

HEALTH SCIENCES



Yumna Albertus

University of Cape Town

Mentor: Prof W Derman

Broad research area: Electromyography and cardiac rehabilitation

Specific research field: Neuromuscular adaptation during rehabilitation programs in patients with chronic cardiac disease

Purpose of study:

Patients with chronic cardiovascular disease are shown to have secondary changes in the skeletal muscle, which can contribute to decreased functional capacity, and exercise intolerance. Previous studies show that supervised exercise rehabilitation programmes are beneficial in the recovery phase of these patients but the exact effect of exercise on skeletal muscle activity is unknown. The purpose of my research is to determine the effect of a medically supervised 12-week exercise program on the EMG activity of the lower legs during the 6-minute walk test, maximal and submaximal contractions. Preliminary data in the 6-minute walk test, patients showed a 19.7% (± 104 m) improvement in distance covered post-rehabilitation ($p=0.00$), whereas the controls did not improve. The EMG activity was not significantly different between the controls and patients nor did EMG change during the 6-minute walk test post-rehabilitation for all muscles. In the MFCV testing patients had significantly lower muscle strength (183 ± 18 Nm) compared to controls (230 ± 38.8 Nm) ($p=0.026$) and their strength significantly improved after 12 weeks of rehabilitation (203 ± 32 Nm) ($p=0.038$). MFCV has shown no difference between controls (3.4 ± 0.5 ms) and patients (3.3 ± 0.8 ms) in the pre-rehabilitation trial during MVCs and dynamic contractions, more testing needs to be completed for the control group. The findings of this study suggest that exercise tolerance improves following rehabilitation, yet EMG remains unchanged. Improvements in functional capacity could therefore be related to factors that relate to muscle efficiency or biomechanical advantage achieved through exercise training. More patients and controls are needed to complete this study and finalise the findings.

Contact: Yumna.albertus@uct.ac.za

Amaal Abrahams

University of Cape Town

Mentor: S Prince

Broad research area: Health sciences

Specific research field: Identifying the molecular basis for treatment resistance in a subset of myasthenia gravis patients of African ancestry

Purpose of study:

Myasthenia gravis (MG) is an auto-immune disease in which complement activation leads to damage at the muscle endplate. Decay accelerating factor (DAF), a complement regulating protein, is important in protecting the endplate against complement-mediated damage. We have previously demonstrated that MG subjects of African or recent African ancestry with the c.-198C>G SNP in the regulatory region of the DAF gene, have an increased risk of developing treatment-resistant extraocular muscle (EOM) dysfunction. Importantly, we showed that the c.-198C>G SNP affects the up-regulation of DAF mRNA and protein levels in response to an immune stress stimulus such as lipopolysaccharide (LPS). We thus hypothesised that the c.-198C>G SNP/mutant may result in lower DAF expression in our MG patients on standard immunosuppressive therapy. To test this, we have investigated the effect(s) of these treatments on DAF expression in MG patients with the c.-198C>G SNP. The results suggest that in MG patients with the c.-198C>G SNP and an additional immune stimulus such as LPS, leads to inadequate DAF upregulation which may be exacerbated when treated with prednisone, the first-line immunosuppressive therapy. Long-term the proposed study will have a direct impact in treating at-risk patients.

Contact: Amaal.abrahams@uct.ac.za

Marique Aucamp

North-West University

Mentor: W Liebenberg

Broad research area: Drug research and development

Specific research field: Solubility differences between different roxithromycin monohydrates

Purpose of study:

To discuss the mechanism and the ability of roxithromycin to form monohydrates from different organic solvents, and to evaluate the physico-chemical properties thereof. Several recrystallisations of roxithromycin were prepared by dissolving roxithromycin in organic

solutions. Acetonitrile, ethyl acetate, dichloromethane and DMSO were used to investigate the recrystallisation products. The forms obtained were analysed and characterised through SXRD, XRPD, DSC, TGA, TM, SEM, Karl Fischer and solubility measurements. It seems as though the monohydrated form is a stable form for roxithromycin. Therefore, it could be concluded that any trace of water within the recrystallisation solvent or the sample as a matter of fact would induce the formation of the more stable monohydrated form. However, although several monohydrated forms were reported in this study it seems as though these forms differ in terms of morphology and other physico-chemical properties. It could be concluded that roxithromycin exhibits the ability to form different monohydrated forms from organic solvents that significantly differ from each other in terms of physico-chemical characteristics

Contact: marique.aucamp@nwu.ac.za

Jacqueline Bracher University of Cape Town

Mentor: Associate Prof D Hendricks

Broad research area: Cancer biochemistry

Specific research field: Insulin receptor isoforms as therapeutic targets in oesophageal cancer

Purpose of study:

Compelling evidence in the literature and results obtained in our laboratory indicate that epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor type I (IGF-I) play critical roles in providing proliferative and survival signals to oesophageal cancer cells. These receptors provide attractive targets in the chemotherapeutic treatment of oesophageal cancer, and numerous inhibitors to EGFR and IGFIR are currently available to test this hypothesis. However, in many cancers insulin receptor isoform A (IR-A) is a splice variant of the classic insulin receptor, and isoform B (IR-B) is overexpressed and heterodimerises with IGFIR, providing survival and proliferative signals. The expression profile of insulin receptor isoforms A and B were examined in our panel of cultured oesophageal cancer cells, using a qRT-PCR-based assay that exploits the relative structures of each isoform. In all cell lines examined, both isoform A and B were present, albeit to differing levels. Since isoform A and B differ through alternative splicing of exon 11, a 36 bp region of the gene, siRNA silencing of the specific insulin receptor isoforms is not possible. However, by knocking-down the insulin receptor in cell lines, which predominantly expresses isoform A or predominantly express isoform B, we can ascertain how

each form contributes to the cancer phenotype. Treatment of our cells lines with insulin receptor siRNA resulted in effective knock-down of the insulin receptor protein up to five days post siRNA treatment, as revealed by western blot analysis. Cell proliferation assays, using MTT reagent, showed that inhibition of insulin receptor expression with siRNA was able to significantly reduce oesophageal cancer cell proliferation, regardless of which isoform was expressed.

Contact: jacquelinebracher@gmail.com

Virginia Davids University of Cape Town

Mentor: Associate Prof M Collins

Broad research area: Oxidative stress and antioxidants

Specific research field: Elucidation of urate biochemistry in red blood cells as an unsuspected endogenous antioxidant strategy

Purpose of study:

We have previously identified the accumulation of various LMW antioxidant substances, particularly high levels of tyrosine and urate in the mammalian red blood cell. These substances are potent antioxidants. Since the red blood cell is able to scavenge damaging reactive species from the surrounding plasma, accumulation particularly of urate in the red blood cells may not only protect the red cell itself but also the peripheral tissues through which blood circulates during episodes or conditions of oxidative stress. Investigation of the mechanism of accumulation of these substances in the red cell is essential to reveal if such an endogenous pathway may be a novel therapeutic target. Preliminary results indicate that, contrary to reports from the 1960s, the red cell does have the enzyme necessary for the formation of urate. We are concurrently investigating whether provision of enzyme substrate may augment the protective response of this antioxidant pathway, particularly since activation of this enzyme appears to be occurring as an adaptive/protective response in conditions associated with oxidative stress. The implications for therapeutic applications in many such conditions (including tuberculosis for which new treatment and preventative approaches are urgently required) might be profound.

Contact: v.davids@uct.ac.za

Andrew Mark Dellis

University of Cape Town

Mentor: D Ross and D Stein

Broad research area: Experimental psychology

Specific research field: Pathological gambling

Purpose of study:

The Diagnostic and Statistical Manual for Mental Disorders (DSM-VI) classifies pathological gambling (PG) as a disorder of impulse control and lists 10 symptoms of which positive endorsement of five indicates a disorder. Recent research focused on the cortical-basal ganglia reward circuit motivates for a classification revision in line with processes implicated in addiction. In this respect, the tools brought forth by economic analysis provide structure to neuroscientific and behavioural observations. Studies using DSM-IV criteria indicate prevalence figures of between 0.4% and 2.0% for pathological gambling in the US and Canadian populations, however, rates for PG in South African samples (including vulnerable populations such as adolescents and the poor) are unknown, as is PG comorbidity. Knowledge of co-occurring disorders increases understanding of etiology and thereby improves prevention or early intervention strategies. Moreover, the severity and impact of disordered gambling are related to current comorbidities as well as vulnerability, with implications for both cost estimation and treatment. While evidence is increasingly available, especially for substance use and mood and anxiety disorders, few general population surveys have been conducted, and surveys outside the US have not commonly focused on comorbidities. Most research is with treatment-seeking samples, which apart from inflating population estimates do not control for socio-economic factors known to influence rates of psychopathology. Relatedly, few surveys have made use of the distinction between 'problem' and 'pathological' gambling or assessed along 'a continuum of risk', notable advances in recent study designs. Beyond survey research, no studies have examined choice behaviour among South African gamblers experimentally, for example eliciting risk and time preferences. Similarly a full appreciation of the dynamics of reward system functioning in PG awaits more rigorous neuroimaging work. Our group is conducting psychological, behavioural, economic and neuroimaging research aimed at interrogating PG in South Africa at a number of levels.

Contact: Andrew.Dellis@gmail.com

Maria Esterhuysen

University of Stellenbosch

Mentor: Prof JH van Wyk and Prof E Hoal

Broad research area: Functional genetics

Specific research field: Epigenetics in TB case control studies

Purpose of study:

Tuberculosis (TB) caused by the infection of *Mycobacterium tuberculosis* (TBM) in humans, is currently the single most evident infectious disease responsible for human deaths. Phagocytosis of the pathogen is mediated by complement molecules, antibodies and specific receptors of the innate immune system. A process which is localised in the alveolar macrophage. Shortly after initial infection the bacilli multiply within unactivated macrophages, however, other macrophages begin to extravasate from peripheral blood at which time lymphocytes begin to infiltrate and recognise the processed mycobacterium as antigen in the context of MHC molecules. This results in T-cell activation and cause cytokines to liberate after which the macrophages are activated to destroy the bacillus. A tenth of infected people develop active disease and a vast number of factors impact this probability. Among those factors, several studies to date have proven that genetic factors contribute to the outcome of TB, with an estimated heritability of 36–80%. On the other hand, even though various linkage and association studies have been executed to attempt finding genetic factors to explain some of the variation in TB susceptibility, to date these candidate genes were not conclusive across various populations. No epigenetic study has to our knowledge been conducted in this regard. DNA methylation, a covalent binding of a methyl group to cytosines during cellular differentiation in mammals, is a stable modification to the classic four letter DNA code, which largely affect the ability of a gene being possible to be transcribed or not. DNA methylation is orchestrated enzymatically by DNMT3a and b at the time stem cells differentiate into a specific cell line. This cell line will then retain its methylation pattern throughout all cellular generations. This 'maintenance' methylation is brought about by DNMT1. 60-90% of CpGs are reported to be methylated throughout the genome, yet unmethylated CpGs can be found clustered in CpG islands most often associated with 5' promoter regions of genes. Hypomethylated CpG islands are generally known to be associated with transcriptional upregulation and are now described to be a significant contributor to altered gene expression. In this study we used a small sample set to compare global methylation between TB cases and controls. Subsequently we studied regional methylation by interrogating the methylation

status of strategically chosen CpGs in 14 475 consensus coding sequences as well as 110 miRNA promoters, using the same sample set on a bead-based chip providing a very comprehensive analysis of gene associated DNA methylation. This chip data was followed up with regional bisulfite sequencing of selected gene targets which proved to be differentially methylated between cases and controls of both male and female matched pairs.

Contact: Mme2@sun.ac.za

Nai-Jen Hsu University of Cape Town

Mentor: M Jacobs

Broad research area: Immunology, neuroscience

Specific research field: An in vitro study of *Mycobacterium tuberculosis* neural infection

Purpose of study:

Pulmonary tuberculosis (TB) is the primary infection of TB, caused by the Gram-positive bacilli *Mycobacterium tuberculosis* (MTB), but the most predominant extra-pulmonary form of TB is TB Meningitis (TBM). Relatively little is known about the pathogenesis, the mechanism underlies the neurological complication and the immunological responses during TBM. Although it is known that microglial cells take up MTB, such cells have a close relationship with neurons, and may induce neuronal damage. Interestingly, another intracellular pathogen, *Listeria Monocytogenes* has been shown in vitro to internalise in neurons. The interaction of MTB directly with neuronal cells remains largely unexplored. An in vitro infection model was adopted to characterise the immunology of TBM in the nervous system. The primary neuron and microglia cultures were cultivated from the hippocampus and cortex of C57Bl/6 17-days old embryos. The primary cultures and neural cell-lines included HT22 (neuronal), Neuro2A (neuronal) and BV2 (microglial), were infected with differing concentrations of laboratory MTB (H37Rv or H37Rv-GFP). At different points in time of infection, the cells were subjected to a Ziehl Neelson or an immunofluorescent stain then analysed using microscopy. A direct association between neuronal cells and the H37Rv at all multiplicity of infection (MOI) has been observed. To verify whether the bacilli are not only associated with the neurons but are also 'internalised' by the neurons, confocal microscopy was employed. The GFP-expressing bacilli were clearly found inside the neuronal cytoplasm labelled by phalloidin marker. In this study we show that neurons can act as target cells for MTB, which is comparable to what is

seen in microglia. Visualisation of neurons actually showing uptake of MTB introduces a new dimension in studying the characteristics of TBM infection. Such neuronal responses may account for the neurological morbidity seen in patients suffering from TBM.

Contact: nai-jen.hsu@uct.ac.za

Dheshnie Keswell University of Cape Town

Mentor: J Goedecke

Broad research area: Obesity and diabetes

Specific research field: Ethnic-specific differences in ectopic fat deposition and the association with insulin sensitivity in black and white South African women

Purpose of study:

Type-2 diabetes is a major health risk and its prevalence continues to increase in the South African (SA) population. One of the major risk factors for diabetes is insulin resistance (IR). The prevalence of IR is higher in black SA women compared to their white counterparts and one of the major contributing factors to IR is visceral adipose tissue (VAT) deposition. Despite the high prevalence of IR in black women, they have less VAT and more peripheral adipose tissue compared to white SA women. This suggests that increased adipose deposition peripherally, and not VAT, may lead to IR in black SA women. Increase adipose tissue together with a decreased capacity to store fat peripherally often results in ectopic fat deposition. Increase intramuscular fat levels increases cytokine and chemokine production that may cause defects in insulin signalling pathways in muscle. This highlights the close intercellular relationship between skeletal muscle and adipose tissue. To date no studies have been performed to assess the mechanisms involved in crosstalk between adipose tissue and skeletal muscle (in terms of insulin signalling) in the South African context. By using adipocytes from black and white South African women and co-culturing them with normal human muscle cells we will explore how adipokines, released from adipocytes, affect the expression of genes involved in insulin signalling in skeletal muscle; whether adipocytes release reactive-oxygen species that affect insulin signalling pathways in skeletal muscle; and determine whether disruption of the insulin signalling pathway in skeletal muscle cells can be rescued.

Contact: Dheshnie.Keswell@uct.ac.za

Wendy Kröger

University of Cape Town

Mentor: S Prince

Broad research area: Molecular and cell biology

Specific research field: The Role of the T-box transcription Factor TBX3 in melanoma progression

Purpose of study:

Melanoma is a highly aggressive tumour, with an incidence increasing faster than any other form of cancer. While it only constitutes 4% of all dermatological cancers, it is responsible for approximately 80% of skin cancer mortality. Malignant melanoma occurs as a result of melanocytes progressing through a series of phenotypically distinct steps, however, a detailed understanding of this process at a molecular level is still lacking. The T-box transcription factor TBX3 has been shown to be overexpressed in several cancers including a subset of melanomas. In this study, we provide compelling evidence that TBX3 plays an important role in the vertical growth phase (VGP) of melanoma progression. VGP is characterised by melanocytes acquiring the ability to proliferate indefinitely, cross the basement membrane and invade the underlying dermis to form tumours. Using an siRNA approach to silence TBX3 in melanoma cell lines representative of VGP and metastatic melanoma, we demonstrate in vitro and in vivo transformation assays that these cells require TBX3 for anchorage independence and tumour forming ability and metastasis. Furthermore using a yeast-two hybrid screen, immunoprecipitation assays and immunofluorescence, we have identified a novel interaction between TBX3 and the transcription factor c-MYB, which has important implications for our understanding of TBX3's oncogenic activity. These results suggest that TBX3 plays an important role in melanoma progression and reveal mechanisms of interfering with TBX3 activity, which may represent therapeutic targets to treat this highly intractable disease.

Contact: Krogerwendy@uct.ac.za

Gail Louw

Stellenbosch University

Specific research field: Activated efflux pumps define the level of rifampicin resistance in *Mycobacterium tuberculosis*.

Purpose of study:

Central dogma suggests that rifampicin (RMP) resistance develops through mutations in the *rpoB* gene of *Mycobacterium tuberculosis*. In this study we challenge this dogma by demonstrating that

activation of efflux pumps, in addition to *rpoB* mutations, which defines the level of RMP resistance. Clinical isolates demonstrating different *rpoB* mutations and representative of three MDR-TB outbreaks, MDR-TB and mono-RMP strains from different genetic backgrounds were selected. The level of RMP resistance was assessed by MIC determination in BACTEC 12B medium (containing 0.0002 to 200 µg/ml RMP). Subsequently, the effect of efflux pump inhibitors (EPIs) on RMP susceptibility was determined by culturing the resistant isolates in BACTEC 12B medium containing 2 µg/ml RMP and either 80 µg/ml reserpine or 50 µg/ml verapamil. To confirm that restoration of RMP susceptibility was due to the presence of EPIs, isolates were initially cultured in the presence of EPI and subsequently serially diluted into BACTEC 12B media containing either RMP (2 mg/ml) only, or RMP and the corresponding EPI. Growth was monitored for nine consecutive days. The level of RMP resistance was found to be highly variable among all RMP-resistant strains analysed, irrespective of genetic background and *rpoB* mutation. It was also observed that RMP susceptibility was significantly restored (53-81% and 61-86% for reserpine and verapamil, respectively) in all RMP resistant isolates. In addition, it was demonstrated that efflux pump activity is restored when the EPI concentration is lowered to below 8 and 5 µg/ml for reserpine and verapamil, respectively. This study suggests that additional biological mechanisms are responsible for defining the RMP MIC. We show that the inhibition of activated efflux pumps significantly restores RMP susceptibility to below the critical concentration. We conclude that the level of RMP resistance is defined by an increase in the binding constant between the mutant *RpoB* and RMP, as well as active efflux.

Contact: gail@sun.ac.za

Mohlopheni Jackson Marakalala

University of Cape Town

Mentor: G Brown

Broad research area: Medical research

Specific research field: The role of Dectin-1 in TB infection

Purpose of study:

Dectin-1 is a C-type lectin-like receptor that binds to β-glucans and has an established protective role against fungal infections. In mycobacterial infections Dectin-1 collaborates with TLR2 to trigger cytokine release and also stimulates IL-12p40 production by dendritic cells in vitro. To explore the role of Dectin-1 during tuberculosis infection, we used an in vivo model in which mice were infected with

100 CFU of *M. tuberculosis*, and at 8 and 16 weeks post infection, the cytokine production, histopathology, bacterial burden and long term survival were assessed in Dectin-1-deficient (Dectin-1 $-/-$) and -sufficient mice (wild type). At both 8 and 16 weeks post infection, the histopathology section from the lungs of both Dectin-1 $-/-$ and wild types showed similar histological features but the bacilli were more abundant in the wild types than in the Dectin-1 $-/-$. Investigation of cytokine profiles with ELISA revealed that there was no significant difference in the IL-12p40, IL-12p70, IL-6, IL-17 and INF- γ levels in Dectin-1 $-/-$ and in wild type mice at both 8 and 16 weeks points. Further cytokine analysis showed that TNF- γ levels were higher in the Dectin-1 $-/-$ mice than the wild types after 8 weeks of the infection. In contrary, IL-10 was found to be significantly higher in the wild types than the knockouts. These data suggests that Th1 response is hampered by the increased IL-10 levels in the wild types and is further supported by the significantly higher bacterial burdens in these mice as compared to Dectin-1 $-/-$ mice.

Contact: molopheni.marakalala@uct.co.za

James J Meiring Stellenbosch University

Mentor: Prof MF Essop

Broad research area: Cardiac metabolism

Specific research field: Hyperglycaemia-induced flux through the hexosamine biosynthetic pathway impairs cardiac contractile function

Purpose of study:

Hyperglycaemia during diabetes increases the risk for cardiovascular diseases. We previously demonstrated that hyperglycaemic conditions activate the hexosamine biosynthetic pathway (HBP), increasing myocardial apoptosis. We now hypothesise that hyperglycaemia-mediated HBP activation impairs contractile function in response to ischemia-reperfusion. We perfused isolated rat hearts with Krebs Henseleit buffer ± 11 mM, 22 mM and 33 mM glucose, respectively (Langendorff system) for 90 minutes, followed by 30 minutes global ischemia and 60 minutes reperfusion. To assess the role of the HBP, perfusions were performed ± 50 μ M DON (HBP inhibitor) at reperfusion. Here the left ventricular developed pressure (LVDP) (as a percentage of baseline function) and significantly decreased under hyperglycaemic conditions (22 mM, 33 mM) following myocardial ischemia. DON administration did not alter functional recovery with 11 mM glucose. However, with 22 mM glucose it enhanced

rate pressure product, velocity of contraction, and LVDP during the reperfusion phase ($p \leq 0.05$ versus matched controls). It also improved LVDP (as a percentage of baseline function) by $42.2 \pm 8.8\%$ at 10 minutes and further by $62.6 \pm 3.8\%$ at 60 minutes of reperfusion. However, DON administration was less effective with higher glucose levels (33 mM) and also displayed delayed cardioprotective effects during reperfusion, such as increased LVDP (as a percentage of baseline function) by $26.2 \pm 4.4\%$ at 30 minutes and $35.6 \pm 3.8\%$ at 60 minutes of reperfusion. We show that HBP activation under hyperglycaemic conditions impairs cardiac contractile function in response to ischemia-reperfusion. HBP inhibition partly confers cardioprotection, although this response is blunted under more severe hyperglycaemic conditions.

Contact: meiringj@sun.ac.za

Lynthia Paul University of Cape Town

Mentor: Associate Prof V Abratt

Broad research area: Molecular mechanisms of antibiotic Resistance in bacteria

Specific research field: The characterisation of novel resistance mechanisms against metronidazole in *Bacteroides fragilis*

Purpose of study:

Metronidazole resistance in the anaerobic human pathogen *Bacteroides fragilis* is generally attributed to the presence of nitroreductases, encoded by nim genes. However, the isolation of highly resistant, nim negative, clinical isolates suggests the presence of other undefined resistance mechanisms. This study identified 3 transposon-associated mutations conferring increased resistance to metronidazole. Two of the mutants contained transposon insertions in genes encoding a hypothetical protein and a nitrile oxidoreductase respectively. A third mutant contained an insertion upstream of a gene encoding a putative flotillin-like protein. The role of flotillin-like proteins in bacteria is unknown, although this protein in *Bacillus subtilis* was shown to be membrane-associated and specifically with the lipid raft fraction of the membrane. This study aims to characterise the nature of metronidazole resistance in a *Bacteroides fragilis* flotillin mutant through mutational and functional studies, as well as phenotypical studies of various flo mutant strains. We hypothesise that flotillin from *Bacteroides fragilis* is membrane associated, and that modulation of flotillin levels affects the sensitivity of this bacterium

to metronidazole either through diminished metronidazole uptake, or altered signal transduction. We showed that (1) the transposon mutant is eight times more resistant to metronidazole compared to the wild type parent, (2) the flo gene is transcribed as part of an operon with a gene encoding a transmembrane protein, (3) the operon was interrupted by the transposon insertion and the flo gene in the mutant is now transcribed from within a weak promoter on the transposon, and (4) deletion of the flo gene conferred increased resistance to metronidazole (using E-tests), but not to the degree that the transposon mutant exhibited. In conclusion, this preliminary study has identified novel genes, in *Bacteroides fragilis*, involved in metronidazole resistance.

Contact: lynthia.paul@uct.ac.za

James Smith University of Cape Town

Mentor: Prof N Phaswana-Mafuya

Broad research area: Cancer research

Specific research field: The oncogenic transcription factor TBX2 is regulated by transforming growth factor β 1

Purpose of study:

The T-box transcription factors, which are well known for their critical roles in embryonic development, have recently been associated with cancer. TBX2 for example, is overexpressed in several cancers and can override the senescence and tetraploidy checkpoints, which normally protect against cancer. Furthermore, we have demonstrated that knocking down TBX2 in melanoma and breast cancer cell lines reverses key features of the cancer phenotype. We hypothesise that an understanding of the signaling pathways that regulate TBX2 levels will lead to a better understanding of its role in cancer and will provide more versatile targets for cancer treatments. This study shows that TBX2 levels are regulated by transforming growth factor β 1 (TGF β 1), which functions as a tumour suppressor and whose signaling pathways are often disrupted during oncogenesis. When WI-38 human embryonic lung fibroblasts were treated with 5 ng/ml TGF β 1, we observed an initial activation of TBX2 mRNA and protein levels (1-8 hours) followed by a robust and sustained repression. This finding was not restricted to normal fibroblasts as it was reproducible in the human metastatic 501 melanoma cell line. The TGF β 1 signal commonly mediates transcription both through activation of the Smad3/Smad4 pathway and through additional transcription factors including the AP-1 member, JunB. Using

promoter-reporter assays, we show that expression of JunB activated a luciferase reporter gene driven by the human TBX2 promoter, whereas Smad3 and Smad4 expression repressed it. Interestingly, coexpression of Smad3 and Smad4 with JunB further increased the activation of the TBX2-luciferase reporter compared to expression of JunB alone. Using sequential 5' deletion and site directed mutagenesis of the TBX2 promoter, we have identified the sites within a region spanning -218bp to +32bp relative to the transcriptional start site that mediate this regulation by Smad3/4 and JunB. Collectively, these findings show for the first time that TBX2 expression is regulated by TGF β 1 and suggest that the ratio between Smad3/4 and JunB activity may be an important determinant of whether TGF β 1 activates or represses TBX2 expression. This study provides novel insight into the regulation of TBX2, which has important implications for our understanding of its role in development and cancer.

Contact: James.smith@uct.ac.za

Cily Tabane HSRC

Mentor: Prof N Phaswana-Mafuya

Broad research area: HIV/AIDS and cultural practices

Specific research area: The relationship between cultural practices of Batswana people and the transmission of HIV/AIDS in Botswana.

Purpose of study:

The aim was to establish the influence of cultural practices of the Batswana on the transmission of HIV/AIDS in Botswana. The research questions included (a) What are the current nature and prevalence of cultural practices of the Batswana in relation to the transmission of HIV/AIDS in Botswana? (b) To what extent do these cultural practices contribute to the spread of HIV/AIDS? (c) What can be done to prevent the problem of HIV/AIDS in relation with cultural practices of Batswana people in Botswana? The empirical research findings of the qualitative and quantitative data confirmed that it is acceptable in Botswana for men to have multiple relationships even after marriage. Polygamy is still a part of the Batswana culture. Children are very important therefore the use of condoms is unacceptable. Prevention strategies do not take cultural practices into consideration. The empirical findings also revealed that the two statements, namely (a) "A man is like a bull and should not be confined to one pasture" and (b) "Men are the only people who can go to the cattle post and this puts women

in subordinate positions" are part of the Batswana culture. These behaviours contribute to the spread of HIV. The family should take responsibility in educating the children about HIV/AIDS. HIV/AIDS prevention and care strategies should take cultural practices of the Batswana into consideration so that the community can cooperate in the fight against HIV/AIDS. The cultural practices should be used in a positive way. For example circumcision schools should not be discouraged and people should be educated on how to prevent HIV transmission at the circumcision schools.

Contact: ctibane@tlabs.ac.za

Pauline van der Watt **University of Cape Town**

Mentor: Dr V Leaner

Broad research area: Cancer research

Specific research field: Identification of nuclear import inhibitors with anti-cancer activity

Purpose of study:

Karyopherin beta 1 (Kpn β 1) is a nuclear transport protein involved in the import of cargo proteins from the cytoplasm into the nucleus. Recently, it was determined that Kpn β 1 expression is elevated in cervical cancer and oesophageal cancer cells compared to normal, suggesting that cancer cells might be more dependent on Kpn β 1 function. In line with this, inhibition of Kpn β 1 expression in cancer cells using siRNA results in cell death via apoptosis, while inhibition in normal cells has only a minor effect on cell biology. As targeted inhibition of Kpn β 1 results in the selective killing of cancer cells and since no drugs currently exist that inhibit Kpn β 1, we propose that the development of Kpn β 1 inhibitors could have therapeutic potential for the treatment of cancer. This study aims to identify and characterise novel small molecule inhibitors of Kpn β 1, and to determine their anti-cancer activity. To identify inhibitors of Kpn β 1 we have performed an in silico screening assay using a library of 14 million chemical compounds to identify molecules that bind Kpn β 1, based on its published three-dimensional crystal structure. The targeted region comprises a protein-binding pocket important for Kpn β 1 function. Compounds that potentially bind this region have been identified and scored according to their predicted binding affinity. Currently, the top-scoring compounds are being investigated. In addition, an assay has been set up to test the compounds, whereby a stable cell line expressing GFP-tagged Karyopherin alpha 2 (Kpn α 2), an adaptor protein often required for Kpn β 1 function was generated. Normally Kpn α 2-GFP is localised

to the nucleus by Kpn β 1, but inhibition of Kpn β 1 results in the cytoplasmic accumulation of Kpn α 2-GFP, which can be visualised using fluorescence microscopy. Using siRNA to inhibit Kpn β 1 the assay has been proved effective. Monitoring the localisation of Kpn α 2-GFP after treatment with the different compounds will enable the identification of effective inhibitors of Kpn β 1. The anti-cancer properties of these compounds will then be investigated.

Contact: Pauline.vanderwatt@uct.ac.za

Jennifer Watermeyer **University of the Witwatersrand**

Mentor: Prof C Penn

Broad research area: Health communication, social sciences

Specific research field: Communication skills training for pharmacists

Purpose of study:

The question of why patients do not take medications poses a long-term challenge to researchers and clinicians. In the HIV/AIDS field high adherence levels are required to ensure treatment success, but are often not achieved. Researchers are increasingly focusing on how communication processes and patient comprehension of medication instructions influence adherence behaviours. Pharmacists have an important role to play in this regard, but communication skills training is a neglected area and existing programmes in developed countries are not necessarily sensitive to the needs of the African pharmacist. The complexity and management of ARV regimens within a multilingual context is vast and these factors are influenced by issues such as patient literacy, poverty, and a mismatch between the language of health professionals and patients. Using an action research approach, this project has involved the development, implementation and evaluation of a communication skills training programme for pharmacists working in multicultural HIV/AIDS contexts across Africa. A workshop and handbook have been developed and to date, the handbook has been distributed to universities, NGOs and clinics throughout Africa. This project underlines the importance of providing ongoing disease- and context-specific communication training using appropriate methods and theoretical frameworks, which address the needs of health professionals working in developing contexts.

Contact: Jennifer.watermeyer@wits.ac.za
