adaptation. This study utilises *Mycobacterium smegmatis* and TiO_2 phosphopeptide enrichment combined with quantitative label free mass spectrometry-based methods to elucidate the mycobacterial response to sub-lethal concentration of vitamin C treatment. After an initial validation of peptide spectra, 164 non-redundant phosphopeptides in 104 proteins were retained with high confidence. The data analysis revealed that 28 peptides were differentially phosphorylated with Vitamin C treatment, including 22 phosphopeptides/phosphoproteins that were found exclusively phosphorylated within treated samples. Interestingly, 18/22 phosphoproteins were transmembrane proteins. In all cases the transmembrane proteins were phosphorylated in their cytoplasmic region, suggesting that in some cases it is likely vitamin C triggers typical signal transduction events in which the protein periplasmic domain perceives environmental signals and the cytoplasmic domain is then phosphorylated. Finally, the diverse nature of phosphorylated proteins involved in signalling (PknG, Thr 72); transport (SecE, Thr 21; SecA, Thr 910); metabolism (GarA, Thr 40) and carbohydrate biosynthesis (rhamnose reductase Thr 151) indicates the extent of such regulatory phosphorylations events and their possible implications at various levels of cellular responses. This study confirms the potential of vitamin C as convenient mean to study aspects of mycobacterial dormancy, including those regulates at post translational level.

P8

SUB-MIC LEVELS OF NITRIC OXIDE AND HYDROGEN PEROXIDE *IN MYCOBACTERIUM SMEGMATIS*: A PROTEOMIC TIME COURSE INVESTIGATION

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The macrophage mediated oxidative burst is known to be an important mechanism for the host defence against mycobacterial infection. Two products of the oxidative burst are hydrogen peroxide and nitric-oxide. Here we have investigated the effects of sub-MIC hydrogen peroxide and nitric-oxide over time via liquid chromatography couple tandem mass spectrometry based proteomics. We have, with high confidence identified and quantified 3333 proteins and shown 176 and 259 proteins are significantly dysregulated for hydrogen peroxide and nitric oxide treatment respectively. Bioinformatics analyses revealed the treatments shared some responses, notably both treatments induced the dosR response, altered signalling via two component systems as well as dysregulated lipid metabolism. The treatments also elicited unique responses in amino acid metabolism their metabolism with hydrogen peroxide treatment resulting in dysregulation of the leucine, isoleucine and valine metabolism, whereas nitric-oxide treatment resulted in dysregulation of glutamate metabolism.

P9

TIME COURSE PROTEOMIC ANALYSIS ON EFFECT OF EXPOSURE TO SUB-LETHAL RIFAMPICIN CONCENTRATION ON MYCOBACTERIUM SMEGMATIS

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Mycobacterium tuberculosis is the causative agent of tuberculosis disease which kills almost 1.5 million people annually. In the last 40 years only one new antitubercular drug has been approved whilst resistance to current treatments, including the frontline drug Rifampicin, is rapidly spreading. Poor penetration of M. tuberculosis - containing granulomas by drugs such as Rifampicin and poor adherence by patients to treatment schedules mean M. tuberculosis infections may regularly face sub-lethal concentrations of drug within the host.

We used the model organism M. smegmatis, and mass spectrometry-based proteomics to show here, for the first time, the effect of sub-lethal concentrations of Rifampicin on the mycobacterial proteome in a time course experiment. We examined the proteome of M. smegmatis treated with 10 µg/mL Rifampicin at 30, 255 and 300 min post-treatment corresponding to early response, onset of bacteriostasis and early recovery.

The data shows that after dampening an initial SOS reponse, the bacteria suppress the DevR (DosR) regulon and upregulate both their transcriptional and translational machinery. A co-ordinated dysregulation in haeme and mycobactin synthesis was also observed alongside strong dysregulation of redox related proteins - possibly to prevent and manage ROS production. Finally, a gradual upregulation of the M. smegmatis specific Rifampin ADP-ribosyl transferase was observed and it is likely this, together with the upregulation of transcriptional and translational machinery, that allows recovery of normal growth.

P10

IDENTIFYING NOVEL CANCER ANTIGENS USING IMMUNOPROTEOMICS

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The Blackburn lab has developed a highly sensitive and selective native cancer-antigen (CT100+) microarray, used to detect autoantibodies in the blood samples of cancer patients. Although technically advanced, the microarray has biological limitations as we observed a random antibody response in 10-20% of patients. Our aim is therefore to use immunoproteomics to identify TSAs which can reproducibly be used for cancer diagnosis and prognosis.
We have an archive of 67 cancer, and paired normal, tissues with corresponding autologous blood plasma samples, from patients with colorectal cancer (CRC) (GSH, South Africa) for identifying novel TSAs. We have developed an immuno-pulldown assay, in which Protein A/G magnetic microbeads are used to capture antibodies from the patient blood plasma, which are used to capture antigens from patient tissue lysates.
Using mass spectrometry, we were able to identify 360 proteins unique to the cancer samples, of which matched to the Tantigen database. Furthermore, 3 of the 8 proteins identified have been reported to induce antibody responses in cancer patients.
In conclusion, we have developed an immuno-pulldown assay that captures and identifies proteins which are unique to cancer tissues, of which several have shown to induce a B-cell response in cancer patients. Although we are able to identify protein candidates of interest, we observe several limitations with the assay, for which we are currently troubleshooting. Once the method is optimised, the newly identified TSAs will be fabricated on to the CT100+ microarray, and validated with patient sera to confirm the presence of cancer-specific antibody response.

P11

THE ORGANOID MODEL FOR PRECISION MEDICINE IN SOUTH AFRICA

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Over the past 250 years cancer has taken the pedestal of interest in the research community. Despite numerous accomplishments cancer continues to be the leading cause of death worldwide. Precision medicine is now emerging as the state of the art approach to cancer therapies. It tailors therapeutic regimes to specific genomic variants identified in patient biopsies. South Africans are gifted with the greatest degree of genetic diversity in the world. Unfortunately this means genetic variants that result in positive or negative prognosis remain largely unidentified. Characterizing novel genomic variants will not only direct us to appropriate therapeutic regimes but will also provide the world with as of yet undescribed tumor genotypes. To undertake precision medicine we are developing the state-of-the-art organoid model in translational research. Organoids, a perfect intermediary between in vivo tumors and monolayer cultures, reliably recapitulate tumor dynamics with the formation of pH, osmolarity, nutrient and oxygen gradients as growth progresses. This model will allow us to characterize tumor mutations, epigenetic alterations, and identify biomarkers. In addition, drug sensitivity screening to predict ideal drugs or drug combinations for specific tumor genotypes will now be possible.

P12

INVESTIGATING THE POTENTIAL OF NUCLEAR TRANSPORT PROTEINS AS CANCER BIOMARKERS BY EXAMINING CANCER CELL SECRETOME AND EXOSOMES

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Previous work in our laboratory has shown that elevated levels of the nuclear transport protein Karyopherin Beta 1 (KPNβ1) associates with cancer cell survival and proliferation. KPNβ1 is the primary importer protein that binds cargo proteins in the cytoplasm and transports them into the nucleus. A predicted 57% of proteins use this protein in nuclear transport. Upregulated proteins in the cell have the potential to be secreted. This study aims to investigate whether KPNβ1 and other transport proteins are secreted in cancer and therefore have therapeutic and diagnostic potential. Western blot analysis showed upregulation of KPNβ1 and exportin 1 (XPO1) in the cell component of cervical and oesophageal cancers compared to normal and transformed cells, additionally that these proteins are secreted and that the level of secretion correlate with cellular levels. KPNβ1 and XPO1 were detected in exosomes, which are secreted vesicles. Exosomes offer added protection to their cargo proteins, making these proteins better candidate biomarkers. Mass spectrometric analysis confirmed these results and identified additional transport proteins in the secretome and exosomes of oesophageal cancer cells. These include KPNβ1, importin (IPO) 5, 7, 9, KPNα2, α4, XPO1, 2, transportin (TNPO) 1 and RAN in the secretome and KPNβ1, IPO7, KPNα2, XPO2, TNPO1 and RAN in the exosomes. Future mass spectrometric analysis will be performed to compare levels of these transport proteins between normal, transformed and cancer cell lines. Our findings from this study suggest that nuclear transport proteins have potential as secreted biomarkers for cancer.

P13

INVESTIGATING SMALL MOLECULES AGAINST NUCLEAR TRANSPORT AS ANTI-CANCER AGENTS

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The Karyopherin superfamily is a group of soluble transport proteins which are involved in nuclear-cytoplasmic trafficking. Studies have also shown their involvement in nuclear pore assembly, nuclear membrane assembly and DNA replication. Since all these cell regulatory functions are critical for normal cell function, dysregulation of Karyopherin proteins may have an impact on cancer cells. In this study, we investigated small molecules for their ability to inhibit Karyopherin B1 (KpnB1), a key nuclear transport protein, in cervical and oesophageal cancer cell lines. A novel small molecule inhibitor Compound 53 (C53) significantly inhibited the proliferation of oesophageal and cervical cancer cell lines. Cervical and Oesophageal cancer cell lines appeared more sensitive to C53 compared to non-cancer cells. The reduction of proliferation in cancer cells associated with apoptotic cell death which was confirmed by Annexin V staining. Our results demonstrate that C53 induces cancer cell death and shows promise for further investigation as an anti-cancer molecule.

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INVESTIGATING THE SPECIFICITY OF THE SMALL MOLECULE INHIBITOR, INI-43, FOR KPNβ1

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Karyopherin β1 (Kpnβ1) is the major nuclear import protein involved in the shuttling of cargo proteins and mRNAs into the cell nucleus. Recent studies have found that Kpnβ1 levels are significantly upregulated in cancer cells, and that targeted inhibition of Kpnβ1 with siRNA results in reduced proliferation and the induction of apoptosis, suggesting that Kpnβ1 is important for cancer cell survival. Inhibitor of Nuclear Import-43 (INI-43) is a small molecule inhibitor identified in our laboratory as having the potential to interfere with the nuclear import function of Kpnβ1. It has been shown to inhibit the nuclear localization of both Kpnβ1 and known Kpnβ1 cargoes such as NFκB and NFAT, as well as inhibit the proliferation of cancer cells of different tissue origins. This study aimed to investigate the specificity of INI-43 for Kpnβ1 by performing experiments to determine whether overexpression of Kpnβ1 was able to rescue cells from the effects of INI-43 treatment. Results show that Kpnβ1 overexpression rescues cell viability, as well as the inhibitory effects that INI-43 has on the nuclear import of NFκB/p65. Overexpression of Kpnβ1 also rescues cells from an INI-43 induced G2/M cell cycle block. In addition, treatment of cells with INI-43 enhances Kpnβ1 degradation. These results suggest that the small molecule inhibitor INI-43 is acting, at least in part, by targeting Kpnβ1.

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INVESTIGATION INTO THE EFFECTS OF AN HIV-1 SUBTYPE C TRANSMISSION MOTIF ON ENVELOPE EXPRESSION, PROCESSING AND VIRAL ENTRY.

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In the majority of Human Immunodeficiency Virus Type-1 (HIV-1) infections only a single variant, termed the transmitted/founder (T/F) virus, is transmitted from the donor. Thus, it has been proposed that selection of this T/F variant at the mucosal barrier occurs and facilitates HIV-1 infection. Envelope (Env), a glycoprotein found on the surface of the virus, has been identified as carrying possible transmission motifs that allow for selection of the T/F as initiates the first interaction between the virus and its target cell receptors. One of the robust transmission motifs identified in subtype B T/F viruses is the absence of a potential N-linked glycosylation site (PNGS) at position 415-417 (HXB2 numbering), which is present during chronic infection viruses and has been associated with immune escape. This study used a subtype C T/F Env clone to investigate the effects of the loss of the PNG at 415-417 on expression, N-glycosylation and viral entry using site-directed mutagenesis (SDM). Western blot results show an increase in expression of mutant Env compared to wild-type (WT). No difference in the levels of high mannose type N-glycans present in the N-glycosylation profile of the mutant Env was observed compared to wild-type, suggesting that this mutation may not lead to increased binding affinity to DC Specific ICAM-3 Grabbing non-integrin (DC-SIGN), the surface lectin present on dendritic cells (DCs). However, the entry efficiency of this PNG mutant was enhanced. These results indicate that the loss of the PNG at position 415-417 during early/acute infection may influence viral fitness and infectivity.

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INVESTIGATION INTO THE ROLE OF THE HIV-1 SUBTYPE B SIGNAL PEPTIDE MOTIF ON SUBTYPE C TRANSMITTED FOUNDER ENVELOPE FUNCTION

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It has been reported that in the majority of transmission cases, that human immunodeficiency virus type-1 (HIV-1) infections are as a result of transmission of only a single variant, [termed the transmitted/founder (T/F)]. This suggests that specific variants could be selected during transmission although the mechanism remains unknown. A study analysed sequences of T/Fs from subtype B variants circulating in the USA and identified a potential transmission motif in the signal peptide of Envelope (Env), the glycoprotein responsible for binding to host receptors (CD4, CCR5 and DC-SIGN) and mediating entry into target cells. A follow-up study suggested that this single amino acid in the signal peptide [histidine at position 12 (H12)] increased expression and secretion of Env resulting in enhanced HIV-1 infectivity. A potential mechanism linking increased Env expression to enhanced viral infectivity is based on the finding of another study that increased Env expression was associated with an alteration in the N-glycosylation profile of Env, such that predominantly high mannose glycoforms are added. The changes in N-glycosylation on Env could alter the binding between Env and host receptors which could then facilitate transmission by increasing the ability of the virus to infect target cells in the genital tract. In this study, a glutamine in the signal peptide of Env of a subtype C T/F variant was mutated by site-directed mutagenesis to either a histidine or alanine at position 12. Western blots showed that after transfection of HEK 293T cells, there was no significant increase in expression of the Env mutants compared to that of wild type. When Env was treated with endo-glycosidases to compare the level of high mannose glycoforms between the mutants and wild-type, there was no difference in N-glycosylation. Furthermore, pseudovirus that carried H12 within the signal peptide did not have enhanced entry efficiency compared to wild type. These results suggests that unlike the histidine at position 12 of subtype B signal peptides, this position does not influence subtype C expression, N-glycosylation or Env entry into target cells. Taken together, these results suggest that transmission motifs in subtype C might differ from those identified in subtype B, suggesting that vaccines that protect against one subtype might not protect against another.

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PHOTOIMMUNOTHERANOSTIC AGENTS FOR TRIPLE-NEGATIVE BREAST CANCER (TNBC) DIAGNOSIS AND THERAPY THAT CAN BE ACTIVATED ON DEMAND.

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A promising new shift in cancer treatment involves the creation of diagnostic and therapeutic compounds called theranostics. Having developed a new antibody format by fusing disease-specific ligands to the SNAP tag, a modified DNA repair enzyme allowing to covalently attach any Benzylguanine modified substrate in a 1:1 stoichiometry in an autocatalytic reaction. In a recent study, antibodies against three different TNBC-associated cell surface antigens i.e. the epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM) and the chondroitin sulfate proteoglycan 4 (CSPG4) were conjugated to a IRDye® 700DX phthalocyanine using SNAP-tag technology. After confirmation of selective binding to different types of triple-negative breast cancer (TNBC) cell lines, the different combination products were found to produce high levels of cytotoxicity in antigen-postive cells, producing IC_{50} values in the nanomolar range (62–165 nM). The robust safety profile of these reagents was further demonstrated by the lack of toxicity caused by the free IR700 dye, even after irradiation.
These results provide a framework for using SNAP tag based antibody fusion proteins to generate e.g. photoimmunotheranostic reagents for selective targeting of TNBC-associated cell surface antigens. With these promising results generated, we will further develop this technology to confirm biological activity in preclinical animal models to confirm diagnostic and therapeutic value.

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SELECTIVE ELIMINATION OF CD64-POSITIVE DYSREGULATED M1-MACROPHAGES ALLOW TREATMENT OF CHRONIC INFLAMMATORY DISEASES.

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Up to today, no disease-specific cell surface antigens have been described that would allow the development of cell specific targeted therapies for most chronic inflammatory diseases. In consequence, current treatment aims to interfere with pro-inflammatory cytokines (e.g. TNF-α) rather than intervening at the primary disease initiation cycle. Recently we show that selective killing of CD64-positive M1 macrophages is resolving cutaneous chronic inflammation in mice and patient biopsies.
In short summary, bacterial Pseudomonas exotoxin A (ETA) or mutants of human angiogenin were genetically fused to H22, a humanized single-chain antibody fragment (scFv) specific for Fc gamma receptor I (CD64). Corresponding H22(scFv)-ETA' and H22(scFv)-Ang constructs selectively killed hCD64tg murine M1 macrophages as well as human pro-inflammatory macrophages (M1Φ). The selectivity for M1 was found linked to the reduced endosomal protease activity in M1Φ as demonstrated by inhibition of endosomal proteases. A transgenic mouse model of chronic cutaneous inflammation was subsequently used to confirm selective elimination of M1Φ *in vivo* after local intradermal injection: $hCD64^+$ CD14 M1Φ were significantly reduced, whereas $CD206^+$ $CD301^+$ M2Φ remained unaffected. Also, a clear M1 specific effect was found *ex vivo*, after treatment of skin biopsies from atopic dermatitis and type II diabetes patients with chronically-inflamed skin.
These data are highlighting that targeting M1Φ through CD64 can be instrumental for the development of novel intervention strategies. We will use the increasing know how to generate tailor made immunodiagnostic and -therapeutic constructs for the treatment of different macrophage-driven diseases.

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SYNTHETIC PEPTIDE ADAPTORS IMPROVING TRANSLOCATION AND CYTOSOLIC ACCUMULATION OF HUMAN CFPs

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Immunotherapy provides a targeted approach to cancer therapy with reduced systemic toxicity, leading to better quality of life for treatment patients. Recent generations of immunotoxins (ITs) involve the use of single chain antibody fragments (scFv), which are internalized more readily due to smaller size, as targeting factor and the use of apoptosis-inducing human enzymes as cytolytic effector, reducing the risk of a neutralizing immunogenic response in human patients. Once bound to the cell-surface “receptor” via the scFv “ligand”, hCFPs are internalized by endocytosis and primarily trapped in endosomes. Lacking the translocation sequences of bacterial toxins, they are unable to cross the endosomal membrane and are subject to endosomal degradation.
The obstacles of a) evading degradation and b) translocating into the cytosol are major limiting factors affecting the cytotoxic efficacy of hCFPs. Several methods have been described for promoting endosomal escape to enhance cytosolic accumulation of cytolytic effector in disease-specific target cells. The use of adaptors consisting of a translocating peptide flanked by pH sensitive cleavable peptides has been shown not only to mediate translocation of hCFPs into the cell, but also to retain them in the target compartments and prevent leakage. We aim to target triple negative breast cancer (TNBC) cells by means of αCSPG4(scFv), conjugated via novel synthetic peptide adaptors to a series of mutant angiogenin variants. These variants will be generated in tight collaboration with Paolo Carloni, using simulations of dynamic protein interactions to identify crucial amino acids and will be designed to enhance angiogenin ribonucleolytic activity.

P20

SELECTIVE ELIMINATION OF KEY IMMUNE EFFECTOR CELLS IN ASTHMA PATHOLOGY BY RECOMBINANT IMMUNOTOXINS

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According to a recent report by the Global Initiative for Asthma (GINA), South Africa has the world’s fourth highest asthma death rate among 5 to 35 years old patients. This may be as a result of non-responsiveness to available treatment and hence highlighting the need for novel treatment strategies. With this project we are aiming to selectively eliminate allergen reactive cells contributing to the pathology of asthma by recombinant immunotoxins. Allergen-reactive cells are defined by the presence of allergen-specific immunoglobulins, such as B-cell receptors (BCRs) or Fc-receptor–bound antibodies. Recombinant immunotoxins containing a protein toxin fused to an allergen or allergen fragment are representing a new approach to selectively eliminate these cells.
For proof of concept, major timothy grass pollen allergen, Phl p 5b was genetically fused to a truncated version of *Pseudosomonas* exotoxin A (ETA’). Specificity and toxicity was confirmed in a reliable *in vitro* B cell model. Allergen-specific and nonspecific B cells were challenged with P5-ETA’, but only the Phl p 5b–reactive B cells were selectively eliminated.
This approach represents an initial step toward a novel therapeutic strategy in the treatment of asthma. Our laboratory in tight collaboration with the group of Frank Brombacher aims to develop novel recombinant fusion proteins for selective elimination of e.g. T-helper 2 cells and allergen specific B cells involved in asthma pathology.

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THE FUNCTIONAL ROLES OF HPV11E6 AND HPV18E6 IN INITIATING CELLULAR TRANSFORMATION

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Although high risk human papillomaviruses (HPVs) are the major risk factors for cervical cancer it has been associated with several other cancers such as head and neck, oral and oesophageal cancers. Since the low risk HPV11 DNA has been shown to be integrated in oesophageal tumours, this study compared the effects of low risk HPV11E6 and high-risk HPV18E6 on cellular gene expression. The E6 genes from HPV11 and HPV18 were cloned into an adenoviral vector and expressed in HaCaT cells. HPV11E6 had no effect on either p21 or p53 gene expression, while HPV18E6 resulted in a marked reduction in these proteins. However, both HPV11E6 and HPV18E6 enabled growth of colonies in soft agar, although the level of colony formation was higher in HPV18E6 infected cells. DNA microarray analysis identified significantly deregulated genes involved in the cellular transformation signalling pathways. These findings suggest that HPV11E6 and HPV18E6 are important in initiating cellular transformation via deregulation of signalling pathways such as PI3K/AKT and pathways that are directly involved in DNA damage repair, cell survival and cell proliferation. Overall we show that low risk HPV11E6 may have similar functions as high-risk HPV18E6, but at a much reduced effect level.

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BisPMB INDUCED CYTOTOXICITY AGAINST OESOPHAGEAL CELLS LINES IS MEDIATED BY CHOP AND ERK1/2

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BisPMB (E,Z)-1,8-(Bis-p-methoxyphenyl)-2,3,7-trithiaocta-4-ene 7-oxide) is a synthetic analogue of the garlic compound Ajoene which is 12 times more active at inhibiting the growth of oesophageal squamous cell carcinoma WHCO1 cells and displays selectivity for cancer cells over normal cells. BisPMB is therefore attractive as a potential cancer therapeutic. Through the use of gene expression microarray analysis, this study observed that bisPMB primarily targets the ER stress pathway in WHCO1 cells. Consistent with this finding, bisPMB was found to induce an increase in the total levels of ubiquitinated proteins and in the expression of ER stress and UPR genes ATF4, Grp78 and CHOP. A decrease in ATF6 90 kDa protein and transient XBP-1 splicing was also observed in WHCO1 cells following bisPMB treatment. siRNA mediated knock-down of CHOP abolished the anti-proliferative effect of bisPMB in WHCO1 cells. In addition to the ER stress pathway, bisPMB altered the expression of MAPK proteins p38, JNK and ERK in WHCO1 cells. The inhibition of JNK and p38 MAPK by chemical inhibitors, SP600125 and SB 203580 respectively, had no effect on bisPMB antiproliferative activity against WHCO1 cells. On the other hand, inhibition of ERK1/2 MAPK by U0126 enhanced the anti-proliferative effect of bisPMB in WHCO1 cells. These results support the hypothesis that ER stress and ERK1/2 MAPK signalling pathways are involved in bisPMB induced cytotoxicity in oesophageal cancer cells.