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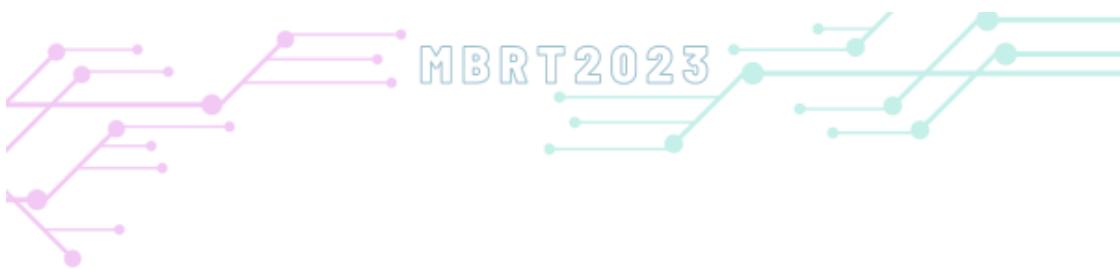


Table of contents:		Page
MBRT: What and who were are		2
MBRT: Aspirational goals and objectives		2
MBRT: Postgraduate research symposium		3
Welcome message:		
MBRT Executives		4
About the MBRT Executives		5
MBRT Champions		7
Keynote speaker		9
Program		10
Sponsor adverts		12
Oral presentation abstracts		15
Poster presentation abstracts		28

MBRT: What and who were are

The Molecular Biosciences Research Thrust (MBRT) was founded to stimulate research and other academic activities in the broad area of health-related molecular biosciences at the University of the Witwatersrand, and to create a vibrant atmosphere in which scientists and students within both the Faculties of Science and Health Sciences can realize their full research potential. The Thrust is governed by the Executives (Dr Adriaan Basson and Dr Nancy Meulenberg) and supported by the Champions (Prof. Mandeep Kaur and Prof. Maria Papathanasopoulos). The Thrust is administered by the WITS Health Sciences Research Office.

MBRT: Aspirational goals and objectives

The research focus of the Thrust is encapsulated in the molecular understanding of common diseases and the health of Sub-Saharan African populations. Groups of researchers and postgraduate students who share common research interests can identify suitable collaborations and collaborators within the University of the Witwatersrand and partner institutions – a hallmark of effective modern science. The primary element binding participants of the Thrust is the use of common technologies and equipment to address research questions, but the vision is that it will foster clusters of researchers working on different aspects of the same theme. In this way, bigger research questions can be tackled, and more research funding can be leveraged for the successful completion of research projects.

MBRT: Postgraduate research symposium

The annual MBRT Postgraduate Research Day is a highlight in our calendars. It is aimed specifically at postgraduate students and postdoctoral fellows in the WITS Faculties of Sciences and Health Sciences to showcase the exciting research taking place across both Faculties, and to provide an opportunity for intra- and inter-Faculty collaborations between postgraduate students, early career researchers and senior scientists.



Welcome message: MBRT Executives

This year has been one of steady progress and great victories. As a country, we celebrated our fourth Rugby world cup win, yet the second half of the game fostered feelings of anxiety, doubt, hope, and ensuing relief. We believe that many of our postgraduate students have run the gamut of those emotions during their studies and we are optimistic that they will also reach the finish line successfully. The MBRT research day is an opportunity for them to showcase their research in an engaging scientific environment in the hopes that this level of engagement will encourage future collaborations.

We are pleased to announce that we received a record number of abstracts featuring research topics ranging from SARS, Alzheimers, Cancer, CBD, Drug and Gene Delivery, Antibody production, to many more. Prof Patrick Arbuthnot will be our plenary speaker this year to give us an exclusive look into the intricacies of building mRNA vaccine technology platforms in middle- to low- income countries. Similar to previous years, we could only accommodate 12 oral presentations, but we encourage you to take the time and engage with the outstanding posters on display (and the respective students) during the poster sessions.

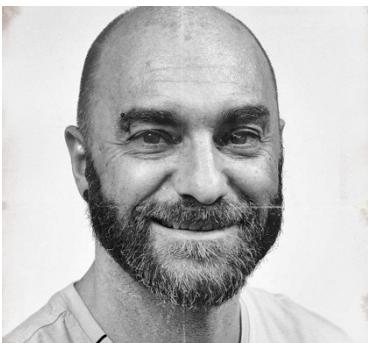
Last but certainly not least, we would like to thank all our sponsors as well as the judges, without whom this day would not be feasible.

The MBRT Executives,

Dr Adriaan E Basson

Dr Nancy Meulenberg

About the MBRT Executives:



Dr Adriaan Basson is a senior lecturer and a researcher in the HIV Pathogenesis Research Unit (Department of Molecular Medicine and Haematology) at the University of the Witwatersrand. He obtained his Ph.D. in Biochemistry at the University of Johannesburg and kicked off his career as research scientist at the

University of Pretoria and the National Institute of Communicable Diseases. His research is focused on HIV-1 drug resistance and HIV-1 cure-related research. He strongly believes that research should make some contribution towards public health concerns, and his research projects are directed towards this goal. Molecular (cloning, sequencing) and in vitro cellular assays (toxicity, anti-viral activity) make up some of the fundamental methodologies that he employs on a daily basis. In addition to his enthusiasm for HIV-1 drug resistance and HIV-1 cure-related research, he also has a strong interest in screening novel antiretroviral drugs from both natural and synthetic sources. He is in ongoing collaborations with organic chemists, both locally and internationally, to investigate novel synthetic compounds as potential antiretroviral drugs.

About the MBRT Executive:



Dr Nancy Meulenberg is a lecturer and researcher in molecular medicine in the HIV Pathogenesis Research Unit, the Department of Molecular medicine and Haematology, University of the Witwatersrand. Her research interests lie in HIV-1 immunogen design and immunogenicity testing in a preclinical setting, as well as nanoparticulate delivery of vaccines. Prior to this, Nancy spent five years at the NICD, mapping

antibody specificities in the sera of HIV-1 infected individuals. To pursue this, she left a promising career at a startup Biotech company (KapaBiosystems) as she believes that a focus on public health issues was a better career choice. Nancy received her PhD from the university of the Witwatersrand, an MSc from the University of Cape Town and her Bachelor's with Distinctions from Boston University. While her studies and career have spanned three continents, Africa is her home as she strives to see healthcare and education strengthened as well as diversity and equity established here. Nancy has co-authored multiple peer-reviewed articles in international journals and she is devoted to supervising and mentoring the next generation of scientists and academics. Her multilingual and culturally-rich heritage make her an excellent candidate for bridging the gap in knowledge discrepancy by increasing exposure of the STEM disciplines in resource-limited settings and by fighting the all too prevalent vaccine hesitancy.

Welcome message: MBRT Champions



Prof. Mandeep Kaur
Sciences



Prof. Maria Papathanasopoulos
Health Sciences

The field of molecular bioscience is moving rapidly. We need to embrace new fields of study and technology driven approaches to ensure that we develop a systems approach to our understanding of biological processes. High impact science requires collaboration, and we all need to develop specific areas of expertise to become valuable and effective collaborators. As researchers, our core value is to perform ethical research to bring new scientific advancements to our country for the betterment of our communities.

We are excited about the student oral and poster presentations. To all the students, please make the most of this exciting opportunity to network and to learn about the scientific questions that occupy the minds of your colleagues. Communicate your science with clarity (i.e.,

just the right amount of interesting background to draw in your audience), present your results with brevity, and focus on the outcomes and potential impact of your research. Make most of this opportunity to connect with fellow researchers, find new collaborations, enrich yourself and learn about new fields.

We wish to thank our able and enthusiastic research day organisers and Executives, Dr Adriaan E. Basson and Dr Nancy Meulenberg, for arranging a rich array of talks from your submissions. We also wish to thank our generous sponsors who have contributed to making this event possible.

Best wishes,
The MBRT champions

Keynote speaker:

“Building mRNA vaccine technology in low- and middle-income countries”



Patrick Arbuthnot is personal professor and director of the Wits/SAMRC Antiviral Gene Therapy Research Unit (AGTRU). After completing medical studies and a PhD, he worked on gene therapy as a post-doctoral fellow at Necker Hospital in Paris. On returning to South Africa, Patrick established the AGTRU, which has now published widely on gene therapy-based methods to counter infection with hepatitis B virus and other pathogens. Recently the team has engaged

with the WHO, Medicines Patent Pool, various governments, philanthropies, and industry to establish an African mRNA vaccine technology platform. Research in the unit is also advancing use of recombinant adenoviral vectors and yeast-expressed proteins as vaccines.

PROGRAMME		
8:15 – 9:00	Check-in for registered attendees	
9:00 – 9:10	Opening/Welcome	MBRT Executives
9:10 – 9:50	Keynote address	Prof. Arbuthnot
9:50 – 10:00	Introduction to IDORI	Caryn McNamara

Oral presentation session 1

10:00 – 10:15	O1: mRNA-mediated delivery of anti-HIV-1 broadly neutralising multispecific antibodies as a potential strategy for HIV-1 prevention	Caitlyn J. O'Connor
10:15 – 10:30	O2: Heat Shock Factor (HSF) expression and its effect on life table parameters in the main African malaria vector <i>Anopheles Funestus</i>	Nerissa Bloch

Poster presentation session 1

Oral presentation session 2		
11:30 – 11:45	O3: The Antimicrobial Potential of Essential Oils against the ESKAPE Pathogens	Keruné Naidoo
11:45 – 12:00	O4: Aortic Haemodynamics in Patients with Heart Failure and the Impact of Blood Pressure Control	Marcus Lebelo
12:00 – 12:15	O5: The effects of ibogaine on myelination in Sprague Dawley rats	Demi Govender
12:15 – 12:30	O6: Synergistic solutions: The potential of repurposing ibuprofen for combatting cutaneous infections	Shivar Simbu

Lunch and group photo

Oral presentation session 3		
13:30 – 13:45	O7: Assessing the Effects of Low-density Lipoprotein Receptor Upregulation on Alzheimer's Disease Hallmarks	Ester Tshipamba

13:45 – 14:00	O8: Unravelling Cell Type-Specific STAT Pathway Utilisation in the Interferon-alpha Response	Darisia Moonsamy
14:00 – 14:15	O9: Neurobehavioral and molecular changes in a rodent model of ACTH-induced HPA axis dysfunction	Farhanah Sallie
14:15 – 14:30	O10: Investigating the role of ancestral derived AAV capsid to deliver transgenes <i>in vitro</i> and <i>in vivo</i>	Ridhwaanah Jacobs

Poster presentation session 2

15:30 – 15:45	O11: Unveiling SARS-CoV-2 Mpro: A Biochemical quest	Mpho Setshedi
15:45 – 16:00	O12: Biorenewable waste products as building blocks for novel Lipid Nanoparticles to deliver RNA vaccines	Dylan Kairuz
16:00 – 16:30	Judges' deliberation	
16:30 – 17:00	Prize giving and closing remarks	



MBRT2023

Celtic Molecular Diagnostics Research Product Portfolio 2023



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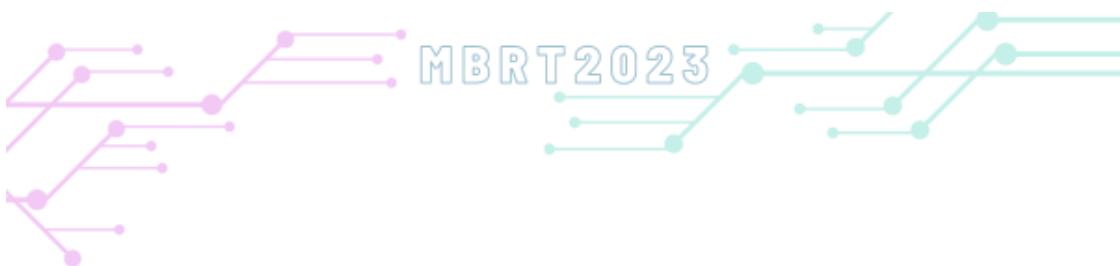


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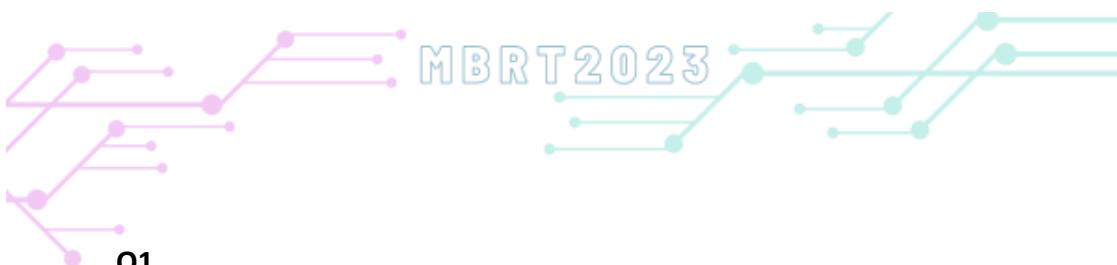
Sample to Insight

QIAGEN is a leading global provider of molecular sample and assay technologies. Our solutions are used in various areas, from life sciences research to clinical diagnostics. QIAGEN's products aid in DNA, RNA, and protein purification, assay development, and molecular diagnostics, serving researchers, clinicians, and labs worldwide. Dedicated to advancing insights and answers, QIAGEN is synonymous with reliability, quality, and transformative science.

Our vision is truly powerful and conveys our ambition to make a difference:
Making improvements in life possible.



Oral presentation abstracts



O1

TITLE

mRNA-mediated delivery of anti-HIV-1 broadly neutralising multispecific antibodies as a potential strategy for HIV-1 prevention

AUTHORS

Caitlyn J. O'Connor¹, Abdullah Ely², Maria A. Papathanasopoulos¹, Mark A. Killick¹

AFFILIATIONS

1HIV Pathogenesis Research Unit, Faculty of Health Sciences, University of the Witwatersrand. ²Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witwatersrand

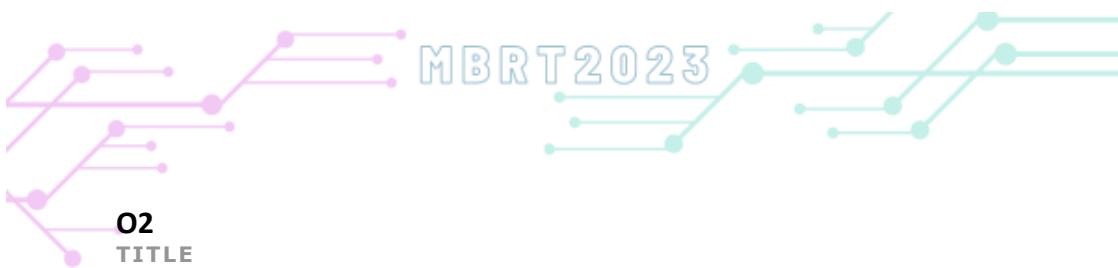
BACKGROUND: HIV-1 broadly neutralising antibodies (bNAbs) show great promise at both reducing viraemia and preventing HIV-1 infection, however, their application may ultimately be limited due to high manufacturing costs and the requirement for combination-based therapies. This project describes the development of *in vitro* transcribed (IVT)-mRNA delivery of multispecific antibodies as a potential cost-effective, passive immunisation strategy against HIV-1.

METHODS: Previously described bispecific (Bi-scFv and Bi-NAb) and trispecific (Tri-NAb) antibodies combining VRC01/PGT121 and VRC01/PGT121/10e08 paratopes, respectively (>95% neutralisation coverage *in vitro* (208 pseudovirus panel), with geometric mean IC₅₀ titres <0.4 µg/mL), were selected for development. Antibody-encoding DNA plasmid constructs, engineered with T7-IVT mRNA transcription compatibility, were generated. Parental (PGT121, VRC01 and 10e08) and multispecific antibodies were expressed from DNA constructs by transient transfection in 293F cells, purified and biochemically characterised. Multispecific antibody-encoding mRNA transcripts were IVT from linearised DNA, purified of dsRNA, enzymatically capped (5' Cap 1 structure), and transfected into 293F cells. Functionality of the purified antibodies and mRNA-transfected cell culture supernatants were assessed *in vitro* against a panel of 17 HIV-1 tier 2 pseudoviruses.

RESULTS & DISCUSSION: Purified multispecific antibodies demonstrated improved neutralisation potency and coverage compared to the parental monoclonal antibodies, as expected. Encouragingly, un-purified mRNA-transfected cell culture supernatants matched the neutralisation coverage of purified antibodies, with sufficient multispecific antibody titres to generate inhibitory dilution factors conferring 50% neutralisation (ID₅₀) >400: Bi-scFv (1963), Bi-NAb (954), and Tri-NAb (475).

CONCLUSIONS: These data support the preclinical advancement of IVT-mRNA encoding multispecific antibodies as a possible passive immunisation strategy against HIV-1 and require empirical determination of whether therapeutic titres are attainable *in vivo*.

ACKNOWLEDGEMENTS: Research funding was provided by the Poliomyelitis Research Foundation (PRF grant 21/71) and the Faculty of Health Sciences, University of the Witwatersrand. Student funding was provided through the Postgraduate Merit Award from the University of the Witwatersrand, the PRF (grant 22/61), and the National Research Foundation (NRF grant PMDS22070230651).



O2

TITLE

Heat Shock Factor (HSF) expression and its effect on life table parameters in the main African malaria vector *Anopheles Funestus*

AUTHORS

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AFFILIATIONS

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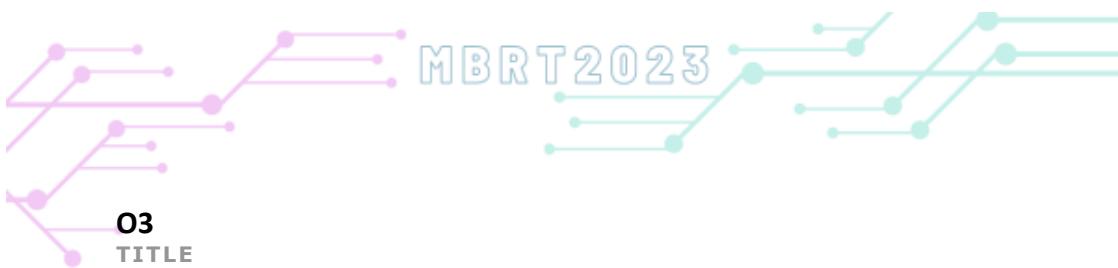
BACKGROUND: *Anopheles funestus* is a major malaria vector in southern Africa and therefore it is essential to control this species. This necessitates studying the underlying factors that influence the biology of this species. In mosquitoes, the transcription factor, heat shock factor (HSF), is a key component in physiological and behavioral processes. However, there is limited information regarding HSF and its role in *An. funestus*. The aim of this study is to investigate the HSF expression in the different life stages of *An. funestus*, and its biological role on life table parameters in this species.

METHODS: The baseline expression of HSF in all life stages of *An. funestus* were investigated using real-time PCR (RT-qPCR). *Anopheles funestus* adults were fed with sugar water containing an optimized concentration of short interfering RNA that targets the HSF gene (HSF-siRNA). The expression of HSF in HSF-siRNA treated *An. funestus* adults was confirmed via RT-qPCR. The phenotypic effect of HSF-siRNA on longevity and reproduction in *An. funestus* was examined.

RESULTS & DISCUSSION: HSF transcript abundance analysis on the four life stages in *An. funestus*, showed that it was significantly lower at the egg and pupal stages compared to the larval and adult stages. The HSF-siRNA knockdown reduced both longevity and reproduction in *An. funestus*.

CONCLUSIONS: In conclusion, HSF is expressed at varying levels in all life stages, and might play a role in developmental processes. HSF plays a role in survival and reproduction, indicating the pleiotropic effect of HSF in *An. funestus*.

ACKNOWLEDGEMENTS: This work is based on the research supported in part by the National Research Foundation of South Africa (Ref Numbers SRUG2203311457). I would like to thank Dr Yael Dahan-Moss and Prof. Lizette Koekemoer for all their guidance and enthusiasm. Thank you to all the staff and students from the department for their assistance and support.



O3

TITLE

The Antimicrobial Potential of Essential Oils against the ESKAPE Pathogens

AUTHORS

Keruné Naidoo, Sandy van Vuuren, Ané Orchard

AFFILIATIONS

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BACKGROUND: It is widely known that antimicrobial resistance is a global phenomenon. Essential oils (EOs) are volatile natural products with a vast number of studies investigating their antimicrobial activity, including those that are resistant to antimicrobials, such as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). It is theorized that EOs can resist the development of resistance due to their multi-component structures, however, studies to validate this are limited.

METHODS: This study investigated the antimicrobial activity of 23 EOs and subsequently the resistance development to five EOs based on a representation of antimicrobial activity ranges in comparison to Ciprofloxacin, Erythromycin, and Gentamicin. Anti-quorum sensing assays and Biofilm inhibition assays were conducted to explore the mechanisms of resistance.

RESULTS & DISCUSSION: Notable results were observed for the antimicrobial (1.00 mg/mL), anti-quorum sensing (> 70% inhibition), and biofilm inhibition studies (> 70% inhibition) for *Origanum vulgare*. *Staphylococcus aureus* ATCC 6538 displayed a pattern of heteroresistance to antibiotics after repeated exposure to the EOs at sub-inhibitory concentrations.

CONCLUSIONS: The MIC of the EOs remained consistent throughout the cycles. These findings indicate that, despite continuous exposure to the EOs, resistance to them has not been observed, thus presenting a highly promising solution to the antimicrobial crisis.

ACKNOWLEDGEMENTS: Phumzile Moerane (Lab technician), NRF Thathuka funding and FRC (2022).

O4**TITLE**

Aortic Haemodynamics in Patients with Heart Failure and the Impact of Blood Pressure Control

AUTHORS

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AFFILIATIONS

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BACKGROUND: In patients with heart failure (HF) both decreases and increases in pulse pressure (PP) are associated with a poor prognosis. If the predominant determinant of decreased aortic PP in HF is systolic dysfunction, then improvements in stroke volume (SV), aortic flow (Q) or forward wave pressure (Pf) with medical management would be beneficial. Alternatively, if hypertension is the primary cause of HF, aortic PP may be increased as a consequence of increases in aortic characteristic impedance (Zc) and backward wave pressure (Pb). Consequently, intense blood pressure (BP) lowering may be required in the chronic management of patients with hypertension-induced HF.

METHODS: Aortic haemodynamics and the impact of controlled BP (SBP/DBP<140/90 mm Hg) were compared between stable HF patients ($n=42$) from Life Flora Hospital, Johannesburg, South Africa and age and sex-matched controls ($n=298$) from a community-based study. Previously captured aortic flow wave images were recreated using Dicom viewer software and a bespoke excel application. Aortic pressure versus time data were extracted from SphygmoCor software and matched according to time with flow in order to separate aortic pressure into its component waveforms. Multivariate regression analysis was used to compare the data in HF patients to that of the controls after adjusting for confounders.

RESULTS & DISCUSSION: The majority (94.1%) of HF patients had mild-to-moderate HF and 69.7% were receiving vasodilators. HF patients had lower peripheral and central BP and Pb ($p<0.005$) and higher HR ($p<0.005$) than controls. After adjusting for confounders including MAP, no differences in aortic haemodynamics were noted. When assessing the impact of BP control, after adjusting for confounders, HF patients with uncontrolled BP had higher Zc ($p<0.005$), Pf ($p<0.05$) and SVR ($p<0.05$) than both HF patients and controls with controlled BP. Moreover, despite similar PP to controls with uncontrolled BP, Zc ($p<0.005$) and SVR ($p<0.05$) were higher in HF patients with uncontrolled BP.

CONCLUSIONS: In conclusion, the lower aortic PP observed in stable patients with mild-to-moderate HF is not due to reductions in systolic function. However, in patients with HF in the absence of controlled BP, aortic stiffness (Zc) and SVR are increased. Hence, BP control is imperative in patients with HF to protect the heart from the detrimental effects of increased afterloads.

ACKNOWLEDGEMENTS: NRF, Oppenheimer memorial trust

05**TITLE**

The effects of ibogaine on myelination in Sprague Dawley rats

AUTHORS

Demi Govender¹, Nancy Meulenberg², Gavin Owen², Tanya Calvey³

AFFILIATIONS

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- Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of the Witwatersrand**
- Department of Human Biology, Faculty of Health Sciences, University of Cape Town**

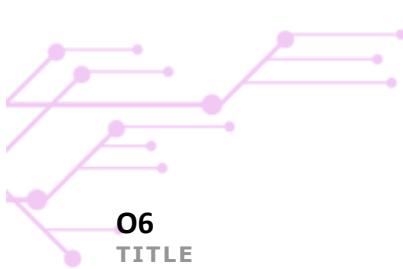
BACKGROUND: The growing opioid epidemic is a worldwide issue which is prevalent in South Africa with the use of opioid cocktails such as nyaope. A possible solution to this problem is the use of psychedelic assisted psychotherapy. Ibogaine is a psychedelic that has been shown to curb addiction cravings and have neuroplastic effects in the brain. Ibogaine is extracted from the root bark of a West African plant and has shown to have neuroplastic effects in the brain. We investigated whether these antiaddictive properties are due to remyelination of the brain's white matter.

METHODS: This study uses qPCR and western blotting to determine how myelin specific proteins and genes such as CNPase (CNP), Myelin Basic Protein (MBP) and Proteolipid Protein (PLP) are affected by morphine (opioids) and ibogaine. The experimental rat groups included a saline, morphine and ibogaine only controls, a combination morphine and ibogaine and a second combination morphine and ibogaine which included a 3 day withdrawal after ibogaine injection.

RESULTS & DISCUSSION: CNP protein was increased in the second morphine ibogaine group ($p<0,0001$) and the CNP mRNA fold expression was increased in the first morphine ibogaine group compared to the second morphine ibogaine group ($p=0,0343$). The 18,5 kDa isoform of MBP had increased expression in the ibogaine control ($p=0,0384$) and second morphine ibogaine group ($p=0,0037$). PLP shows increased protein expression in the second morphine ibogaine group when compared to the first group ($p=0,0464$). There is decreased PLP mRNA expression in the ibogaine control group when compared to morphine control ($p=0,0033$), first morphine ibogaine ($p<0,0001$) and second morphine ibogaine groups ($p=0,003$).

CONCLUSIONS: Ibogaine may cause remyelination following demyelination by morphine. A consistent trend in the data shows that the myelin proteins were increased after the 3 days after administration of ibogaine following chronic morphine administration compared to 1 day after administration of ibogaine. This suggests that remyelination takes between 24-72 hours before it begins to produce new myelin around the axons due to ibogaine. These results also show that CNP and MBP increase in expression earlier than PLP and are good markers for early remyelination. This is consistent with increase in CNP mRNA expression for CNP seen in the first morphine ibogaine but not the second group revealing an immediate effect on mRNA but a delay in protein expression.

ACKNOWLEDGEMENTS: Funding Bodies: The International Society for Neurochemistry (ISN) and the National Research Foundation.

**06****TITLE**

Synergistic solutions: The potential of repurposing ibuprofen for combatting cutaneous infections

AUTHORS

Shivar Simbu, Ané Orchard, Maryna van de Venter, Sandy van Vuuren

AFFILIATIONS

Pharmaceutical Microbiology (Department of Pharmacy and Pharmacology)

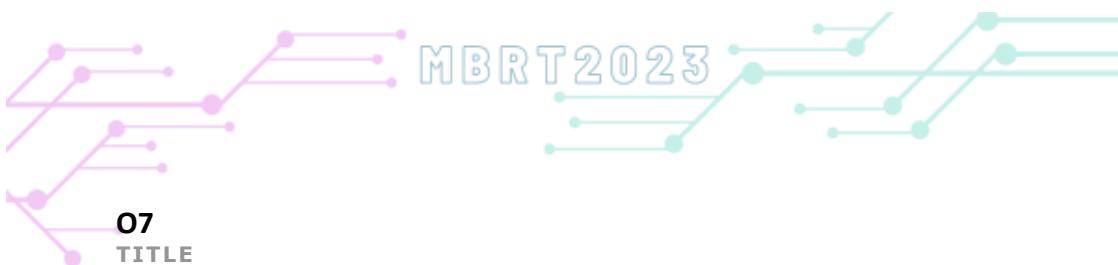
BACKGROUND: The increasing occurrence of multi-drug resistant (MDR) pathogens in skin infections has limited the available therapeutic options. Repurposing non-antibiotics represents an emerging strategy that can help fast-track the drug development process. Ibuprofen represents a class of repurposed drugs called non-antibiotics which demonstrate antimicrobial activity. This study aimed to investigate the interactive properties of combining eight conventional antimicrobials with ibuprofen against common skin pathogens.

METHODS: Materials: Eight conventional antimicrobials (amoxicillin, ciprofloxacin, erythromycin, tetracycline, gentamicin, meropenem, miconazole and nystatin) and ibuprofen. Reference strain pathogens (*Staphylococcus aureus* (ATCC 25923 & ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), *Cutibacterium acnes* (ATCC 11827) and the yeast *Candida albicans* (ATCC 10231). Methods: The antimicrobial analysis was conducted using the broth microdilution method, to determine the minimum inhibitory concentrations (MIC) and fractional inhibitory concentrations (FIC). The cytotoxicity analysis was conducted on HaCAT keratinocytes using the MTT cytotoxicity assay. The anti-inflammatory analysis was conducted on lipopolysaccharide (LPS) activated RAW 264.7 macrophages using the LPS-NO induced assay.

RESULTS & DISCUSSION: For the antimicrobial analysis, four synergistic interactions (ΣFIC 0.33 - 0.50) were identified between ibuprofen and conventional antimicrobials, with the combination of amoxicillin and ibuprofen demonstrating the highest degree of synergy ($\Sigma FIC = 0.33$) against *Cutibacterium acnes*. For the cytotoxicity, none of the combinations demonstrated a cytotoxic effect (cell viability of 93.6-100%). For the anti-inflammatory results, none of the combinations reduced nitrite production however, no antagonistic interaction was observed.

CONCLUSIONS: The combination strategy of ibuprofen and conventional antimicrobials can pave the way for developing new combination therapies to treat MDR infections. However, further studies of the underlying mechanisms supported by animal efficacy experiments are required for the development of safe drug combination usage.

ACKNOWLEDGEMENTS: Dr Luanne Venables, Mrs Anna Hattingh and Mrs Phumzile Moerane. National Research Foundation (NRF) and Faculty Research Committee (FRC).

**07****TITLE**

Assessing the Effects of Low-density Lipoprotein Receptor Upregulation on Alzheimer's Disease Hallmarks

AUTHORS

Tshipamba, E.K., Bignoux, M.J., Otgaar, T.C., Ferreira, E.

AFFILIATIONS

National Research Foundation

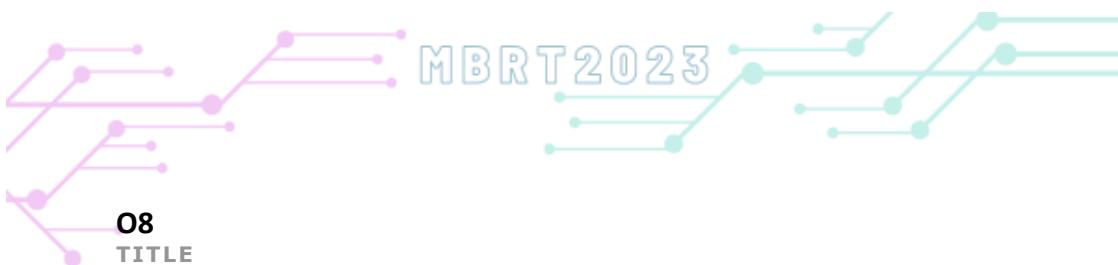
BACKGROUND: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by cognitive decline and the formation of amyloid beta (A β) plaques and neurofibrillary tau tangles in the brain. Currently, there is no effective treatment for AD. Recent studies have shown a link between hypercholesterolemia (elevated low-density lipoprotein (LDL) cholesterol commonly due to mutations in the LDL receptor (LDLR) gene) and AD, such as the influence of high cholesterol on A β production and the altered cholesterol homeostasis characteristic of the AD brain. Hence, this study aimed to investigate the effect of LDLR upregulation on a hypercholesterolemic-AD cell model and AD-related protein expression.

METHODS: A squalene synthase inhibitor (SSI) was used to upregulate LDLR and inhibit cholesterol production in HEK293 and U87 cells. The MTT and alamar blue cell viability assays assessed SSI's cytotoxicity and its ability to protect cells from LDL and A β protein after 24-hour exposure. Western blot analysis was performed to measure the effect of SSI on amyloid precursor protein (APP) and phosphorylated glycogen synthase kinase 3 β (p-GSK3 β) levels, both associated with AD pathology.

RESULTS & DISCUSSION: The results showed that LDLR upregulation significantly increased APP and p-GSK3 β expression in a dose-dependent manner, suggesting a possible reduction in A β formation and tau hyperphosphorylation downstream. Importantly, LDLR upregulation with SSI was non-toxic and rescued the hypercholesterolemic-AD cell model after 24-hour exposure.

CONCLUSIONS: Hence, squalene synthase inhibition could have therapeutic potential for AD by modifying key cellular markers and enhancing cell viability in a hypercholesterolemic-AD cell model, potentially addressing the early pathogenic changes associated with the disease.

ACKNOWLEDGEMENTS: This research has been funded by the National Research Foundation (NRF). The author has been funded by the Postgraduate Merit Award from the University of the Witwatersrand.

**O8****TITLE**

Unravelling Cell Type-Specific STAT Pathway Utilisation in the Interferon-alpha Response

AUTHORS

Moonsamy, D and Gentle, NL

AFFILIATIONS

University of the Witwatersrand

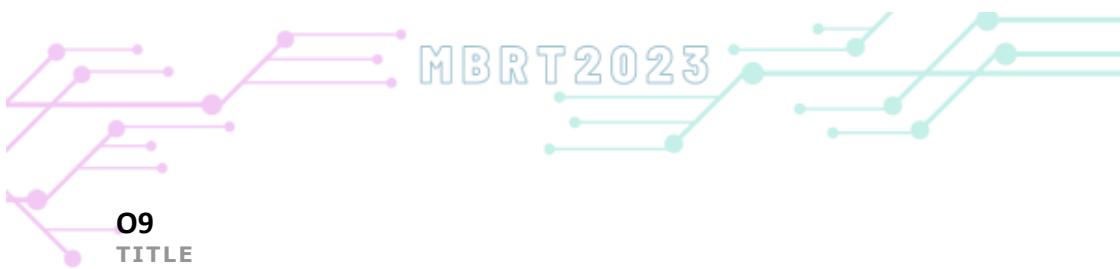
BACKGROUND: Interferon- α (IFN- α) is an antiviral cytokine that induces cell-type-specific immunomodulatory effects in immune cells. While a canonical pathway, using STAT1, STAT2 and IRF9, is well-described, it is increasingly recognised that distinct cell types employ non-canonical STAT pathways to achieve the cell-type-specific response. Yet, the capacity of different cell types to utilise specific STATs and the correlation with their response is unclear. Thus, we investigated whether baseline expression patterns of STAT signalling components reflect specific response signatures in distinct immune cell types to provide insight into the determining factors potentially driving cell type-specific responses.

METHODS: Leveraging single-cell RNA-sequencing data from untreated and IFN- α -stimulated PBMCs, we characterised baseline expression patterns and performed differential gene expression analysis across distinct cell types identified using a graph-based clustering approach. Bulk RNA-sequencing data from knockout studies and ChIP-sequencing data were employed to validate STAT pathway usage.

RESULTS & DISCUSSION: NK and CD8+T cells were characterised by high STAT4 expression and displayed a shared response signature, encompassing cytolysis-associated genes directly regulated by STAT4. STAT6 and IFNAR expression uniquely characterised monocytes, which exhibited a cell-type-specific signature incorporating genes that prime monocytes for activation/differentiation. Furthermore, these genes operated independently from the canonical pathway and harboured functional STAT6 binding sites, demonstrating potential usage of the STAT6 pathway.

CONCLUSIONS: Our findings highlight an association between baseline STAT expression and the usage of specific STAT pathways in driving cell-type-specific immunomodulatory signatures. Furthermore, we describe a novel role for STAT6 in the monocyte response to IFN- α . This work has implications for understanding cell-type-specific dysregulation in autoimmune/inflammatory diseases.

ACKNOWLEDGEMENTS: I would like to Acknowledge my supervisor (Dr Nikki Gentle), the National Research Foundation, the members of FunGenics, and friends

A decorative graphic at the top of the page features a series of colored dots (pink, light blue, and teal) connected by thin lines, creating a branching, tree-like or network-like pattern.

09

TITLE

Neurobehavioral and molecular changes in a rodent model of ACTH-induced HPA axis dysfunction

AUTHORS

Farhanah N Sallie, Leandrie Pienaar, Andrea Lubbe, Sanelisiwe Xhakaza, William MU Daniels, Aletta ME Millen, Sooraj Baijnath

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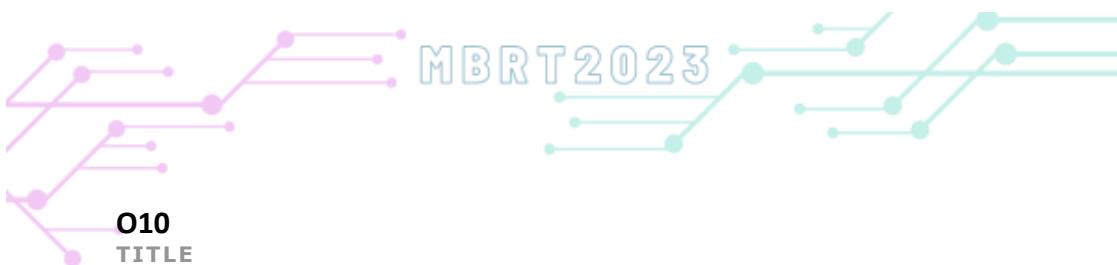
BACKGROUND: Hypothalamic-pituitary-adrenal (HPA) axis dysregulation is linked to the pathophysiology of depression. Administration of adrenocorticotrophic hormone (ACTH) is associated with a depressive-like phenotype in rodents, however neurobehavioral and molecular evidence to support these findings are lacking.

METHODS: Male Sprague-Dawley rats ($n=30$) were randomly assigned to the control ($n=10$); or ACTH groups ($n=20$) that received saline (0.1ml) or ACTH (100 μ g/day), respectively, for two weeks. Thereafter, rats in the ACTH group were divided into saline-treated ($n=10$; 0.2ml) or imipramine-treated ($n=10$; 10mg/kg) groups, for four weeks. Neurobehavioral changes were assessed using the forced swim test (FST), the sucrose preference test (SPT) and the open field test (OFT). mRNA expression of neurotrophic factors in different brain regions were measured using RT-PCR.

RESULTS & DISCUSSION: Neither ACTH nor imipramine treatment affected FST parameters. In the OFT, ACTH administration decreased the locomotor activity, which were not ameliorated by imipramine treatment. ACTH administration resulted in increased sucrose consumption compared to controls, while co-treatment with imipramine resulted in an increased water consumption compared to controls and ACTH treated rats. ACTH-treatment resulted in differential expression of BDNF and CREB across different brain regions compared to controls.

CONCLUSIONS: ACTH administration decreased locomotor activity, increased hedonia, with no changes in behavioural despair. Imipramine treatment did not alter the ACTH-induced neurobehavioral changes. Differential regional gene expression of neurotrophic factors suggests varied pathophysiological processes may be involved in HPA axis dysregulation-induced depressive like symptoms.

ACKNOWLEDGEMENTS: The authors would like to thank Ziphlo Zwane for his technical assistance during this study. The authors would like to thank Peptide Science Laboratory of The University of KwaZulu-Natal for the synthesis of ACTH. The authors would also like to thank the staff at the WRAF for their care and technical assistance with animal experiments. Lastly, the authors would like to thank the National Research Foundation (South Africa) and the University of the Witwatersrand for funding this study.



O10

TITLE

Investigating the role of ancestral derived AAV capsid to deliver transgenes *in vitro* and *in vivo*

AUTHORS

Jacobs,R., Ely, A., Bloom, K., Arbuthnot, P and Maepa, B.

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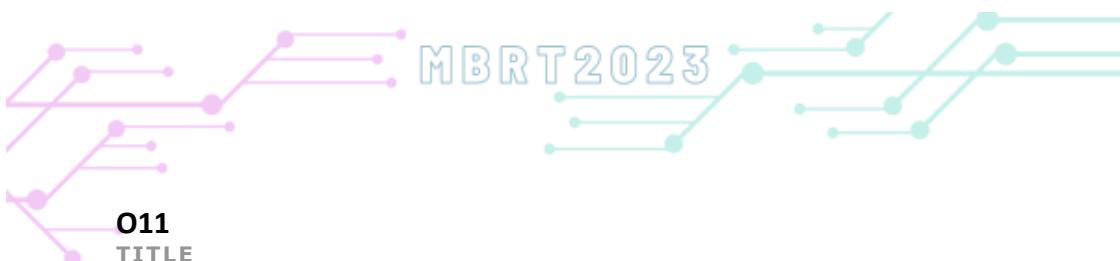
BACKGROUND: As the field of gene therapy evolves and expands, the search for suitable delivery tools is bound to widen. Presently, Adeno-associated viruses (AAVs) has demonstrated great potential as gene delivery vehicles. The attractive nature of this viral vector has resulted in five AAV-enabled gene therapies receiving FDA approval. A major disadvantage is that 30-60% of the population have neutralizing antibodies (nAbs) against naturally occurring AAV serotypes (e.g AAV8 and AAV2) prompting an immune response and subsequent clearance before a therapeutic benefit can be observed. Strategies to evade the immune system has been investigated and one such strategy is evolutionary lineage analysis. This strategy has led to the discovery of Anc 80, the predicted ancestor of AAV 1,2,8 or 9, but is more closely related to AAV 8.

METHODS: This study investigated the efficacy of Anc 80 as a delivery vehicle compared to AAV 2 *in vitro* and AAV 8 *in vivo*. As well as determining whether Anc 80 is neutralized by AAV 8 specific antibodies *in vivo*. Using Huh 7 cells, a luciferase assay was conducted to determine the expression of the luciferase (Fluc) encapsulated in either AAV 2 or Anc 80.

RESULTS & DISCUSSION: Expression of Fluc was observed with AAV 2 Fluc or Anc 80 Fluc as compared to its respective controls. *In vivo*, HBV transgenic mice were injected with either AAV 8 Fluc or Anc 80 Fluc, greater expression in mice was observed with Anc 80 Fluc. Expression with either AAV8 Fluc or Anc 80 Fluc persisted for 3 months. Assessing neutralization of Anc 80 by AAV 8 antibodies, HBV transgenic mice were injected with either AAV 8 empty or Anc 80 Empty or saline. Once suitable AAV antibody titers were obtained, mice we reinjected with either AAV 8 Fluc or Anc 80 Fluc or saline. Data shows that Anc 80 is partially neutralized by AAV 8 specific antibodies.

CONCLUSIONS: These results provide valuable information to the field of gene therapy as well as explores the possibility of using ancestral capsids to evade the immune system.

ACKNOWLEDGEMENTS: This research has been funded by SAMRC. Author/s has been funded by NRF, PRF and SAMRC.

**O11****TITLE****Unveiling SARS-CoV-2 Mpro: A Biochemical quest****AUTHORS****Mpho Setschedi and Prof Yasien Sayed****AFFILIATIONS****NRF**

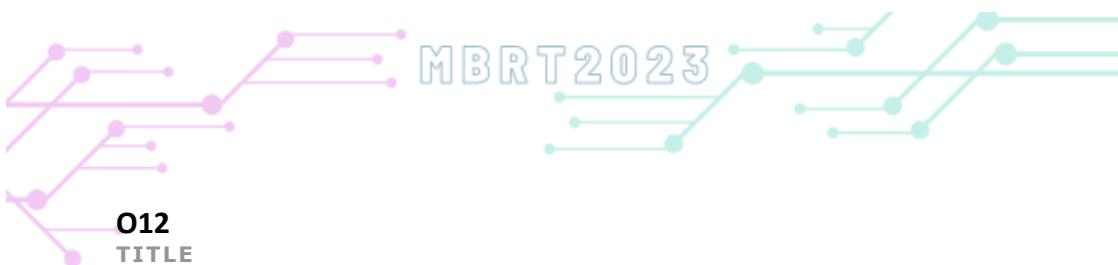
BACKGROUND: On January 2020, the world Health Organization (WHO) announced COVID-19 as a public health emergency of international concern and on March 2020, declared severe acute respiratory syndrome coronavirus (SARS-CoV-2) a pandemic. The main protease of SARS-CoV-2 (Mpro) has emerged as a pivotal target in the development of antiviral drugs to combat the COVID-19 pandemic. The Mpro, which is essential in processing RNA translation, is an attractive drug target. Despite the huge number of research studies reported so far, the development of safe and effective drugs able to block the viral infection is still lacking and represents a major goal for the scientific world.

METHODS: This study presents a comprehensive biochemical characterisation of the Wuhan SARS-CoV-2 Mpro, shedding light on its structural and functional properties.

RESULTS & DISCUSSION: We have shed light on the catalytic mechanism, substrate specificity, and allosteric regulation of Mpro. Far-UV Circular dichroism spectroscopy enabled us to unravel the secondary structure content of Mpro. Enzyme kinetics studies unveiled the catalytic prowess of Mpro, providing a detailed analysis of its substrate binding and turnover rates. We characterized key kinetic parameters, such as K_m and V_{max} , shedding light on the enzyme's substrate specificity and efficiency. Inhibition studies further enriched our understanding of Mpro's potential as a therapeutic target.

CONCLUSIONS: We explored a range of compounds and elucidated their inhibitory effects, offering a foundation for the development of antiviral drugs. This biochemical quest has deepened our understanding of SARS-CoV-2 Mpro, offering valuable insights for future drug development and a potential roadmap for combatting COVID-19.

ACKNOWLEDGEMENTS: Prof Yasien Sayed

**O12****TITLE**

Biorenewable waste products as building blocks for novel Lipid Nanoparticles to deliver RNA vaccines

AUTHORS

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AFFILIATIONS

¹Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, Infectious Diseases and Oncology Research Institute (IDORI), University of the Witwatersrand. ²Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand

BACKGROUND: The COVID-19 pandemic has highlighted the benefits of developing an mRNA platform for rapid response to combat current pathogens and future pandemics. mRNA synthesis technology is a versatile platform allowing strong cell and humoral immune responses to vaccine antigens. However, the mRNA drug substance requires a delivery system, such as lipid nanoparticles (LNPs), to be effective by preventing RNA degradation and acting as an adjuvant. LNPs comprise of an ionisable or cationic lipid, cholesterol, phospholipid, and a PEGylated lipid. Traditionally, ionisable lipids are derived from petroleum sources, and require multiple purification steps, making them costly to synthesize. The PEGylated lipids also potentially have side effects. Identifying novel lipids, and lipid sources for safer and more sustainable mRNA-LNP vaccine production remains important.

METHODS: Here, we have formulated mRNA-LNPs using novel ionisable lipids derived from Cashew nutshell, an abundant, biorenewable waste product. Simple solvent injection and microfluidics methods were used to formulate LNPs. LNP size and encapsulation efficiency were assessed using dynamic light scattering and RiboGreen assay respectively.

RESULTS & DISCUSSION: Single- and multiple-component LNPs of diameters <150 nm were successfully formulated. LNPs encapsulated mRNA with high efficiency (<90%) and show strong transgene expression when used to deliver mRNA in cell culture experiments. Successful in vivo delivery of mRNA after intramuscular and intradermal injection emphasizes potential of the novel lipids for use in vaccines.

CONCLUSIONS: The data generated in this work has laid the groundwork for evaluation of novel LNP formulations using empirical design of lipid chemical structures and combinations for improved vaccine delivery.

ACKNOWLEDGEMENTS: This research and authors have been funded by South African Medical Research Council, Medicines Patent Pool, National Research Foundation, and Poliomyelitis Research Foundation.

Poster presentation abstracts

Poster number	First name(s)	Surname	Abstract Title:
1	Jayne	Polley	IgA1 allelic variation improves neutralization and Fc receptor binding of a potent SARS-CoV-2 antibody
2	Diandre	Gomez	Investigating the diagnostic performance of the ThermoFisher TaqPath Covid-19, Flu A/B, RSV Combo Kit for use in the South African Public Health Sector.
3	Richard	Graca	Structural Characterisation of Klebsiella pneumoniae Antimicrobial Resistance Enzyme: Aminoglycoside (3') (9) Adenyltransferase
4	Teigra	Green	Pan-genome dynamics and phylogenomic analysis of the Enterobacter hormaechei species complex.
5	SARJAN	PATEL	Formulation of a thermo-responsive nasal gelling system for the treatment of opioid use disorder.
6	Hyeonah	Byun	Assessment of the <i>In Vitro</i> Phenotypic Drug Susceptibility of HIV-1 Subtype C Drug Resistant Variants to Islatravir
7	Jason	Futter	Development of immunoreagents and assays for immunological surveillance of respiratory syncytial virus
8	Danielle	Martin	Design of soluble HIV-1 Env trimers from highly neutralisation-resistant HIV-1 strains for the isolation of broadly neutralising antibodies.
9	Keila	Neves	Hepatitis B virus (HBV) surface antigens delivered <i>in vitro</i> and <i>in vivo</i> using recombinant adenoviruses for immunisation against HBV infection.
10	Oliver Khan	Fonguh	Functional consequences of novel IgG3 allelic variation on HIV broadly neutralizing antibody CAP256-VRC26.25
11	Aviwe	Matsha	Investigating the foraging behaviour of entomopathogenic nematodes cultured <i>in vitro</i>
12	Sichumiso Zimi	Gqeba	Evaluating PLGA Encapsulated Recombinant LRP as a treatment for Cardiovascular Disease
13	Mistral	Sebastian	Investigating the DNA Methylation Status of the PXDN and PXDNL Promoter Regions in OSCC Cell Lines
14	Lorato	Mokoto	The design and synthesis of solvated encapsulated HIV-1 protease inhibitor - Lopinavir
15	Kiyasha	Padarath	Comparison of the proteome of Huh-7 cells transfected with replication-competent different (sub)genotypes of Hepatitis B Virus prevailing in and outside sub-Saharan Africa
16	Marushka	Soobben	In Silico Exploration of CB1 and CB2 Receptor Interactions comparing CBD and CBD Diacetate: A Comprehensive Computational Study
17	Rethabile J	Mokoena	Investigating potential plasma nephrotoxicity biomarkers associated with first-line antiretroviral therapy regimens in people living with HIV (PLHIV) in South Africa.
18	Mbali	Nkosi	Epidemiological characteristics and rates of Pneumocystis jirovecii pneumonia in South Africa from 2018 to 2022
19	Risuna	Maswanganyi	<i>In Vitro</i> Characterization of a Single and Combination rtk33Q Mutation Isolated from HIV-infected Individuals with Occult Hepatitis B in Botswana
20	Rutendo	Ndemera	Antibodies produced in a cost-effective fungal expression system neutralizes SARS-CoV-2 variants
21	Thandeka	Malinga	Characterisation of the genetic variation in pharmacogenes involved in anti-tuberculosis drug metabolism across African populations
22	Carl	Belger	Characterizing the gut microbiome of lions in Etosha National Park
23	Tiffany Shenay	Smith	Obligate heterodimeric TALEN-encoding mRNA attenuates hepatitis B virus replication in cultured mammalian cells
24	Tshele	Mokhantso	Exploring Structural Dynamics and Drug Resistance Implications in a Novel HIV-1 Subtype C Protease Hinge-Region Variant from South Africa
25	Akeel	Valli	Targeting the dimer interface of the Schistosoma glutathione transferase enzyme for the novel treatment of human schistosomiasis
26	Nday Lucie	Sungu	Isolation and characterization of mycobacteriophages Bora and Wildflower
26	Qondubuhle	Dube	Expression & Purification of an AIRU-B12 antibody against SARS-CoV-2 using an alternative recombinant protein expression system
27	Dintle	Mototo	Designing an Experimental Model to Probe UPR Mechanisms in Breast Cancer
28	Melissa	Nyalungu	Potential interaction between the SARS-CoV-2 nucleocapsid protein and the forkhead domain of FOXP2
30	Zak	Kayat	Probing a link between the MRN complex and DNA damage associated lncRNAs, DDSR1 and HITTERS

Poster number	First name(s)	Surname	Abstract Title:
31	Dineo	Mashamaite	Investigating a potential interaction between the DNA binding domains of Pax6 and FOXP2
32	Nikita	Reddy	<i>In Vitro</i> Phenotypic Doravirine Susceptibility of Prevalent HIV-1 Subtype C NNRTI-Resistance Mutations
33	Mpho	Motjuwadi	Antibody dynamics during prolonged SARS-CoV-2 infection in people living with HIV and HIV-uninfected individuals
34	Ingrid	Smit	Cystic fibrosis: An update on the variant profile and carrier frequency in the Black South African population
35	Ofentse Thoriso	Lesito	Validation of the LumiraDx point-of-care for diabetes diagnosis and monitoring.
36	Phuti Jason	Mphaki	Prevalence of HPV in OSCC Patients Attending the Charlotte Maxeke Johannesburg Academic Hospital
37	Sibusiso	Radebe	Molecular characterisation of High-Grade B-cell Lymphomas with rearrangements in the MYC and BCL6 genes
38	Tasneem	Farhad	Insertion of transgenes into the adenovirus genome through homologous recombination
39	Jessica	Hurwitz	Overexpression and purification of the vitamin D receptor for protein-protein interaction studies.
40	Keenan	Ikking	Exploring Cell-Specific Isoform Usage Using Long-Read Sequencing Data
41	Keiran	McInnes	FOXP3 and SARS-CoV-2 Nucleocapsid: A Clot to Uncover
42	Oboikanyo	Mokoka	Cassava Mosaic Disease recovery is associated with phytohormones, South African cassava mosaic virus titre, and viral effector-proteins.
43	Raymond	Hartman	Structural characterization and crystallization of the TBR1 DNA binding domain in the absence and presence of DNA and FOXP2:
44	Shiven	Naidoo	Using ChIP-Seq and Gene Expression Microarray data to explore transcriptional dysregulation of PXDN in cardiovascular diseases
45	Shweta	Tooray	Unveiling the biochemical pathway between Type 2 Diabetes Mellitus and early Alzheimer's disease
46	Simphiwe	Hlatshwayo	DNA damage response lncRNA's DDSR1, TUG1, PANDA, and ANRIL expression changes in response to neocarzinostatin in human oesophageal squamous carcinoma cell lines.
47	Thabelo	Mulenga	Exploring the Structure, Function and Stability of Glutathione Transferases Engineered from Intra- and Inter-class Consensus Sequences: How Forgiving is Nature?
48	Thokozile	Makhanya	Investigating the regulation of PXDN expression by the early growth response 1 (EGR1) transcription factor in the context of human fibrotic diseases
49	Yi Fan	Xu	Early epigenetic modulations guide the differentiation of monocytes into macrophages.
50	Sasha	Moonsamy	Long non-coding RNA (lncRNA) PANDA as a potential target for combination chemotherapy in oesophageal squamous cell carcinoma (OSCC) cell lines.
51	Reubina	Wadee	PD-L1 in a South African Cohort of Endometrial Carcinomas
52	Tasvi	Daya	Investigating the effects of cholesterol-depletion on pancreatic cancer and drug resistance <i>in vitro</i> and <i>in vivo</i>
53	Vivash	Naidoo	Computational modelling of Tunicamycin C interactions with potential protein targets: perspectives from inverse docking with molecular dynamic simulation
54	Bontle	Masango	Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans
55	Adam James	Berry	Candidate genetic variation associated with the immune response to P2-VP8 Rotavirus vaccination in South African Infants
56	Alicia	Joshua	Investigating the effects of antioxidants on the transcriptional activity of YY1 and FOXP3
57	Jessica Sian	Brothwell	Autism Spectrum Disorders: Speaking through interactions
58	Neo	Padi	Targeting Glutathione Transferases: A Computational Approach to Discovering Antischistosomal Porphyrin Compounds
59	Olamide	Jeje	Obtaining High Yield Recombinant Enterococcus faecium Nicotinate Nucleotide Adenylyltransferase for X-Ray Crystallography
60	Reabetswe	Maake	The expression and biophysical characterisation of Klebsiella pneumoniae adenylyltransferase

Poster number	First name(s)	Surname	Abstract Title:
61	Phil	Ubanako	Molecular Docking and <i>in vitro</i> validation of Imiquimod as a potential Tankyrase 2 Inhibitor in Colorectal Cancer
62	Sanelisiwe	Duze	Evaluation of the Xpert® Carba-R assay in detecting the blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP-1 gene sequences associated with carbapenem-non-susceptibility.
63	Maryam	Hashmi	Structural and functional studies of hemoglobin from the greater flamingo (<i>Phoenicopterus roseus</i>) through in-silico approach

P1**TITLE**

IgA1 allelic variation improves neutralization and Fc receptor binding of a potent SARS-CoV-2 antibody

AUTHORS

Jayne Polley^{1,2}, Bronwen E. Lambson^{1,2}, Penny L. Moore^{1,2,3}, Simone I. Richardson^{1,2}

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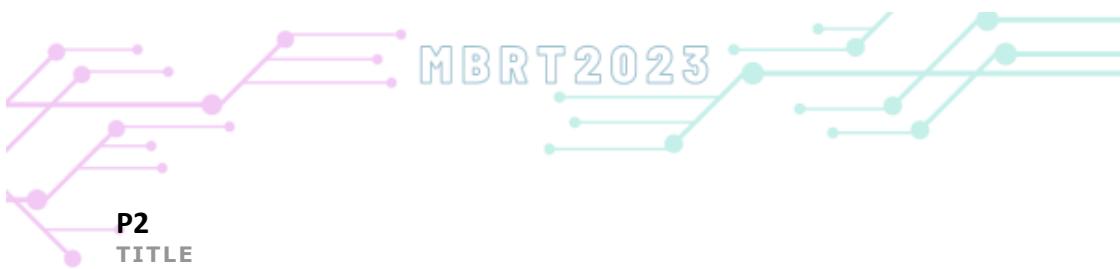
BACKGROUND: Antibodies possess allelic variation within the constant region which can alter antigen binding and Fc effector functions. We previously identified seven unique IgA1 variants from a group of African individuals, but the functional effects of this variation are unknown. This study's aim was to determine whether IgA1 allelic variants of a potent SARS-CoV-2 antibody impact neutralization or binding to the SARS-CoV-2 spike or FcαR.

METHODS: We used the 084-7D antibody, isolated from a Beta-infected individual, that previously showed significantly greater neutralization potency as an IgA1 variant compared to IgG1. We produced this antibody as seven unique allelic variants as well as IgA1 and IgG1. Neutralization was assessed using an eight-virus panel of SARS-CoV-2 variants of concern. Beta spike and FcαR binding were assessed using enzyme linked immunosorbent assays.

RESULTS & DISCUSSION: 084-7D IgG1 showed substantially decreased binding to the Beta spike compared to IgA1*01 and the seven variants, with significantly improved binding observed for T320A/D428E, R392H/L451M and F411Y/L451M variants relative to IgG1. Substantial, virus-specific differences in neutralization were observed, with IgA1 and the variants being between 12.2 and 292.7-fold more potent than IgG1. Overall, F411Y/L451M and K126E/L451M showed the greatest neutralization potency compared to IgA1 with the R392H variant showing the lowest. FcαR binding showed a significant decrease in binding for K126E/L451M relative to IgA1, with the other variants exhibiting no significant differences.

CONCLUSIONS: IgA1 allelic variation has a nuanced but significant impact on neutralization and binding to antigen and Fc receptors, suggesting natural genetic diversity can be harnessed to engineer antibodies against respiratory disease.

ACKNOWLEDGEMENTS: We thank Alaine Marsden who identified the allelic variation which formed the foundation of this study, as well as Strauss van Graan, Qiniso Mkhize, Thrishantha Pillay, Thamara Naidoo, Donald Mhlanga, Sinethemba Bhebhe, Sashkia Balla and Nelia Manamela for their assistance in training.



P2

TITLE

Investigating the diagnostic performance of the ThermoFisher TaqPath Covid-19, Flu A/B, RSV Combo Kit for use in the South African Public Health Sector

AUTHORS

Diandre Ronaldo Gomez, Dr. Riffat Munir, Prof. Lesley Scott, and Prof. Wendy Stevens

AFFILIATIONS

Wits Diagnostic Innovation Hub

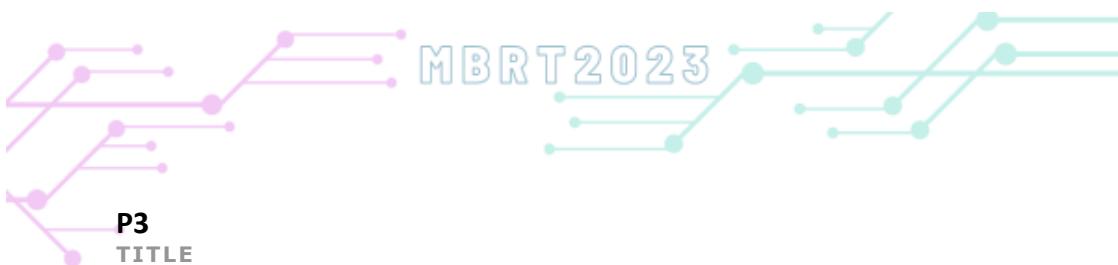
BACKGROUND: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A/B, and respiratory syncytial virus (RSV) are highly prevalent and are associated with the global increase in morbidity and mortality. These co-circulatory infectious agents have similar clinical presentations which makes diagnosis and differentiation between them difficult. Co-infections between these infectious agents have been observed, however, the extent of these cannot be properly assessed due to testing limitations. Therefore, the aim of this study was to evaluate the analytical performance of the TaqPath™ Covid-19, Flu A/B, RSV Combo Kit to simultaneously detect SARS-CoV-2, influenza A/B, and RSV.

METHODS: This was achieved by determining the precision, accuracy (sensitivity, specificity, and agreement), and limit of detection (LoD) of the assay. Results obtained on standard-of-care assays were used as the comparator.

RESULTS & DISCUSSION: Overall, the TaqPath was able to generate reproducible results and achieved a sensitivity of 97.5% (95% CI: 92.7%-99.5%) and a specificity of 100% (95% CI: 86.8%-100%). A Cohen Kappa value of 0.9326 (0.8574-1.0079) was determined for the assay suggesting a very good agreement. The LoD of the assay was <100 cp/ml.

CONCLUSIONS: Altogether, these results suggest that the assay has an excellent potential to be used for clinical diagnostic purposes in detecting SARS-CoV-2, influenza A/B, and RSV. Furthermore, the assay can be added to the 'arsenal' of limited molecular assays that can simultaneously diagnose common, co-circulatory respiratory viruses.

ACKNOWLEDGEMENTS: Prof. Wendy Steven, Prof. Lesley Scott, Wits Diagnostic Innovation Hub Clinical Trials Lab, Dr. Riffat Munir.

**P3****TITLE**

Structural Characterisation of Klebsiella pneumoniae Antimicrobial Resistance Enzyme: Aminoglycoside (3'') (9) Adenylyltransferase

AUTHORS

Richard Graca and Dr Ikechukwu Achilonu

AFFILIATIONS

Protein Structure-Function Research Unit

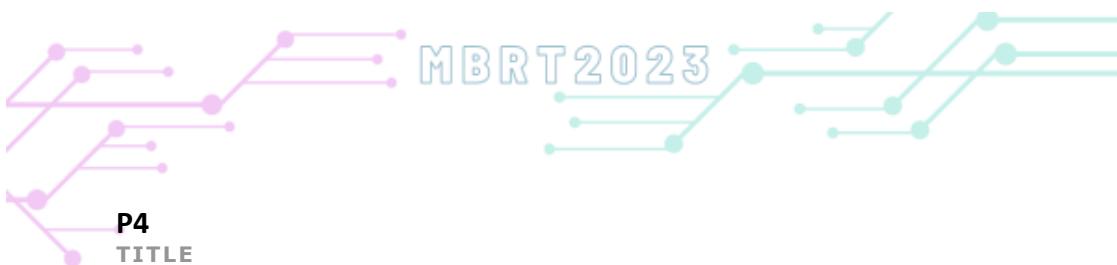
BACKGROUND: Misuse of antibiotics has led to the growing health crisis presented by antimicrobial resistance (AMR). Alternate treatment options to antibiotics are therefore required due to the decline in the discovery of novel agents since the 1950's. These include the use of adjuvant drugs intended to inhibit different AMR mechanisms found in various infections. This investigation aimed to begin characterising an enzyme thought to contribute to AMR in Klebsiella pneumoniae, and to confirm the functionality of a recombinant form of this protein for future studies.

METHODS: Recombinant K. pneumonia 3'' aminoglycoside adenylyltransferase (RKAAT) was expressed in a T7 E. coli expression host and purification made use of immobilised metal affinity chromatography. Once purified, the protein underwent preliminary structural characterisation, before undergoing functionality and stability assessments. Circular dichroism (in the far ultraviolet range) characterised the secondary structure, while intrinsic and extrinsic fluorescence studies, using 8-Anilinonaphthalene-1-sulfonic acid (ANS) assessed the tertiary structure.

RESULTS & DISCUSSION: RKAAT showed predominantly α -helical secondary structure and was found to be expressed in a folded conformation. Fluorescent residues are likely found in hydrophobic regions of the protein. Additionally, the protein was found to contain hydrophobic regions available for ANS binding, possibly associated with the binding site. Moreover, the ANS studies showed catalytic functionality when performed with both RKAAT substrates, though higher precision is needed for thermodynamic assessment. Thermal shift assays showed a stabilising effect of magnesium, seen most notably in ATP containing samples.

CONCLUSIONS: This study established that RKAAT is likely a functional protein, suitable for future research applications.

ACKNOWLEDGEMENTS: Dr Achilonu as supervisor.



P4

TITLE

Pan-genome dynamics and phylogenomic analysis of the *Enterobacter hormaechei* species complex

AUTHORS

Teigra Green and Pieter De Maayer

AFFILIATIONS

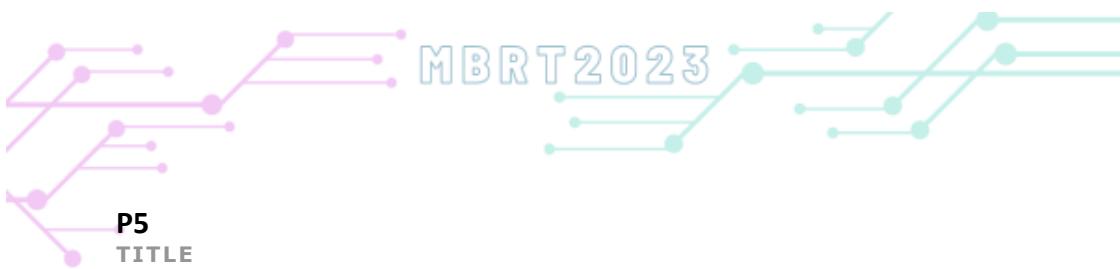
Professor Pieter De Maayer's Lab

BACKGROUND: The *Enterobacter hormaechei* complex currently comprises of five distinct subspecies, namely, *E. hormaechei* subsp. *hormaechei*, *E. hormaechei* subsp. *hoffmannii*, *E. hormaechei* subsp. *xiangfangensis*, *E. hormaechei* subsp. *oharae*, and *E. hormaechei* subsp. *steigerwaltii*. Yet modern pan-genomic evaluations have revealed that this species classification is incorrect, and that the complex is currently in a state of 'taxonomic chaos' and disarray, primarily due to the use of low-resolution analytical methods. The clinical relevance of the *E. hormaechei* species complex has also been significantly underestimated, largely due to the taxonomic obscurity of the species complex, hindering accurate isolate identification for precise treatment. Moreover, few studies have been conducted into their pathogenic-associated factors, despite their role in health-associated diseases.

METHODS: This study used high resolution bioinformatic tools (including Prodigal, BUSCO, FastANI, GGD, IQ-TREE, OrthoFinder, PanGP, and eggNOG-mapper) to analyse the pan-genome of 250 *E. hormaechei* complex isolates in order to develop an accurate taxonomic framework, as well as determine species and subspecies specific genes and elucidate their function.

RESULTS & DISCUSSION: Our analysis established *E. hormaechei*, *E. hoffmannii*, and *E. xiangfangensis* as three distinct species, with *E. xiangfangensis* being comprised of three subspecies, namely *E. xiangfangensis* subsp. *xiangfangensis*, subsp. *oharae*, and subsp. *steigerwaltii*. All species and subspecies exhibited an 'open' pan-genome with the ability to acquire substantial amounts of exogenous genes, contributing to the rapid colonization of novel hosts and environments. Proteins unique to distinct species or subspecies were functionally annotated and their potential role in pathogenesis is discussed.

CONCLUSIONS: In conclusion, the ubiquitous and versatile nature of the *E. hormaechei* species complex led us to employ an in-depth comparative pan-genomic analysis. This was the optimal approach to elucidate its phylogenetic relationships and to outline genomic elements of both species and subspecies. This study emphasized the importance of using high resolution analytical techniques for precise differentiation of closely related species and subspecies. Within this species complex, which falls into five distinct clades, we confirmed the existence of three distinct species: *E. hoffmannii*, *E. xiangfangensis*, and *E. hormaechei*. Moreover, *E. xiangfangensis* has been further categorized into three subspecies, namely *E. xiangfangensis* subsp. *xiangfangensis*, *E. xiangfangensis* subsp. *oharae*, and *E. xiangfangensis* subsp. *steigerwaltii*. All species and subspecies displayed an 'open' pan-genome, accentuating their adaptability and potential for diverse habitats and lifestyles. This study also introduced exploration into the pan-genome dynamics of *E. xiangfangensis* subsp. *oharae*, which may serve as a novel research domain. The distribution of COG functional categories within the *E. hormaechei* species....



P5

TITLE

Formulation of a thermo-responsive nasal gelling system for the treatment of opioid use disorder

AUTHORS

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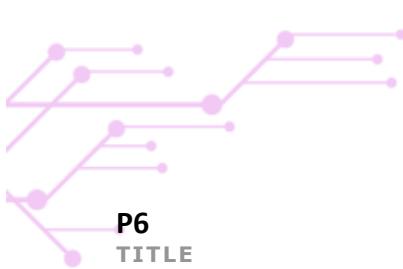
BACKGROUND: In 2022, CDC reported approx. 85 000 deaths due to opioid use disorder (OUD) in the United States. The drugs used in the treatment of OUD have major drawbacks in its bioavailability and residence time. However, this can be resolved by using the injectables, implants and sublinguals with a sustained or controlled drug delivery system but, when it comes to cost, patient compliance and availability, this can be a significant concern. To overcome this, a sustained release thermo-responsive nasal spray containing buprenorphine and naloxone has been formulated for the intermediate and long-term treatment of OUD that will help in reducing the cost, improving the bioavailability of the drugs and increasing patient compliance

METHODS: Polymeric polycaprolactone containing solid lipid nanoparticles of buprenorphine and naloxone were formulated using the double emulsification technique which were then evaluated for their zeta size, zeta potential and drug release profile. The thermo-responsive gel containing Pluronic f-127 and hydroxypropyl methyl cellulose was formulated using the cold method and was evaluated using the rheometer for the rheological properties

RESULTS & DISCUSSION: The thermo-responsive polymeric solid lipid nanoparticles of buprenorphine and naloxone with size and zeta potential range of 150-170 nm and (-18)- (-20) mV were formulated which showed thermo-responsive nature and drug release profile at 34 °C and over a period of 7 days under simulated human nasal conditions, respectively.

CONCLUSIONS: The success in development of this novel system will help in the intermediate and long-term treatment of OUD by reducing the cost, improving the bioavailability of drugs and increasing the patient compliance.

ACKNOWLEDGEMENTS: The research has been funded by SARCHI-ADHOC NRF and the FRC of the University of the Witwatersrand.

**P6****TITLE**

Assessment of the In Vitro Phenotypic Drug Susceptibility of HIV-1 Subtype C Drug Resistant Variants to Islatravir

AUTHORS

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BACKGROUND: Many first-line antiretroviral therapy regimens include the use of two nucleoside reverse transcriptase inhibitors (NRTIs), to which multiple common NRTI mutations are highly resistant. Islatravir (ISL) is a first-in-class NRT-translocation-inhibitor with a long intracellular half-life and a high genetic barrier to resistance. Although preliminary ISL drug-resistance data is available for HIV-1 subtype B, this is still lacking for subtype C, the most nationally prevalent HIV-1 subtype. Hence, we investigated the in vitro phenotypic drug susceptibility of HIV-1 subtype C NRTI drug-resistant variants against ISL.

METHODS: HIV-1 RT sequences obtained from routine HIV-1 genotypic drug resistance testing at the NHLS were scrutinized for the most prevalent NRTI drug resistance mutation (DRM) combinations ($n = 20$). These mutation combinations, as well as the single DRMs from which they are constituted, were introduced into an HIV-1 subtype C gag-pol expression vector. The resulting pseudoviruses produced were then assessed for susceptibility to ISL.

RESULTS & DISCUSSION: Common NRTI mutations K65R and M184V were susceptible and conferred low-level resistance to ISL, respectively. Mutations containing D67N in combination with two or more mutants had high-level resistance to ISL, as did A114S/M184V. The hypersusceptible nature of K65R to ISL, and high-level resistance of A114S/M184V to ISL, have been found in literature, and our fold change values of mutant strains are comparable.

CONCLUSIONS: Our data show that ISL exhibits a resistance profile distinct from that of approved NRTIs, as well as correspond to recent literature discoveries. Results from this study will inform local HIV clinicians on the potential application of ISL for treating HIV-1 infections in our current setting.

ACKNOWLEDGEMENTS: This research has been funded by grant #21/66 from the Poliomyelitis Research Foundation. The student has been funded by grant #22/44 from the Poliomyelitis Research Foundation.

P7**TITLE**

Development of immunoreagents and assays for immunological surveillance of respiratory syncytial virus

AUTHORS

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BACKGROUND: Respiratory Syncytial Virus (RSV) is a leading cause of infant and child (< 5 years) mortality worldwide, particularly in low-and-middle-income countries. The roll-out of maternal RSV pre-F vaccines in South Africa highlights the need for local immunological surveillance, which is currently lacking for this virus. Here we describe the development of immunoreagents and assays for RSV F protein immunological surveillance.

METHODS: We expressed stabilised pre-F protein constructs for RSV A and B strains and purified these using nickel-affinity and size-exclusion chromatography. We expressed several anti-RSV mAbs (ADI-15560, Clesrovimab, CR9501, MPE8, Nirsevimab, and Palivizumab) and purified them using protein A affinity-chromatography. Pre-F constructs and mAbs were validated for purity and function using SDS-PAGE and ELISA. Lastly, we generated RSV F vesicular stomatitis virus pseudoparticles and evaluated infection by testing 27 conditions, varying cell type, cell concentration and incubation times.

RESULTS & DISCUSSION: In-house anti-RSV mAbs bound successfully to the RSV pre-F constructs, with Nirsevimab showing high preference for the quaternary conformation of pre-F protein as expected. No RSV pseudovirus infection was observed in Hep-2 and A549 cells under the conditions tested. However, virus infection was detected in Vero cells, with the highest titres at 5×10^6 cells/plate after 48 hours.

CONCLUSIONS: Overall, we show the successful production of functional anti-RSV mAbs and RSV F pseudoparticles. These will be utilized to assess the impact of RSV F escape mutations that may arise in response to vaccine roll-out.

ACKNOWLEDGEMENTS: This research has been supported by the Global Immunology and Immune Sequencing for Epidemic Response (GIISER) program of the Bill and Melinda Gates Foundation (BMGF). Additional support has been granted from the National Research Foundation (NRF) through Prof Moore's South African Research Chairs Initiative (SARChI), the Poliomyelitis Research Foundation (PRF) and a Wits Postgraduate merit award (PMA).

P8**TITLE**

Design of soluble HIV-1 Env trimers from highly neutralisation-resistant HIV-1 strains for the isolation of broadly neutralising antibodies

AUTHORS

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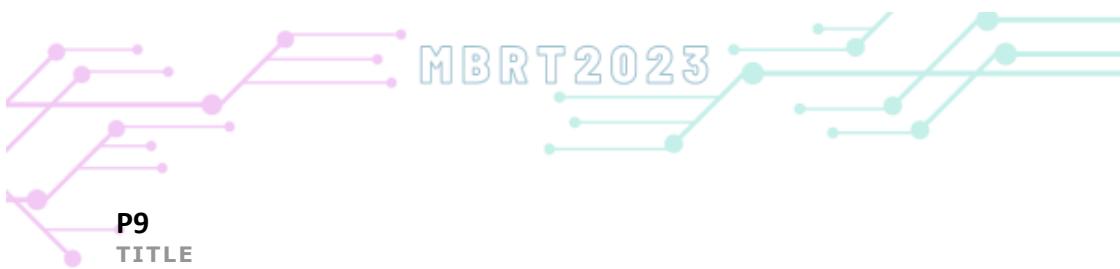
BACKGROUND: Emerging resistance to broadly neutralising antibodies (bNAbs) in circulating HIV-1 variants may compromise the efficacy of bNAbs used as prophylactics, or elicited by a future vaccine. A potential solution is new bNAbs with novel epitope specificities or binding mechanisms. This project aims to use soluble envelope (Env) trimers derived from difficult-to-neutralise virus strains to isolate novel bNAbs from HIV-1 infected donors.

METHODS: From 224 African transmitted-founder viruses, previously screened against eight bNAbs, the five most resistant viruses were selected for further characterisation using an expanded panel of 18 bNAbs in a pseudovirus neutralisation assay. Env trimers, for each of these isolates, were designed with various stabilising mutations. Once expressed, trimers were purified by nickel affinity and size exclusion chromatography, then characterised using SDS-PAGE and antibody binding assays to confirm they were in the correct conformations. Plasma from CAPRISA donors with >30% neutralisation breadth was screened for neutralisation activity against each isolate.

RESULTS & DISCUSSION: Three of the five isolates were resistant to more than 12/18 bNAbs tested ($IC_{50} > 50 \mu\text{g/mL}$), with geometric mean titres above 9 $\mu\text{g/mL}$. Yields of purified trimers averaged 200 μg , however, optimisation to increase yields is ongoing. Plasma from participant CAP287 neutralised one of the most resistant isolates (ID50: 55496), FRESH1388, which is only sensitive to V3-glycan bNAbs.

CONCLUSIONS: Env trimers from difficult-to-neutralise strains may provide highly selective sorting baits, enabling the isolation of novel bNAbs , from participants with cross-reactive plasma neutralisation activity. Screening suggests potent and broad antibodies, possibly targeting the V3-glycan epitope, may be isolated from CAP287 using the FRESH1388 trimer.

ACKNOWLEDGEMENTS: The AIRU unit, my supervisors, and funding from the NRF and the IAVI IIR grant.



P9

TITLE

Hepatitis B virus (HBV) surface antigens delivered in vitro and in vivo using recombinant adenoviruses for immunisation against HBV infection

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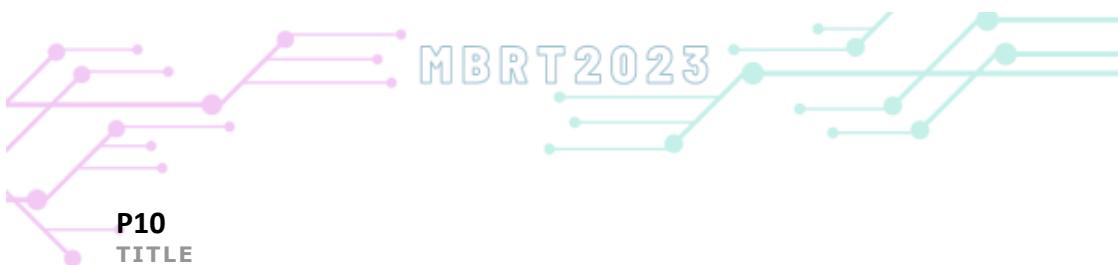
BACKGROUND: Hepatitis B virus (HBV) infection is a global health problem affecting almost 300 million people. Immunisation remains one of the most effective methods of HBV prevention. The HBV surface antigen (HBsAg) is an important component of anti-HBV vaccines because of its presence on the viral envelope and interaction with neutralising antibodies (NAb). Current vaccines against HBV, which deliver only the HBV small surface antigen (S-HBsAg) as a purified peptide in a 3-dose regimen, are often less effective in people over the age of 40 and in immunocompromised individuals and are limited to mostly humoral immunity. Adenoviruses (AdVs) serve as attractive candidates for anti-HBV vaccine design because of their ability to produce both cell-mediated and humoral immune responses, and their scalability to meet vaccine demand. Therefore, this study was conducted to compare expression and immunogenicity between the HBV small and large (L-HBsAg) surface antigens following gene delivery using recombinant AdVs.

METHODS: Recombinant AdVs were produced encoding either the S-HBsAg, L-HBsAg or Firefly luciferase (FLuc).

RESULTS & DISCUSSION: Infection of Human Embryonic Kidney 293T (HEK293T) cells with purified recombinant AdVs showed dose-dependent levels of S-HBsAg in both supernatant and lysate samples. However, as expected, significantly higher levels of L-HBsAg were detected in lysates when compared to supernatant samples, because the L-HBsAg peptide is not naturally secreted. Intramuscular administration of recombinant AdVs carrying the FLuc encoding sequence to BALB/c mice showed significant FLuc expression at days 1 and 3 after injection with expression decreasing by day 7, as expected. Stimulation of isolated splenocytes of mice injected with S-HBsAg and L-HBsAg expressing AdVs showed HBV-specific interferon- γ (IFN- γ) responses.

CONCLUSIONS: These data and ongoing research demonstrate the value of AdV-delivered S-HBsAg and L-HBsAg and AdVs as anti-HBV vaccine vectors.

ACKNOWLEDGEMENTS: The South African Medical Research Council. The Poliomyelitis Research Foundation. The Faculty Research Committee.

**P10****TITLE**

Functional consequences of novel IgG3 allelic variation on HIV broadly neutralizing antibody CAP256-VRC26.25

AUTHORS

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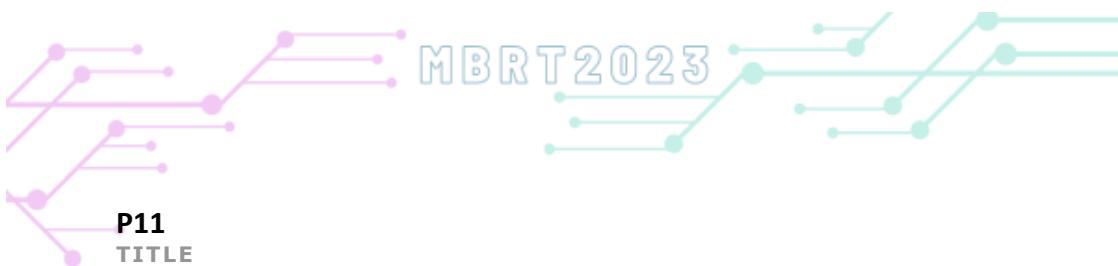
BACKGROUND: Broadly neutralizing antibodies (bnAbs) isolated from HIV-1 individuals have shown promise in prevention. We have previously shown that the IgG3 isotype significantly improved neutralization potency of CAP256-VRC26.25 over the IgG1 constant region that is generally used. However, there is significant allelic diversity inIGHG3, especially in the African population, of which the functional relevance is unknown. This study aimed to assess whether allelic variation in IgG3 could further improve neutralization potency.

METHODS: CAP256-VRC26.25 bNAb was engineered and expressed as 12 different IgG3 allelic variants, including two novel variants, as determined from previous IGHG3 sequencing data from the CAPRISA 002 cohort. These were tested for neutralization against an eight-virus panel and compared to the IgG1 version.

RESULTS & DISCUSSION: Overall, the IgG3 versions of CAP256-VRC26.25 showed improved neutralization compared to IgG1 version. Further, there were significant differences between allelic variants, with IgG3*04, which differs only by a shorter hinge, showing significantly lower potency than IgG3*01. IgG3*15 was also significantly lower than IgG3*01, despite having the same hinge length. IgG3*15 showed reduced potency compared to IgG3*14 from which it only differs at N392K, a mutation that removes a potential N-linked glycosylation site. IgG3*01 and IgG3*13 were the most potent. Overall, hinge length and amino acid differences in the constant region impacted neutralization.

CONCLUSIONS: This study shows that IgG3 allelic variation can be used to alter the neutralization potency of HIV bnAbs.

ACKNOWLEDGEMENTS: I am grateful to the following people for training me - Donald Mhlanga, Strauss van Graan, Sinethemba Bhebhe, Tandile Modise and Qiniso Mkhize, and an NIH U01 grant under H3 Africa to the Antibody Immunity Research Unit.

**P11****TITLE**

Investigating the foraging behaviour of entomopathogenic nematodes cultured in vitro

AUTHORS

Aviwe Matsha and Tiisetso Lephoto

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National Research Foundation (NRF)

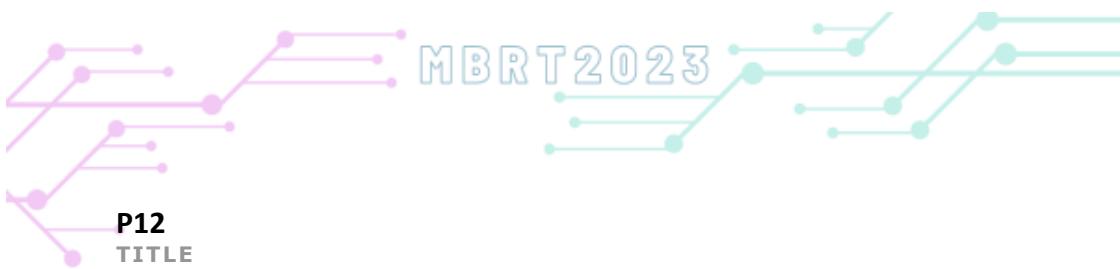
BACKGROUND: Entomopathogenic nematodes (EPNs) are obligate insect parasites that are used as biocontrol agents against economically devastating pests. However, despite their effectiveness, many EPNs do not reach the biopesticide market. This is because, upon emergence or inundative release into soil, EPNs are exposed to biotic and abiotic factors which can cause deviations in their foraging behaviour thus reducing their efficacy towards their target hosts. Therefore, the accurate classification of EPNs is critical and should be investigated by exposing EPNs to signals known to affect their foraging behaviour in nature. Therefore, this study aims to investigate the foraging behaviour of entomopathogenic nematodes cultured in vitro.

METHODS: EPNs were isolated from the soil using soil baiting and white traps. Isolated EPNs were identified molecularly by amplification and sequencing of the 18S rDNA region and morphologically through microscopy. Bacterial symbionts were isolated using selective media and identified molecularly by amplification and sequencing of the 16S rDNA region and morphologically through microscopy. The foraging behavior of EPNs was determined using the 2D-sand assay and vertical columns exposed to varying moisture and temperature levels. Pluronic-gel was used to observe their response to host-associated signals.

RESULTS & DISCUSSION: Isolated nematodes belong to the genus *Cruznema* and are entomopathogenic. Isolate bacteria belongs to the genus *Enterobacter* and shares a mutualistic relationship with the nematodes. Nematodes exhibit an intermediate-like foraging behaviour. Moisture affects the foraging behaviour.

CONCLUSIONS: EPNs were successfully identified, and classified into an appropriate foraging group.

ACKNOWLEDGEMENTS: NRF and Dr. Tiisetso Lephoto.



P12

TITLE

Evaluating PLGA Encapsulated Recombinant LRP as a treatment for Cardiovascular Disease

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AFFILIATIONS

National Research Fund, Technology Innovation Agency Seed Fund and Protein Structure-Function Research Unit

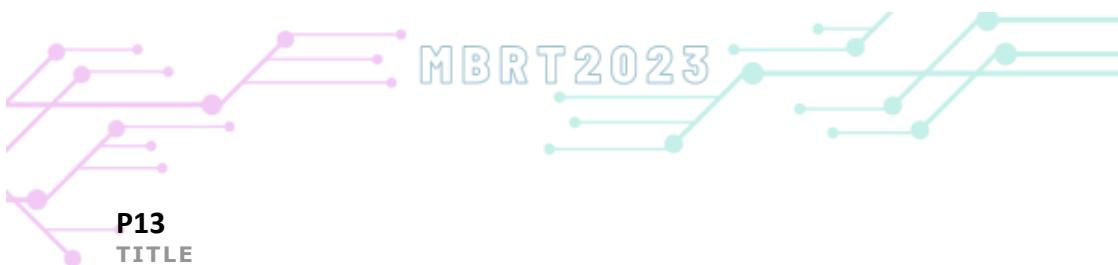
BACKGROUND: Cardiovascular diseases (CVD) are a classification of heart disorders that affect the heart and/or blood vessels and account for 18.56 million deaths worldwide. In South Africa, CVD is one of the leading causes of death with a 17% mortality rate. A frequent cause of most CVDs is atherosclerosis, a disease characterized by chronic inflammation and the accumulation of fats and cholesterol. A potential biomolecular marker that can be used for the treatment of CVD is the 37 kDa laminin receptor precursor/67 kDa laminin receptor (LRP/LR), a transmembrane receptor that has been shown to mediate cardioprotective effects in atherosclerosis. Therefore, the aim of this project was to evaluate PLGA nanoparticle encapsulated LRP as a treatment for cardiovascular disease.

METHODS: LRP was overexpressed using *E. coli* and purified using IMAC. Characterization was done using western blotting, spectroscopy, and binding affinity assay. Thereafter, LRP was encapsulated into PLGA nanoparticles and characterized using scanning electron microscopy, dynamic light scattering and leakage test. To assess encapsulated LRP as a treatment for CVD, U937 cells were differentiated with PMA and treated with 100 µg/ml of oxidized-LDL to form atherosclerotic cell culture models. MTT assay was used to determine the effective dose of encapsulated LRP treatment.

RESULTS & DISCUSSION: The overexpressed protein was confirmed to be LRP, α -helical and correctly folded. Protein encapsulating nanoparticles were spherical with a narrow size distribution and nanoparticles release the protein. MTT assay showed that 75 µg/ml encapsulated protein significantly increases cell viability.

CONCLUSIONS: Protein was successfully overexpressed, characterized, and encapsulated. LRP rescues cells from atherosclerosis.

ACKNOWLEDGEMENTS: Dr van der Merwe, Dr Otgaar and members of the Cell Biology and Signaling Research Lab.

**P13****TITLE**

Investigating the DNA Methylation Status of the PXDN and PXDNL Promoter Regions in OSCC Cell Lines

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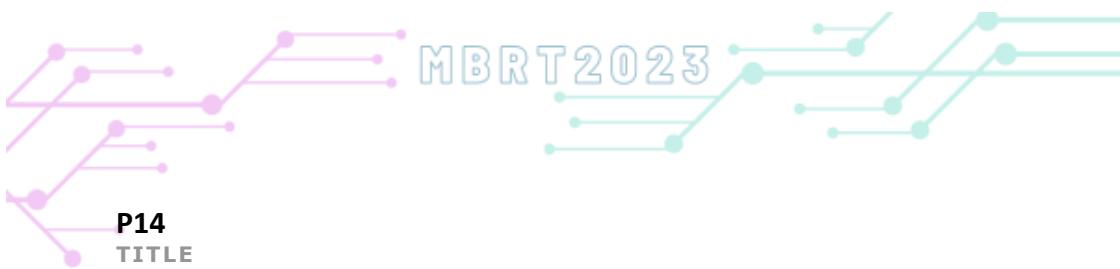
BACKGROUND: Oesophageal squamous cell carcinoma (OSCC) is the most prevalent form of oesophageal cancer in South Africa. Aberrant DNA methylation is a well-established epigenetic mechanism involved in cancer, including OSCC. This study focuses on DNA methylation of peroxidasin (PXDN) and peroxidasin like protein (PXDNL). PXDN consolidates the basement membrane through collagen IV unit oligomerization, influences epithelial- mesenchymal transition and correlates with poorer prognosis in various cancers. PXDNL expression also correlates with poor cancer prognosis and may possess a role in ECM consolidation. To date, no studies pertaining to the DNA methylation status of the PXDN and PXDNL promoter regions and the expression of PXDN and PXDNL in OSCC have been carried out.

METHODS: PXDN and PXDNL localisation was observed using immunofluorescence microscopy (IF); expression of PXDN and PXDNL quantified using western blot and the DNA methylation status of the PXDN promoter was assessed using methylation specific PCR.

RESULTS & DISCUSSION: IF and western blot results indicate that both cell lines showed varying degrees of PXDN and PXDNL expression. In addition, methylation specific PCR has shown that the promoter regions are differentially methylated across both cell lines.

CONCLUSIONS: This study may warrant further investigation of PXDN and PXDNL expression in OSCC to determine whether a correlation exists between expression and prognosis and whether these proteins could be utilised as novel markers for diagnosis and therapeutic intervention.

ACKNOWLEDGEMENTS: I would like to acknowledge God for His wisdom and strength, Prof. Mavri-Damelin for her support and guidance, my family and friends as well as my GH516 labmates for their unwavering encouragement and love. I would also like to acknowledge the NRF for their financial support of this project.

**P14****TITLE**

The design and synthesis of solvated encapsulated HIV-1 protease inhibitor - Lopinavir

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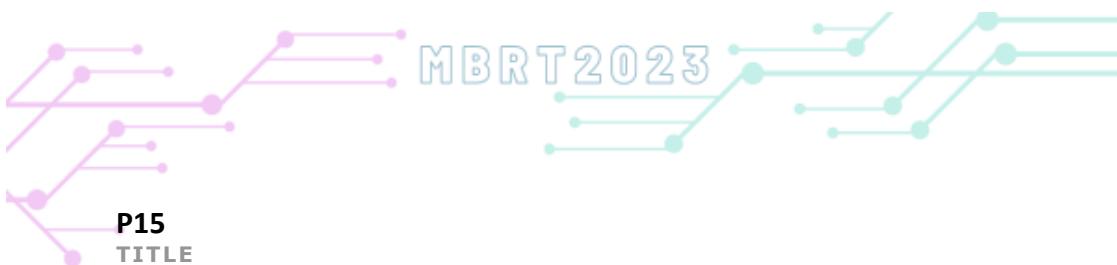
BACKGROUND: Protease inhibitors represent a class of drugs that are indispensable in the successful treatment of HIV/AIDS and form part of highly active antiretroviral therapy (HAART). The presence of many different types of mutations in the HIV-1 protease has resulted in a significant resistance towards protease inhibitors (PIs). Amongst the plethora of approaches that are being investigated in the development of PIs, there has been a surge in research interest towards the reformulation and modification of existing PIs. This is because researchers have found it more efficient to do this rather than design new drugs.

METHODS: Solvation synthesis, slow evaporation crystallisation, single crystal x-ray diffraction.

RESULTS & DISCUSSION: This work describes the synthesis and evaluation of the structural integrity of a variety of encapsulated PIs using several solvents. Eight compounds were synthesised in total. Of this number, six were synthesised using six-membered ring solvents and all crystallised in an orthorhombic ($P212121$) space group. Two compounds were synthesised using linear solvents that resulted in a monoclinic ($C2$) and triclinic ($P1$) space group, respectively.

CONCLUSIONS: The successful synthesis methods described here will be followed by evaluating their activity utilising enzyme kinetics and inhibition studies against the South African wild-type subtype C HIV-1 protease.

ACKNOWLEDGEMENTS: National Research Foundation, G.N.T Mokoto, IB Setschedi



P15

TITLE

Comparison of the proteome of Huh-7 cells transfected with replication-competent different (sub)genotypes of Hepatitis B Virus prevailing in and outside sub-Saharan Africa

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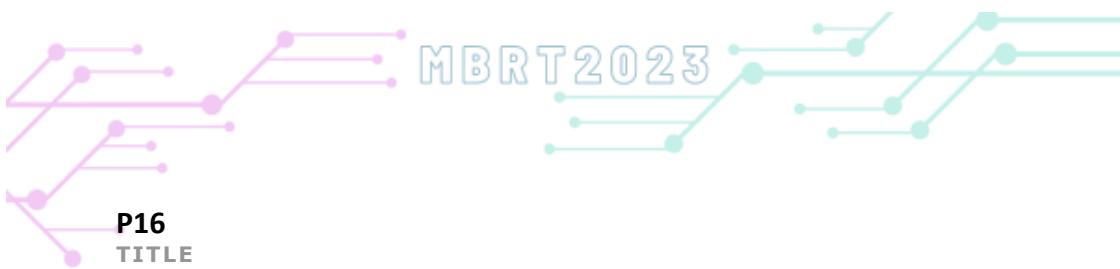
BACKGROUND: Hepatitis B Virus (HBV) is classified into nine genetically distinct genotypes, A to I, and at least 35 subgenotypes. In sub-Saharan Africa (sSA), (sub)genotypes A1, D3, and E prevail. Genetic variations between (sub)genotypes can contribute to differences in HBV pathogenesis and progression to hepatocellular carcinoma (HCC). Individuals infected with subgenotype A1 have a 4.5-fold increased risk of HCC compared to those infected with other (sub)genotypes. The effect of (sub)genotypes on protein expression and host signalling has not been studied.

METHODS: We used mass spectrometry to analyse the proteome of Huh-7 cells transfected with replication-competent clones of (sub)genotypes prevailing in sSA compared to A2, prevailing outside Africa.

RESULTS & DISCUSSION: Proteomic analysis, 5 days post-transfection, revealed significantly differentially expressed proteins between sSA (sub)genotypes compared to A2 ($p<0.05$). These differentially expressed proteins were classified into the top 10 pathways shared between (sub)genotypes A1, A2, D3 and E (apoptosis, angiogenesis, ubiquitin-proteasome, T-cell activation, inflammation, Wnt, p53, RAS, integrin signalling pathway and CCKR signalling). Amongst the top 10 pathways, proteins involved in inflammation, T-cell activation and apoptosis were significantly decreased in sSA (sub)genotypes compared to A2. Furthermore, RAS proteins were significantly upregulated (1.5-fold increase) in A1 compared to other (sub)genotypes. The downregulation of pro-inflammatory cytokines and apoptotic pathways helps promote the survival of HBV-infected hepatocytes, possibly leading to persistence in sSA (sub)genotypes compared to A2.

CONCLUSIONS: Two of the main cellular pathways involving RAS proteins are the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K). Both MAPK and PI3K signalling pathways regulate cell growth, motility, survival, and metabolism. The upregulated RAS proteins: guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 (GNB1), Rho-related GTP-binding protein (RhoC), Ras-Associated Protein 1 (Rap1) have been documented as oncproteins in various cancers and could contribute to the increased hepatocarcinogenic potential of A1. Ongoing future work will involve confirming the differentially expressed proteins *in vitro*.

ACKNOWLEDGEMENTS: This research has been funded by a grant from the Cancer Association of South Africa (CANSA). Author/s has been funded by a Bursary from the National Research Foundation (NRF) and the Poliomyelitis Research Foundation (PRF).

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P16

TITLE

In Silico Exploration of CB1 and CB2 Receptor Interactions comparing CBD and CBD Diacetate: A Comprehensive Computational Study

AUTHORS

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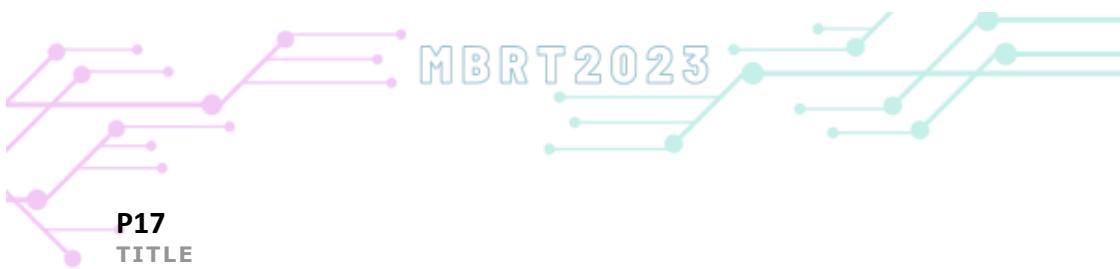
BACKGROUND: In the swiftly changing landscape of cannabis-derived compounds, acetylated cannabinoids such as cannabidiol diacetate (CBDDA) emerged as popular choices due to their stronger effects. However, despite their prevalence on the market, detailed research on their pharmacological effects remained limited, especially concerning CB1 and CB2 receptors. Since there are increasing reports of adverse reactions associated with acetylated cannabinoids, deciphering their behaviour at the molecular level has become imperative. This study aims to bridge this knowledge gap by focusing on CBDDA and its comparison with natural CBD in terms of receptor interactions. CBDDA, recognised for its potency compared to traditional CBD, holds promising potential as a biopharmaceutical product, making it a focal point of this study.

METHODS: Endocannabinoid receptor interactions of known agonists, inverse agonists, and antagonists were explored, comparing the effects of CBD and CBDDA using bioinformatics, molecular docking, and dynamics simulations. Simulations within a POPC and TIP3P membrane faithfully replicated physiological conditions. Following this, a high-throughput virtual screening referencing CBD diacetate was conducted.

RESULTS & DISCUSSION: The study revealed that CBDDA exhibits stronger interactions with CB1 and CB2 receptors compared to CBD. Through HTVS, quebrachamine and SCHEMBL11774734 emerged as the top hits for CBC1 and CBC2, respectively. The identification of optimal hits in HTVS, with CBDDA as a reference, enhances our understanding of the therapeutic potential of this compound.

CONCLUSIONS: These findings not only elucidate the specific interactions of CBD and CBDDA with CB1 and CB2 receptors but also provide a foundation for assessing the safety and efficacy of acetylated cannabinoids.

ACKNOWLEDGEMENTS: Family :) CHBC PS-FRU WITS PMA.

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Investigating potential plasma nephrotoxicity biomarkers associated with first-line antiretroviral therapy regimens in people living with HIV (PLHIV) in South Africa

AUTHORS

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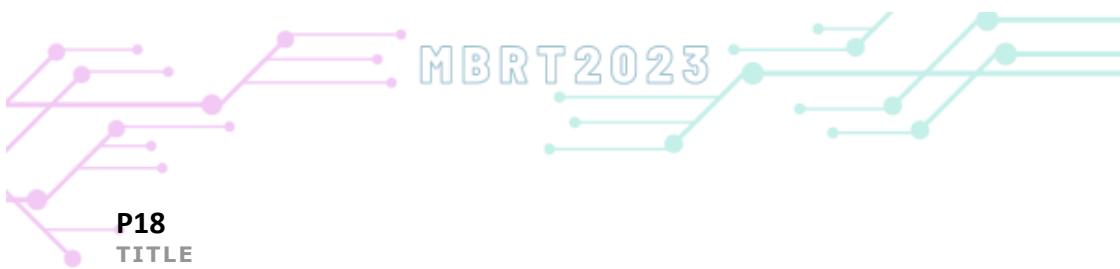
BACKGROUND: South Africa contributes to 20% of the global HIV burden and hosts the largest antiretroviral therapy program worldwide. ARVs used for HIV treatment can cause nephrotoxicity, which can progress into acute kidney injury (AKI) in patients^{1, 2}. With early detection, this side-effect is reversible. However, current serum creatinine tests are unreliable for early detection in the South African population³. Therefore, identifying potential protein biomarkers in plasma using mass spectrometry-based proteomics, can help with early detection of ARV-associated AKI in PLHIV in South Africa.

METHODS: 186 plasma samples (104 AKI; 82 non-AKI) were compared with an in-house sample preparation protocol, using magnetic beads⁴ for automated on-bead sample clean-up and protein digestion. The peptides were analysed with the Evosep One coupled to a Sciex TripleTOF® 5600 mass spectrometer, using a SWATH-data acquisition method. Data were processed using Spectronaut™ 17. An unpaired t-test was used to determine differentially abundant proteins identified between the AKI and non-AKI groups. Differentially abundant proteins with a fold-change ≥ 1.5 , at an FDR of 1% were considered candidate markers. Enrichr was used to identify enriched biological processes and pathways associated with the candidate markers (ranked by adjusted p-value, $p \leq 0.01$).

RESULTS & DISCUSSION: Seventeen candidate protein markers were identified. Thirteen proteins showed increased abundance, while 4 proteins showed decreased abundance in the AKI group compared to the non-AKI group. The GO biological processes of the candidate markers identified showed enrichment of processes involved in immune response, haemostasis, programmed cell death and regulation of cytoskeletal organization.

CONCLUSIONS: Candidate markers participate in enriched biological processes that correspond to known pathophysiological and cellular mechanisms contributing to AKI⁵, suggesting that the proteins identified can be further validated for distinguishing the AKI from the non-AKI group for biomarker discovery.

ACKNOWLEDGEMENTS: The authors would like to thank the PHRU for patient recruitment, sample collection and patient data management, as well as the NRF and the CSIR for funding this study.



P18

TITLE

Epidemiological characteristics and rates of *Pneumocystis jirovecii* pneumonia in South Africa from 2018 to 2022

AUTHORS

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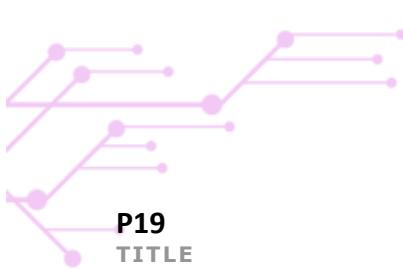
BACKGROUND: *Pneumocystis jirovecii* is the most common pathogen causing infections in immunocompromised patients and makes them susceptible to *P. jirovecii* pneumonia (PJP). It is responsible for high mortality and morbidity rates, however, the rate of PJP is not well-documented in Africa. Hence, this study aimed to investigate the epidemiological characteristics and rates of PJP in South Africa from 2018 to 2022.

METHODS: We performed a five-year retrospective study of patients with suspected *P. jirovecii*. The study used data that was previously collected by Infection Control Services, National Health Laboratory Service after they performed the laboratory diagnostics. The infection rates were calculated and related risk factors were analysed.

RESULTS & DISCUSSION: From 2018 to 2022 a total of 8110 patients' results were retrieved and 8059 met our inclusion criteria. The positivity infection rate from 2018 to 2022 were 32.66%, 29.93%, 34.02%, 24.98%, and 25.78% respectively. This study showed higher PJP infection rates in young children from zero-to-10-year-olds, and adults from 31- to 40-year-olds. Female patients and the intensive care unit also had higher infection rates. South Africa has higher PJP frequency estimates. Further investigation is required in order to identify the underlying causes of the increased infection rates of PJP in South African female patients and in the mentioned age groups.

CONCLUSIONS: The epidemiology of PJP in South Africa is comparable to previous studies in some respects though there are variables unique to our country. Our results hold significant implications for public health planning. As we move forward, it is imperative to develop targeted PJP prevention strategies.

ACKNOWLEDGEMENTS: A special thanks to both my supervisors Dr. Michelle Lowe and Mr. Keegan Hoog for their constructive criticism and support, and NHLS as well for allowing us to use their data.

 P19**TITLE**

In Vitro Characterization of a Single and Combination rtK333Q Mutation Isolated from HIV-infected Individuals with Occult Hepatitis B in Botswana

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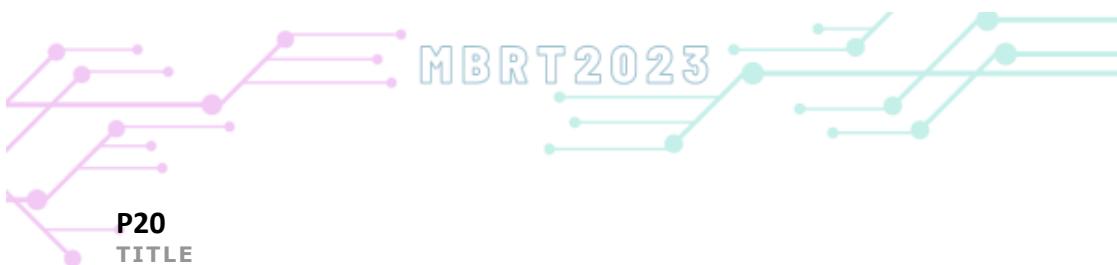
BACKGROUND: Occult HBV infection (OBI) is HBV infection with undetectable serum HBV surface antigen (HBsAg), but detectable HBV DNA. OBI can occur due to structural changes caused by detection escape mutations within the S gene, leading to the inability of current serological tests to detect the infection. Mutations affecting viral gene expression also contribute to OBI as they can lead to reduced/undetectable HBsAg expression. OBI can reactivate, is transmissible, and a risk factor for cancer. There is a paucity of data in functional analysis of mutations associated with OBI especially in Africa. The study aim was to conduct functional characterization of rtK333Q, and combination OBI-associated mutations (rtS332N_rtK333Q_sA194V) found in subgenotype A1 of HBV.

METHODS: Plasmids containing various strains of subgenotype A1 were used to transiently transfet Huh7 cells. Transfected cells were harvested and subcellularly fractionated to determine the extracellular expression of HBsAg using ELISA and to determine the HBV DNA viral load using qRT-PCR.

RESULTS & DISCUSSION: The unpaired t-test has shown a significant decrease in HBsAg expression for the single and combination mutation ($p\text{-value} = 0.0001$) of reverse transcriptase mutations compared to both A1 wildtypes. In addition, the combination mutation showed more significant decrease ($p\text{-value} = 0.04120$) in HBsAg expression over time compared to the single mutation ($p\text{-value} = 0.3643$). There was no observed change in HBV DNA levels in mutants versus the wildtype.

CONCLUSIONS: The mutants contribute to the HBsAg expression phenotype but do not affect viral load. Reasons underlying the insignificant changes in viral load remains unclear and a possible area for further investigation.

ACKNOWLEDGEMENTS: CANSA, Wellcome Trust.



P20

TITLE

Antibodies produced in a cost-effective fungal expression system neutralizes SARS-CoV-2 variants

AUTHORS

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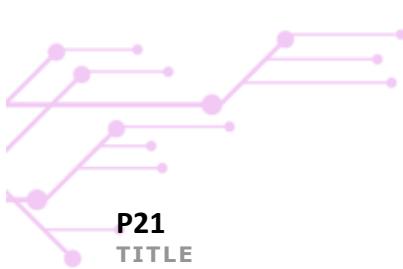
BACKGROUND: The COVID-19 pandemic, caused by the highly mutable SARS-CoV-2 and its emerging viral variants, highlighted the urgent need for effective and efficiently produced therapeutics, especially in low-middle-income countries, where accessibility remains a challenge. Neutralizing antibodies targeting the Receptor Binding Domain (RBD) on the virus's Spike protein are vital in treating the disease. While some antibodies have shown promise, most are produced in costly mammalian cell systems, posing challenges for resource-limited regions. This study explores the potential of a cost-effective fungal expression platform, Myceliophthora thermophila (C1), to produce binding and neutralizing SARS-CoV-2 antibodies.

METHODS: Gene cassettes encoding the heavy and light chains of the AIRU-A6 antibody were introduced into C1 to enable integration into the genome and expression of AIRU-A6 antibody. 946-A6 was expressed and purified from supernatant using Protein A agarose affinity chromatography. SDS PAGE analysis and Western blot against the Fc region of the antibody to identify the antibody. ELISA and Neutralization assays against Omicron, Delta, and Wildtype SARS-CoV-2 RBDs were performed.

RESULTS & DISCUSSION: A6 heavy and light chain constructs were cloned into C1 expression cassettes and transformed into the C1 cell line. 600ug of A6 antibody was purified from 20ml of culture (>90 % purity). SDS-PAGE and immunoblot analysis confirmed the presence of heavy and light antibody chains. Binding assays demonstrated C1-produced A6 has comparable binding and neutralization to the mammalian produced antibody, at an IC₅₀ of 0.03ug/ml.

CONCLUSIONS: We show retention of potency and breadth of a fungal-based antibody. The C1 platform shows promise as a cost-effective therapeutic antibody production platform.

ACKNOWLEDGEMENTS: Sashkia Balla, Dale Kitchin, Penny Moore, Patrick Arbuthnot, Dayadic.

 P21**TITLE**

Characterisation of the genetic variation in pharmacogenes involved in anti-tuberculosis drug metabolism across African populations

AUTHORS

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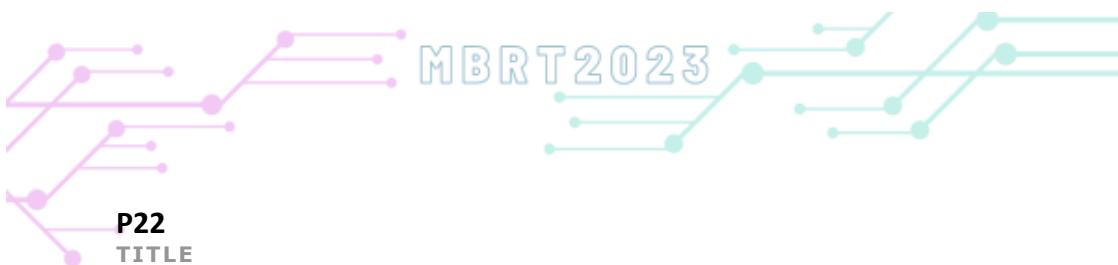
BACKGROUND: Tuberculosis (TB) is an infectious disease affecting many African countries. Although TB is treatable, drug resistance and adverse drugs reactions (ADRs) such as anti-TB drug-induced liver injury are a major health problem. These ADRs can be attributed to variants in pharmacogenes involved in anti-TB metabolism. The distribution of star alleles (haplotypes) that influence anti-TB drug metabolism, is unknown in many African ethnolinguistic groups. This hinders the establishment of precision medicine in high TB-burden African countries.

METHODS: This study used 794 high-depth whole genome sequence datasets representative of eight Sub-Saharan African (SSA) population groups. Data sources included the 1000 Genomes Project and H3Africa AWI-Gen. CYP2E1, NAT1, NAT2, GSTM1 and GSTT1 star alleles were called from the WGS data using StellarPGx. Novel star allele-defining variants were annotated using the Ensembl Variant Effect Predictor.

RESULTS & DISCUSSION: We present both common and rare star alleles influencing anti-TB drug metabolism across various SSA populations, in comparison to other global populations. Among the different SSA populations, relatively common key star alleles were identified such as NAT1*10, GSTM1*0, and GSTT1*0 which had frequencies above 50%. In addition, The NAT2*5B, had the highest star allele frequency among other slow NAT2 acetylators in the SSA population (21.1%), this was comparable to other global populations, excluding the East Asian population (3.7%). Seven novel haplotypes were present at frequencies >1%, while others were relatively rare.

CONCLUSIONS: This study provides insight into the distribution of star alleles relevant to anti-TB drug metabolism across various African populations. Furthermore, it provides a foundation of implementing African-based pharmacogenetic testing.

ACKNOWLEDGEMENTS: We would like to thank the study participants and data providers. The study forms part of the AGORA-TM project which is funded by Genomic Research Approach for Diversity and Optimising Therapeutics (GRADIENT) project, a partnership between GlaxoSmithKline, Novartis, and the South African Medical Research Council. T.M was partially funded by the Wits Postgraduate Merit Award and the Post-Graduate Research Fellowship affiliated with the Sydney Brenner Institute for Molecular Bioscience (SBIMB).

**P22****TITLE****Characterizing the gut microbiome of lions in Etosha National Park****AUTHORS****Carl Belger^{1,2}, Robyn Hetem², Natalie Smyth¹, Dylan Maghini^{1,3}, Jakob Wirbel³, Scott Hazelhurst¹****AFFILIATIONS**

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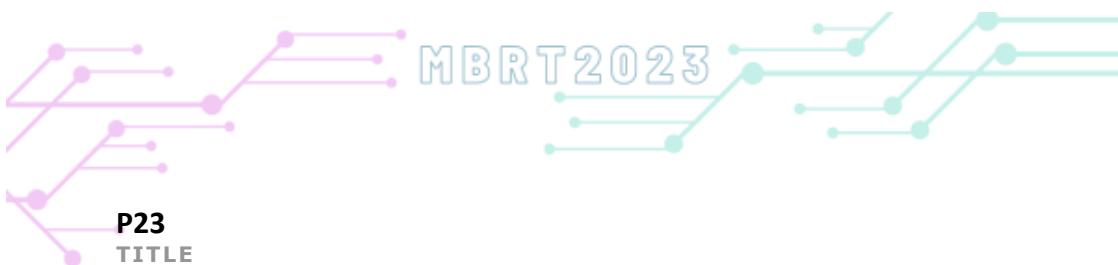
BACKGROUND: Microbial communities in the gut are linked to key health factors such as diet and disease. Large carnivores, remain mostly unexplored. Categorizing microbiomes of species of conservation concern may aid conservation efforts. New tools include faecal microbiome transplants (FMTs) and disease-marking bacterial species. Only one study has previously investigated the gut microbiome of lions. These three lions in Asia had high abundance of Fusobacteria, with Sutterella and Collinsella also present.

METHODS: 23 lions were darted in Etosha National Park, Namibia. Faecal samples were collected. Lion sex, age, pride composition and pregnancy status were noted. DNA was extracted from the samples and transported to South Africa for shotgun sequencing.

RESULTS & DISCUSSION: The lion faecal samples contained a large number of unclassified reads. The most abundant phyla were Bacteroidetes and Proteobacteria. We found that Fusobacteria was not the most abundant genus as previous literature would suggest. Male and female lions differed in their microbiome composition. A difference between sexes is not unexpected as lions often feed hierarchically, with the strongest males eating the most nutritious parts first, followed by females. Current data suggest Clostridium fallax as a key differentiator between sexes as well as an unknown genus-level genome bin (GGB) from the firmicutes phylum.

CONCLUSIONS: The high number of unidentified bacteria in the microbiome of free-living lions, highlights the need for further study. Our data also suggest that the gut microbiome of female lions is significantly different from that of male lions, possibly due to differences in feeding order and feeding habits.

ACKNOWLEDGEMENTS: This research is supported in part by the National Research Foundation of South Africa (Grant number 145976). Thanks to the Leibniz Institute for Zoo and Wildlife Research for providing access to their lion captures and to Stephenie Periquet from Ongava Research Centre, Namibia for access to laboratories and storage.

**P23****TITLE**

Obligate heterodimeric TALEN-encoding mRNA attenuates hepatitis B virus replication in cultured mammalian cells

AUTHORS

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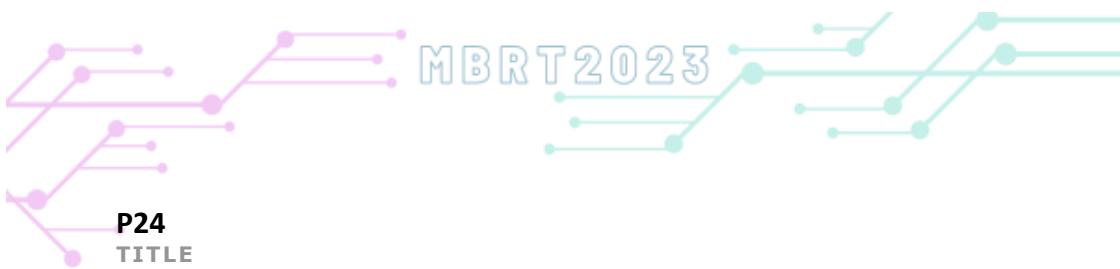
BACKGROUND: Infection with hepatitis B virus (HBV) remains a global health concern as current therapies rarely eliminate the virus from chronic carriers. Upon infection the viral genome is transported to the nucleus and repaired to covalently closed circular DNA (cccDNA). cccDNA serves as a stable replicative intermediate for the transcription of viral RNA, resulting in the persistence of infection. Gene editing targeted against cccDNA offers the means to mutate and permanently inactivate HBV replication. Previously generated obligate heterodimeric transcription activator-like effector nucleases (TALENs) targeted against conserved viral regions have demonstrated great therapeutic potential. These TALENs consist of a DNA-binding motif which is attached to a second-generation nuclease domain of FokI for improved specificity. TALENs function as left and right monomers that cleave complementary strands of targeted DNA when in close proximity. To improve clinical translation of HBV-targeting nucleases, TALEN DNA templates were *in vitro* transcribed to produce TALEN-encoding mRNA.

METHODS: TALEN-encoding sequences were cloned into an mRNA expression cassette containing all necessary elements to produce mRNA transcripts. Liver-derived cells were transfected with TALEN-encoding mRNA and expression was assessed by immunocytochemistry. Anti-HBV effects of TALEN-encoding mRNA were assessed by measuring hepatitis B surface antigen (HBsAg) levels. Cell viability was then assessed by the MTT metabolic assay.

RESULTS & DISCUSSION: Immunofluorescence staining demonstrated successful expression of TALEN-encoding mRNA. Cells that were treated with TALEN-encoding mRNA resulted in significantly reduced HBsAg levels compared to control samples. Furthermore TALEN-encoding mRNA did not induce cell-mediated toxicity.

CONCLUSIONS: TALENs play a significant role in advancing HBV therapy as cccDNA is targeted and permanently inactivated. Studies using lipoplexes containing TALEN-encoding mRNA are underway in a murine model of HBV replication.

ACKNOWLEDGEMENTS: This research has been funded by the National Research Foundation (NRF), the Poliomyelitis Research Fund (PRF), and the Medical Research Council (MRC).

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P24

TITLE

Exploring Structural Dynamics and Drug Resistance Implications in a Novel HIV-1 Subtype C Protease Hinge-Region Variant from South Africa

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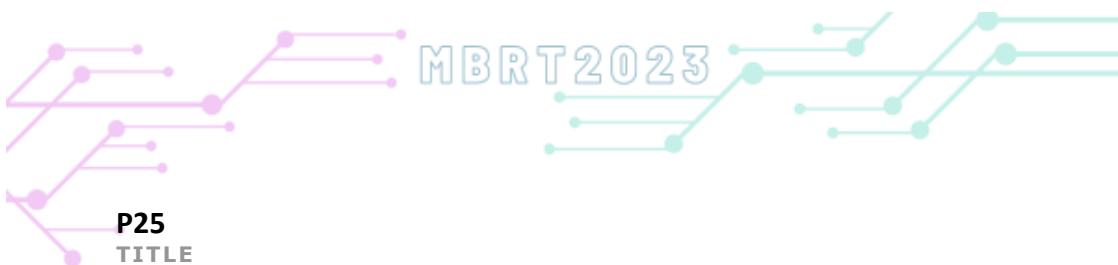
BACKGROUND: HIV-1 subtype C is responsible for the majority of HIV-1 infections globally, with the HIV-1 subtype C protease (PR) accounting for at least 90% of South African HIV-1 infections. However, currently available HIV-1 protease inhibitors (PIs) are designed based on the structure of the less dominant HIV-1 subtype B PR. The continuous emergence of amino acid mutations in the HIV-1 subtype C PR significantly alters the structure and dynamics of this enzyme, reducing the effectiveness of FDA-approved PIs.

METHODS: This study aimed to elucidate the impact of non-active site mutations on the structure and molecular dynamics of HIV-1 protease by analysing a novel South African HIV-1 subtype C hinge region variant, N37T↑V+10. This variant contains 10 naturally occurring polymorphisms (+10) as well as a substitution (N→T) and insertion (↑V) at the 37th position.

RESULTS & DISCUSSION: The assessment of the secondary structure using far-UV circular dichroism revealed that the enzyme is predominantly β-stranded. SE-HPLC results approximate the protein size to 22 kDa, consistent with previous reports. A thermal shift assay indicated reduced thermal stability for N37T↑V+10 compared to the wildtype PR. Molecular dynamics simulations investigate the stability and drug interactions of key regions on the PR. The results suggest that the insertion and substitution in the hinge region enables the variant to sample more open conformations, potentially contributing to the drug resistance mechanism. Furthermore, the structure of N37T↑V+10 displayed increased dynamics in critical regions, including the flaps, hinges, and fulcrum, that are associated with enhanced enzymatic activity.

CONCLUSIONS: Collectively, our data provides insights into how non-active site mutations, along with naturally occurring polymorphisms, affect the structure and conformational stability of key regions in the PR, potentially reducing drug binding and contributing to drug resistance.

ACKNOWLEDGEMENTS: We would like to thank the National Research Foundation and the Council of Scientific and Industrial Research for financial assistance.

**P25****TITLE**

Targeting the dimer interface of the Schistosoma glutathione transferase enzyme for the novel treatment of human schistosomiasis

AUTHORS

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AFFILIATIONS

None

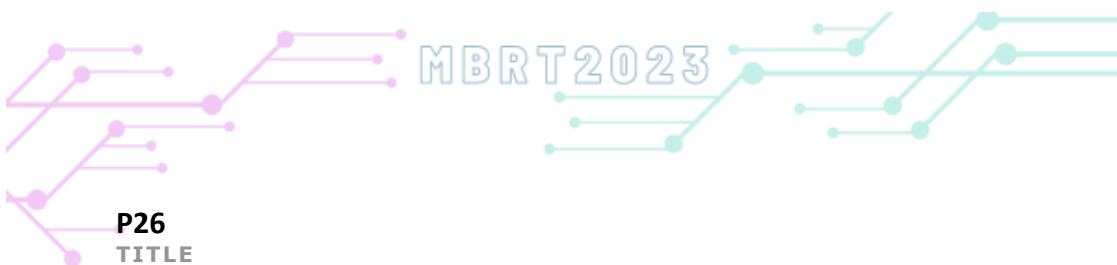
BACKGROUND: The disease burden that human schistosomiasis exerts upon impoverished peoples in the developing world is often underestimated, especially considering reports of inadequacies in current treatment regimes. Therefore, the design of novel antischistosomal drugs is a critical research avenue. Our laboratory has observed discriminative binding of small molecule inhibitors to the dimer interface of the Schistosoma glutathione transferase enzyme (GST), establishing it as an attractive drug target.

METHODS: We begin our investigation by performing a comprehensive structural characterisation of the dimer interface of Schistosoma GST families compared to human GST counterparts. Next, we generate a pharmacophore of the putative target binding site, this is a three-dimensional model containing the necessary chemical features for ligand interaction. Finally, we demonstrate a novel approach to assess the capability of our pharmacophore model to retrieve lead candidates bearing desirable drug-like properties.

RESULTS & DISCUSSION: Comparative structural investigation of the dimer interface revealed aromatic- and sulfonate-stabilising residues present exclusively in Schistosoma GST isoforms, which confers the selective binding ability. These were identified to anchor certain drug molecules, contributing to stable binding. We furthermore developed pharmacophore models for two variants of Schistosoma GST. These models displayed the ability to retrieve ligand candidates which, upon subsequent computational analysis, exhibited tendencies toward selective binding. Finally, our assessment of drug-resolving capabilities revealed a minimum 92% retrieval rate for drug-like candidates from a vast ligand database.

CONCLUSIONS: Our work illustrates the capabilities of computational modelling to evaluate binding site architectures, as well as predict potential ligands. Most importantly, we demonstrate the value of the pharmacophore model for rapidly prioritising high-quality candidates for the purpose of rational drug design.

ACKNOWLEDGEMENTS: Dr Ikechukwu Achilonu (Supervisor), National Research Foundation(Funding), Wits Postgraduate Merit Award (Funding).



P26

TITLE

Isolation and characterization of mycobacteriophages Bora and Wildflower

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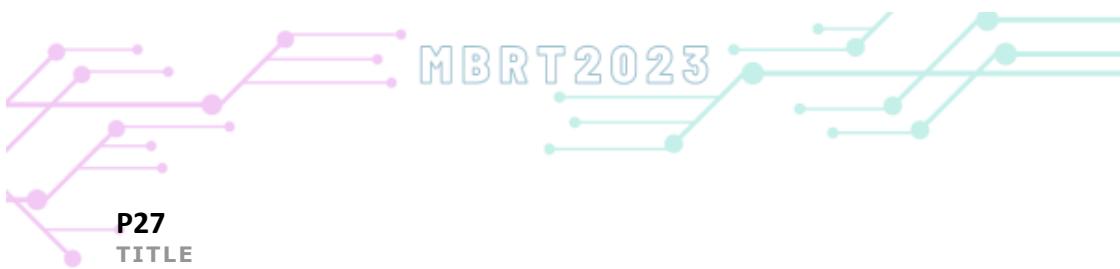
BACKGROUND: The emergence of antimicrobial resistance (AMR) in the clinical setting, including tuberculosis (TB), is of increasing concern. Bacteriophages offer possible alternative treatment options. Preliminary clinical trials have shown promise in treating patients infected with drug-resistant *Mycobacterium abscessus*. To prevent bacterial strains from becoming resistant to mycobacteriophages, viral cocktails can be co-administered. Herein, we sought to isolate new mycobacteriophages.

METHODS: Mycobacteriophages were isolated by resuspending soil samples in MP buffer, followed by purification through 0.22 µm filters. Resultant lysates were used to infect *Mycobacterium smegmatis* and phages were identified as clear plaques on bacterial lawns of *M. smegmatis*. High titre stocks were prepared for transmission electron microscopy (TEM) and DNA extraction for whole genome sequencing. Following sequencing, genomes were annotated to identify putative open reading frames. Previous evidence linked phage resistance to resuscitation-promoting factor B (*rpfB*) in *Schaalia odontolytica*. Consequently, the infectivity of Bora was tested in *M. smegmatis* strains with or without resuscitation-promoting factors (*rpf*s).

RESULTS & DISCUSSION: Bora and Wildflower were obtained from outdoor and indoor soil samples, respectively. TEM revealed Siphoviridae morphologies for both and genome annotations placed classified both as Cluster O members. Bora's genome contains 71494 nucleotides which putatively encodes 119 genes. Infection of Bora in *M. smegmatis* lacking all four *rpf*s resulted in a ten-fold decrease of plaque-forming units, which was complemented by *Mycobacterium tuberculosis rpf* genes.

CONCLUSIONS: We successfully isolated two lytic mycobacteriophages from soil. Phylogenetically, Bora and Wildflower are classified as cluster O members. Bora's infectivity decreased in the absence of *rpf*s thus confirming a link between these enzymes and mycobacteriophages.

ACKNOWLEDGEMENTS: Inqaba for sequencing, UCT microscopy unit for TEM images, and Debbi and Dan for annotation.



P27

TITLE

Expression & Purification of an AIRU-B12 antibody against SARS-CoV-2 using an alternative recombinant protein expression system

AUTHORS

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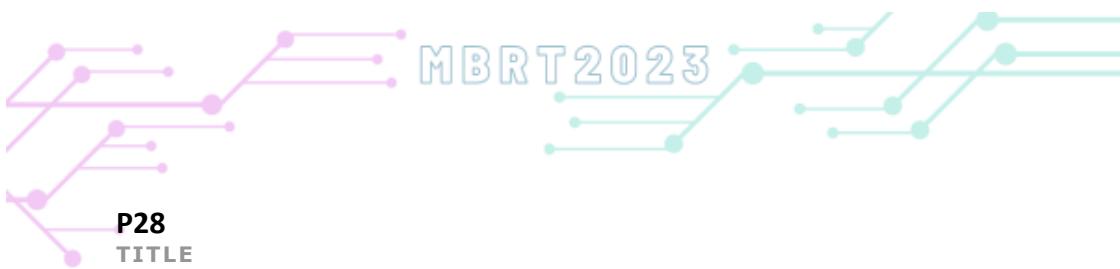
BACKGROUND: Severe acute respiratory syndrome coronaviruses (SARS-CoVs) are members of the genus Betacoronavirus and subgenus Sarbecovirus and can infect humans. Antibodies with remarkable breadth and potency targeting SARS cov 2 were isolated from human clinical trial participants. These antibodies may form part of a therapeutic strategy targeting current and future coronaviruses. Antibody therapy is expensive, and therefore unattainable in low-to-middle-income countries. A filamentous fungus, C1, is a promising cost-effective and up-scalable alternative for antibody production. The aim of the study is to produce 946-B12, a potent SARS-CoV-2 antibody, in C1.

METHODS: The gene sequences of the heavy and light chains of the AIRU-B12 antibody were integrated into the C1 genome. The antibody was produced at lab-scale and purified using Protein A resin-based chromatography. SDS-PAGE analysis and Western Blot were used to detect the heavy and light chain fragments and the presence of the antibody respectively.

RESULTS & DISCUSSION: B12 was secreted into the culture supernatant after transformation into C1 and purified using Protein A column chromatography. SDS-PAGE analysis showed that the heavy and light chain fragments migrated at the expected sizes of 75 and 25 kDa, respectively. Western blot analysis confirmed the presence of B12 using an anti-Fc antibody. Quantitation analysis showed that ~2 mg of protein was produced from a 100 mL culture, at 85% purity. These results demonstrate the ability to produce and purify a broad SARS-CoV-2 neutralizing antibody in an alternative recombinant protein production system.

CONCLUSIONS: Given AIRU-B12's ability to neutralize multiple Sarbecoviruses, it can potentially play an important role in pandemic preparedness.

ACKNOWLEDGEMENTS: AGTRU, AIRU



P28

TITLE

Designing an Experimental Model to Probe UPR Mechanisms in Breast Cancer

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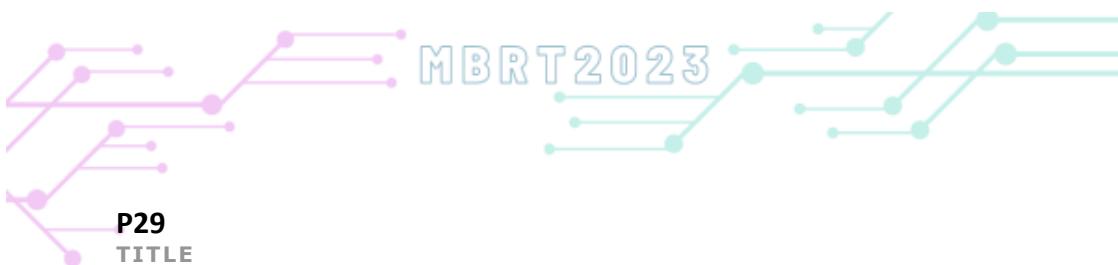
BACKGROUND: A newly discovered adaptive mechanism, UPR, has been observed to promote breast cancer tumorigenicity by enabling cells to survive in stressful environments. The known habitual stressors in breast cancer TME are hypoxia and acidosis; and these, along with intrinsic stressors lead to failures in ER folding capacities and accumulation of misfolded proteins in the ER lumen, a condition known as ER stress. Consequently, UPR is activated to restore ER homeostasis and promote cell survival. The three axes, that are canonically activated, to effect this are crosslinked and also interact with other signaling pathways, thus making it difficult to map out a route preferred to maintain cancers. In this current study we aimed to establish a model for probing UPR mechanisms using a mimetic of hypoxia.

METHODS: This was done by treating MCF7 cells with varying concentrations of a hypoxia-mimetic agent, CoCl₂, at different timepoints and then determining cell viability using an MTT assay.

RESULTS & DISCUSSION: Current data shows differential responses to the mimetic, whilst also showing possible resistance to its cytotoxic effects at low concentrations (nM to uM ranges). This is consistent with other literature findings, where the agent is observed to result in cellular changes that enable cancer cell survival.

CONCLUSIONS: CoCl₂ may be an ideal agent for probing hypoxia mediated changes in expression profiles of the different UPR components and will be important in elucidating the role played by the UPR in breast cancer progression.

ACKNOWLEDGEMENTS: The Carnegie Engabling Grant and School of MCB for project funding.

**P29****TITLE**

Potential interaction between the SARS-CoV-2 nucleocapsid protein and the forkhead domain of FOXP2

AUTHORS

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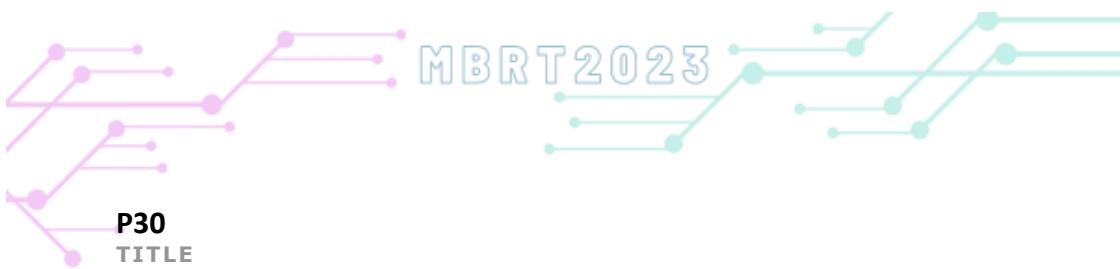
BACKGROUND: Research suggests that SARS-CoV-2 can cause neurological problems in over 30% of those infected. The SARS-CoV-2 nucleocapsid (NC) protein can cross the blood-brain barrier and interact with host proteins in the central nervous system. FOXP2 is a transcription factor that is associated with speech and language development, and mutations in its DNA binding domain have been linked to language and cognition impairments. The interaction between the SARS-CoV-2 NC protein and the forkhead domain of FOXP2 may have a role in the neurological problems found in some patients.

METHODS: Both proteins were overexpressed in E.coli cells and purified using immobilized metal affinity chromatography and size exclusion chromatography. Structural characterization was done using far-UV circular dichroism and intrinsic tryptophan fluorescence to confirm protein fold. DNA binding studies were conducted for both proteins using electrophoretic mobility shift assays (EMSA). Protein-protein interactions were assessed using molecular docking, as well as a protein-protein pull-down assay.

RESULTS & DISCUSSION: NC was shown to bind non-specifically to Nelson DNA, the same DNA sequence the FOXP2 FHD binds to, thus suggesting that it might compete with the FOXP2 FHD for DNA binding. Molecular docking predicted that an NC-FOXP2 FHD interaction is highly likely. This interaction was verified in vitro using a protein-protein pull-down assay which showed that the two proteins interact.

CONCLUSIONS: NC interacts with FOXP2 FHD in vitro, potentially causing speech and cognition complications after viral infection. Further research is required to confirm whether this also occurs under physiological conditions.

ACKNOWLEDGEMENTS: I want to express my gratitude to Dr. Sylvia Fanucchi, my supervisor, for her constant support and to Keiran Mcinnes, my mentor, for his invaluable guidance. Additionally, I would like to thank my friends and colleagues at the PSFRU.



P30

TITLE

Probing a link between the MRN complex and DNA damage associated IncRNAs, DDSR1 and HITTERS

AUTHORS

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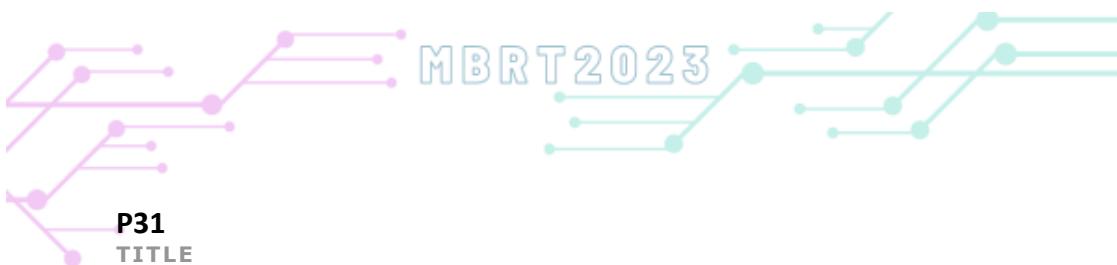
BACKGROUND: Oesophageal squamous cell carcinoma (OSCC) is one of the most common causes of cancer-related deaths and commonly affects black males. Cisplatin is commonly used as a treatment for this cancer, however, the exact mechanisms by which cells may respond to this drug and develop resistance are poorly understood. Cisplatin causes DNA damage through DNA double-strand breaks (DSBs), which are recognised by the DNA repair effector complex, MRE11-RAD50-NBS1 (MRN).

METHODS: This study aimed to determine if long non-coding RNAs (lncRNAs) DNA damage sensitive RNA-1 (DDSR1) and HERPUD1 intronic transcript of endoplasmic reticulum stress (HITTERS), and then determine whether DDSR1 has an association with the MRN complex, using HITTERS as a positive control. It was also sought to determine the relative expression levels of γH2AX, NBS1, and MRE11, which are key factors in measuring the level of DNA damage repair and response. This was carried out on three oesophageal cancer cell lines (SNO, WHCO1, and WHCO5), by performing RT-PCR and western blot analysis on the RNA and protein extracts, respectively. RNA immunoprecipitation (RIP) analysis was performed, however, it required a greater sample population.

RESULTS & DISCUSSION: It was found that HITTERS and DDSR1 were expressed in all three cell lines. We also found that WHCO1 cells respond as expected to DNA damage, with SNO cells going against the norm. Results for WHCO5 cells were inconclusive.

CONCLUSIONS: The research outlined serves as a proof of concept for further studies and contributes to our understanding of DNA damage repair pathways in the context of cancer treatment.

ACKNOWLEDGEMENTS: Sharma et al (2015). Dr Rupal Jivan. GH700 lab



P31

TITLE

Investigating a potential interaction between the DNA binding domains of Pax6 and FOXP2

AUTHORS

Dineo Mashamaite and Sylvia Fanucchi

AFFILIATIONS

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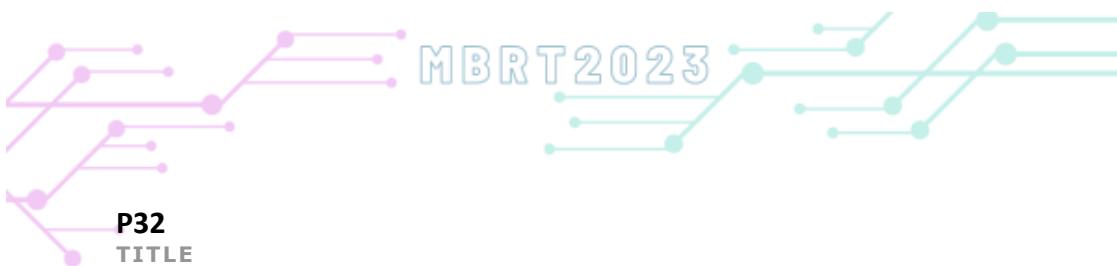
BACKGROUND: The Paired Box 6 (Pax6) and the Forkhead Box P2 (FOXP2) proteins are both transcription factors that regulate parts of the embryonic brain that are responsible for cognitive function, language, and speech. The Pax6 transcription factor is hypothesised to interact with the FOXP2 transcription factor which is responsible for the regulation of speech and language genes. Impaired Pax6 is associated with autistic behaviors, speech, and language abnormalities while decreased expression levels of FOXP2 are associated with autism spectrum disorders since speech and language impairment are considered characteristic features of autism spectrum disorder. Thus, the dysfunction and genetic impairment of the Pax6 transcription factor influences the expression of the FOXP2 transcription factor. The aim of this study is to determine whether the Pax6 paired domain interacts with the FOXP2 forkhead domain.

METHODS: The proteins were expressed in T7 E.coli cells and purified using immobilised metal affinity chromatography. Their secondary and tertiary structures were assessed using circular dichroism and fluorescence spectroscopy respectively and DNA binding studies were conducted using electrophoretic mobility shift assay in the presence of each protein's cognate DNA sequence. Finally, molecular docking and pull-down assay were used to determine if Pax6 PD and FOXP2 FHD were able to form an interaction.

RESULTS & DISCUSSION: The molecular docking and pull-down assay results suggest a possible interaction between the Pax6 paired domain and the FOXP2 forkhead domain. Further studies such as fluorescence anisotropy can be used to further confirm the interaction between the DNA binding domains of the two proteins.

CONCLUSIONS: There is a possible interaction between Pax6 paired domain and the FOXP2 forkhead domain. Therefore, the dysfunction of the Pax6 transcription factor may affect the expression levels of the FOXP2 transcription factor which may explain the cognitive, language and speech disorders observed in autism spectrum disorder patients.

ACKNOWLEDGEMENTS: My supervisor Dr Fanucchi, Keiran McInnes, Jessica Brothwell and the members of the PSFRU community.

**P32****TITLE**

In Vitro Phenotypic Doravirine Susceptibility of Prevalent HIV-1 Subtype C NNRTI-Resistance Mutations

AUTHORS

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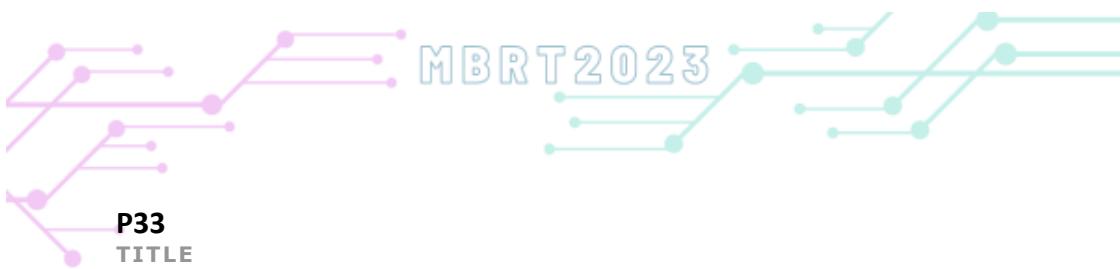
BACKGROUND: HIV remains prevalent in South Africa, with 7.5 million people living with HIV-1. The rising prevalence of antiretroviral drug resistance warrants novel antiretroviral drugs, effective against drug resistant variants. Doravirine (DOR) is a novel NNRTI with an improved safety profile that requires only a single daily dose and has shown activity against prevalent NNRTI drug resistance mutations. To gauge the impact of NNRTI drug resistance mutations on DOR in South Africa where HIV-1 subtype C is prevalent, this study assessed the in vitro phenotypic drug susceptibility of DOR against prevalent HIV-1 subtype C drug-resistant variants.

METHODS: Replication-defective HIV-like pseudoviruses containing the most prevalent NNRTI drug resistance mutant combinations ($n=20$), as well as the single mutations ($n=15$) they contain, were generated and screened in a single-cycle in vitro phenotypic assay for susceptibility to DOR.

RESULTS & DISCUSSION: The V106M and Y188L single mutants displayed high-level resistance to DOR. The K101P, K103N, V179D and Y181C single mutants remained susceptible. These results are largely in agreement with predicted responses, apart from V106M, Y181C and F227L. Most pseudoviruses with more than one mutation ($n=17$) displayed high-level DOR resistance. The K101P/K103N mutant remained susceptible. Results for majority of the combination mutant profiles are in agreement with the predicted responses, except for the K103N/A98G mutant.

CONCLUSIONS: DOR is effective against prevalent single NNRTI mutations (K103N, Y181C) in vitro. The majority of combination mutants displayed high-level resistance. This phenotypic assessment should be repeated in patient samples to understand their clinical impact on DOR susceptibility in South Africa.

ACKNOWLEDGEMENTS: This project was funded by the University of the Witwatersrand Health Sciences Faculty Research Council (FRC) and Poliomyelitis Research Foundation (PRF).

P33**TITLE**

Antibody dynamics during prolonged SARS-CoV-2 infection in people living with HIV and HIV-uninfected individuals

AUTHORS

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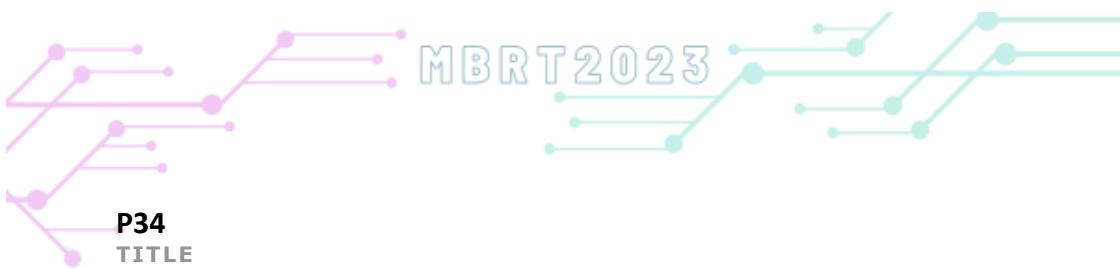
BACKGROUND: Prolonged SARS-CoV-2 infection occurs frequently in immunocompromised individuals, though it is not unique to this group. Prolonged infection has been shown to result in SARS-CoV-2 diversification, however the impact of the immune system on this is not known. Therefore, we aimed to characterise and compare SARS-CoV-2 antibody quality, quantity, and kinetics during prolonged SARS-CoV-2 infection in people living with HIV (PLWH) and in HIV-uninfected individuals.

METHODS: PLWH (n=18) and HIV-uninfected (n=28) individuals, who were hospitalised between May to December 2020 with severe COVID-19 and then shed SARS-CoV-2 for up to 6-weeks (7- to 77-days) post-hospitalisation were selected for this study. A Luminescence-based multiplex assay was used to quantitatively test longitudinal serum samples for binding to spike proteins of SARS-CoV-1 and three SARS-CoV-2 variants (D614G, Beta, and Omicron BA.1).

RESULTS & DISCUSSION: Despite infection with D614G prior to the emergence of variants, all individuals mounted cross-reactive spike binding antibodies, including to SARS-CoV-1. In the first week post-symptom onset (PSO) all participants had low binding titres that went on to peak between 29- and 42-days PSO, and subsequently waned. At the peak response PLWH had between 4- to 7-fold lower median titres to all four spike proteins compared with HIV-uninfected individuals, and this trend was mirrored at all time points after 7 days PSO.

CONCLUSIONS: Suboptimal antibody responses in PLWH with prolonged SARS-CoV-2 infection is likely to result in SARS-CoV-2 diversification and the development of novel variants. Thus, ongoing SARS-CoV-2 genomic and immunologic surveillance is imperative to limit the impact of future outbreaks by highly divergent variants.

ACKNOWLEDGEMENTS: This research has been supported by the Global Immunology and Immune Sequencing for Epidemic Response (GIISER) program and the Global Grand Challenges program, of the Bill and Melinda Gates Foundation (BMGF). Additional support has been granted from the National Research Foundation (NRF) through Prof Moore's South African Research Chairs Initiative (SARChI), and the Poliomyelitis Research Foundation (PRF).

A decorative graphic at the top of the page features stylized tree branches in pink and teal, branching out from the left and right sides towards the center, with small circular nodes at the ends of the branches.

P34

TITLE

Cystic fibrosis: An update on the variant profile and carrier frequency in the Black South African population

AUTHORS

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AFFILIATIONS

University of the Witwatersrand; National Health Laboratory Service

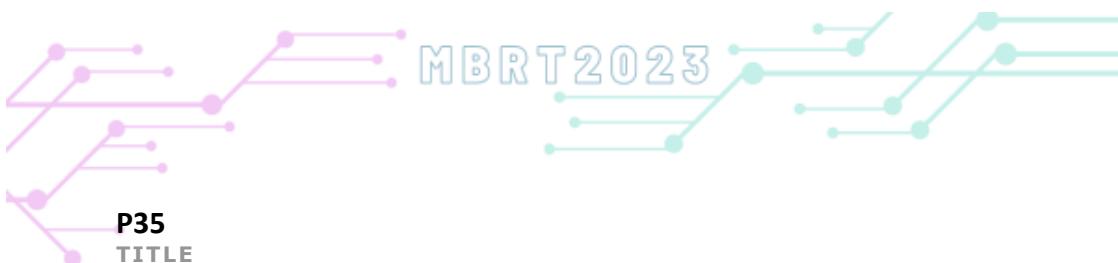
BACKGROUND: Cystic fibrosis (CF) is an autosomal recessive disorder caused by pathogenic variants in the CFTR gene. In the Black South African population, limited research has been conducted on the genetics of CF, and molecular testing is frequently the only way a diagnosis can be made. At the NHLS, the Elucigene CF50 kit is used to test for the 3120+1G>A variant, the only common and unique variant known in the Black population, and other common European CF variants. Recent studies in the Division of Human Genetics show evidence of other recurrent CFTR variants, and these and other variants could be further explored using NGS data generated in the Division, to update the CFTR variant profile and revise the carrier frequency of CF in the Black population.

METHODS: Data on 395 unaffected individuals was used for the identification, annotation, prioritisation, and classification of CFTR variants using the ACMG guidelines. Pathogenic and likely pathogenic variants were used to estimate the carrier frequencies and birth rates for both CF and the 3120+1G>A variant.

RESULTS & DISCUSSION: The estimated CF carrier rate was consistent with literature (1 in 36), and the 3120+1G>A variant accounted for 36.4% of CF alleles, which is less than previously reported (46%). The recurrent variants from recent studies were not detected, indicating possible limitations in sequencing or sample size. Three novel likely pathogenic variants (c.3392T>C, c.3038C>G, and c.2594G>C) were identified in one individual each, which could potentially be African-specific.

CONCLUSIONS: This study highlights the value of carrier screening using routinely generated NGS data, for the identification of novel, potentially African-specific variants in CFTR, as well as an updated estimation of the carrier frequency of CF and the 3120+1G>A variant. The findings suggest the presence of other common CF-causing variants in the Black population, and the identification of novel variants not currently included in commercial panels, will allow for targeted molecular testing.

ACKNOWLEDGEMENTS: I would like to thank Ms Clarice Smal and Ms Fahmida Essop for continuous support and guidance throughout this project, as well as Prof Amanda Krause for her valuable input and advice.

**P35****TITLE**

Validation of the LumiraDx point-of-care for diabetes diagnosis and monitoring.

AUTHORS

Ofentse Lesito, Ngalula Kone, Riffat Munir, Jaya George

AFFILIATIONS

Wits Diagnostic Innovation Hub (WDIH)

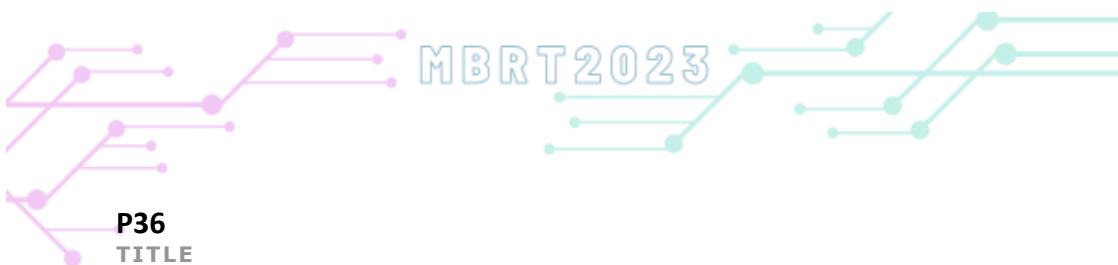
BACKGROUND: There is a need for wider access and easier testing systems for diabetes diagnosis and monitoring. In this study, the LumiraDx point-of-care analyzer is evaluated for the measurement of HbA1c.

METHODS: Detection of HbA1c by LumiraDx is based on immunoassay. CLSI protocols were used as guidelines in the validation. The precision, accuracy, linearity, and lot-to-lot variation was assessed in the laboratory using a combination of quality controls and remnant clinical specimens selected to cover the measuring range of the assay (4.0 – 14.0%) and the diagnostic cut-off point of 6.5%.

RESULTS & DISCUSSION: For the 5-day precision analysis, LumiraDx showed within-run and between-run coefficient of variation (CV) of 0.5 – 0.9% and 0.7 – 1.1%, respectively. For the 1-day precision analysis, within-run and between-run CV was acceptable and consistent with manufacturer's claim for HbA1c of 4.7%, 6.0%, 6.2% and 7.1%. Linearity over the measuring range of 4.6 – 11.5%HbA1c was acceptable with a slope of 0.99 and intercept of 0.48. There was a good correlation between the Bio-Rad HPLC method and LumiraDx. The Concordance correlation coefficient was 0.98. Bland-Altman showed mean difference of 0.2% HbA1c (95% CI: -0.04 to 0.16% HbA1c). Passing-Bablok regression showed $r=0.979$, slope of 1.0 (0.96 – 1.05) and y-intercept of 0.1 (-0.23 – 0.39). For the lot-to-lot variation analysis, a concordance correlation between the Bio-Rad and LumiraDx measurements using three test strip lots yielded a concordance correlation coefficient of >0.95 for each lot.

CONCLUSIONS: The LumiraDx analyzer performed well overall for the measurement of HbA1c in the laboratory.

ACKNOWLEDGEMENTS: This research has been funded by the Wits Diagnostic Innovation Hub (WDIH).

**P36****TITLE**

Prevalence of HPV in OSCC Patients Attending the Charlotte Maxeke Johannesburg Academic Hospital

AUTHORS

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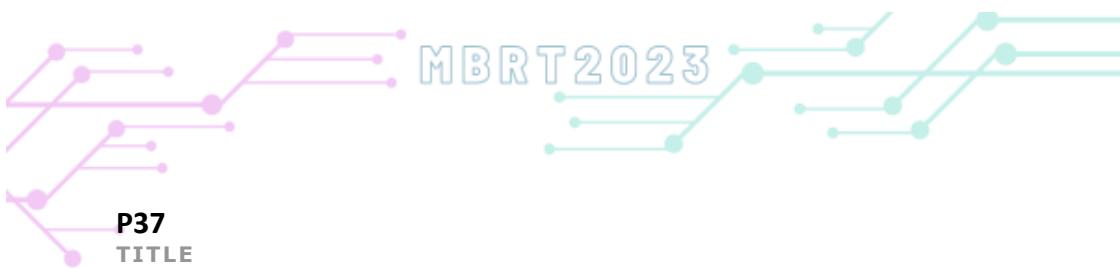
BACKGROUND: Oral squamous cell carcinoma (OSCC) is the most common type of tumour in the oral cavity, accounting for more than 90 % of all oral cancers, and it occurs in the mucosal lining of the oral cavity. The human papilloma virus (HPV) has been identified as a risk factor for developing OSCC. HPV-positive OSCC cases have recently been reported to be increasing worldwide. However, the prevalence and impact of HPV-positive OSCC in Africa and sub-Saharan Africa (particularly South Africa) is unknown. Therefore, the aim of the study was to assess the prevalence of HPV infection in patients with OSCC at the Charlotte Maxeke Johannesburg Academic Hospital.

METHODS: Twenty-nine formalin-fixed paraffin embedded tissue samples of patients with OSCC were assessed for the presence of 15 high-risk HPV subtypes using the AmpFire® HPV Multiplex Assay/Kit (Atila BioSystems).

RESULTS & DISCUSSION: Following analysis, 62.07% (18/29) of patients with OSCC were found to be positive for HPV high-risk subtypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 59, 66, 68). The most prevalent HPV subtypes were HPV45, HPV59, and HPV31, which were positive in 27.59% (8/29), 24.14% (7/29), and 20.69% (6/29) of cases, respectively. Interestingly, only 5.56% (1/18) of the HPV-positive cases were positive for HPV18.

CONCLUSIONS: In our context, OSCC is slightly associated with HPV18. OSCC may arise through other high-risk HPV subtypes, the most prevalent being HPV45 and HPV59.

ACKNOWLEDGEMENTS: Pumza Magangane and Mulalo Molaudzi for assistance and guidance in the lab. Pumza Magangane for assistance in rationalizing results and write-up. Peers who provided moral support.



P37

TITLE

Molecular Characterisation of High-Grade B-cell lymphomas with rearrangements in the MYC and BCL6 genes

AUTHORS

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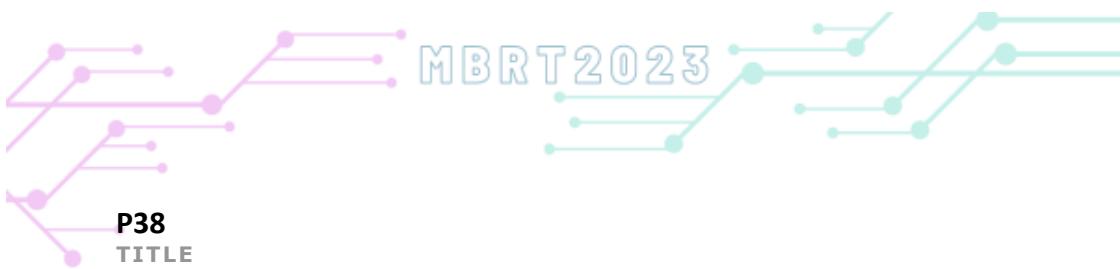
BACKGROUND: Diffuse large B-cell lymphomas are the genetically and molecularly heterogeneous aggressive most common subtype of NHLs in the adult population found to account for approximately 40% of all B-cell lymphomas and more than 80% of all aggressive lymphomas worldwide. DLBCL harbouring MYC, BCL2 and/or BCL6 translocations were reported by studies using fluorescent in situ hybridisation (FISH) to account for approximately 10% DLBCL and were therefore called "double-hit" lymphoma (DHL) or "triple-hit" lymphoma (THL).

METHODS: We conducted a study to investigate the proteomic and microRNA expression profile of single-hit lymphomas (MYC+ or BCL6+) and double-hit lymphomas (DHL) (both MYC+ and BCL6+). We employed MALDI IMS for proteomic analysis and RT-PCR for microRNA analysis. To validate our findings, we utilised immunohistochemistry (IHC) on proteomics data and statistical analysis on the microRNA-related data.

RESULTS & DISCUSSION: We identified 12 proteins that were differentially expressed between DHL and single-hit DLBCL. These proteins belonged to the cytoskeletal family of proteins including keratin, actin, histones and pyruvate kinase. All the proteins could differentiate between two groups with at least an area under the curve (AUC) of 0,8. We also identified 11 microRNA by RT-PCR which included miR-221-5p, -205-5p, -21-5p, -196a-5p, -375, -451a, -146a-3p, -21-3p, -491-59, -155-5p, and Let-7d. Let-7d correlated with the expression of MYC and KRAS in DHL.

CONCLUSIONS: DHL proved to have an aggressive genotype compared to single-hits and null-hits, respectively. We observed an upregulation of pathways involved in cellular proliferation.

ACKNOWLEDGEMENTS: National Health Laboratory Service Research Thrust, National Research Foundation, University of Cape Town Hair and Skin Research Laboratory for MALDI IMS instrument.



P38

TITLE

Insertion of transgenes into the adenovirus genome through homologous recombination

AUTHORS

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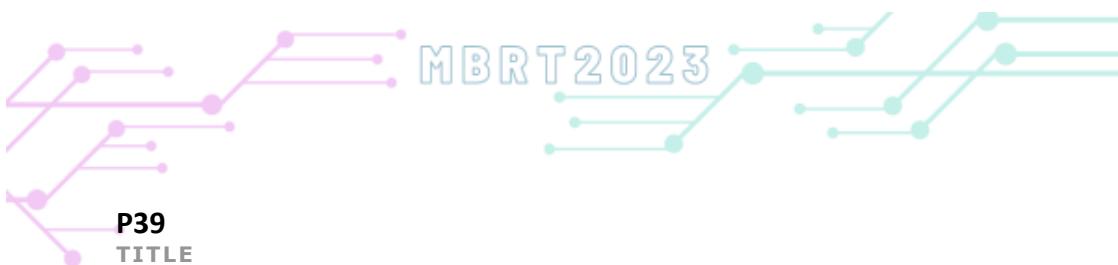
BACKGROUND: Adenoviruses are a common viral vector used in both vaccines and gene therapy development. Helper dependent adenoviral (HDAd) vectors are modified adenoviruses that have had all viral genes removed, and thus have a large carrying capacity for therapeutic genes. However, traditional ligation techniques are inefficient in generating large DNA constructs. This study aims to design a homologous recombination-dependent system that will allow for easy insertion of transgenes into the HDAd genome.

METHODS: A shuttle plasmid was designed to contain two regions of homology with the HDAd genome to facilitate homologous recombination between the two plasmids. To test the functionality of this shuttle plasmid, anti-HBV clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) or nanoluciferase encoding sequences were inserted into the shuttle.

RESULTS & DISCUSSION: The degree of HBV surface antigen knockdown due to the anti-HBV CRISPR/Cas9 sequence was assessed in HBV antigen expressing cells using an ELISA. The level of nanoluciferase expression from the shuttle plasmid was assessed using a luciferase assay.

CONCLUSIONS: The shuttle will then be used to insert these transgenes into the HDAd genome. This study will mitigate one of the major drawbacks of using HDAd by improving the efficiency of a key step in their production, thereby streamlining the process, and offering potential for expanding future applications of HDAds in gene therapy.

ACKNOWLEDGEMENTS: This research has been funded by grant # 120383 from the South African National Research Foundation. Author/s has been funded by a bursary from The Poliomyelitis Research Foundation.

**P39****TITLE**

Overexpression and purification of the vitamin D receptor for protein-protein interaction studies.

AUTHORS

Jessica Hurwitz, Dr Sylvia Fanucchi, Dr Vanessa Meyer

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PSFRU, School of MCB

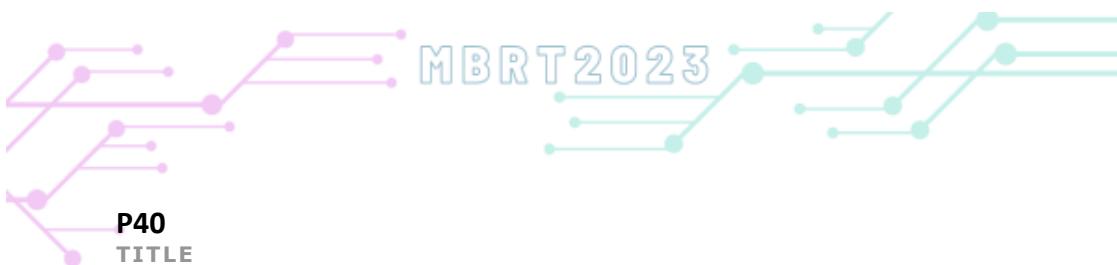
BACKGROUND: The immune system needs to be regulated to avoid autoimmune diseases. This regulation is brought about by transcription factors, forkhead box P3 (FOXP3) and the vitamin D receptor (VDR), causing the differentiation of regulatory T cells (Tregs). VDR and vitamin D upregulate the expression of FOXP3. However, whether these two proteins cooperatively regulate the immune system through direct interaction following FOXP3 expression is yet unknown. Understanding these two proteins and their roles in various autoimmune disorders could open therapeutic avenues.

METHODS: An in silico study was done using Maestro-Bioluminate, to observe if it was possible for the DNA-binding forkhead (FHD) domain of FOXP3 to bind to the ligand binding domain (LBD) of VDR. To perform in vitro binding studies of FOXP3 FHD and VDR LBD, the proteins need to be overexpressed in an E. coli host. Expression trials using variable conditions and cell lines have been performed for VDR LBD.

RESULTS & DISCUSSION: VDR LBD was found to bind at helix 1 and helix 3 (the DNA binding helix) of the FOXP3 FHD, in silico. FOXP3 FHD has been successfully overexpressed in T7 E. coli cells. VDR LBD is overexpressed in the insoluble state in five out of six trials, with the final trial having overexpression in the soluble state after chaperones were added.

CONCLUSIONS: A potential interaction between these two proteins has been identified which will be confirmed in vitro, unpacking the mechanism of association between FOXP3 and VDR in immune regulation.

ACKNOWLEDGEMENTS: Acknowledgements go to my supervisors, Dr Fanucchi and Dr Meyer, to the PSFRU, and to the school of MCB.



P40

TITLE

Exploring Cell-Specific Isoform Usage Using Long-Read Sequencing Data

AUTHORS

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AFFILIATIONS

MCB

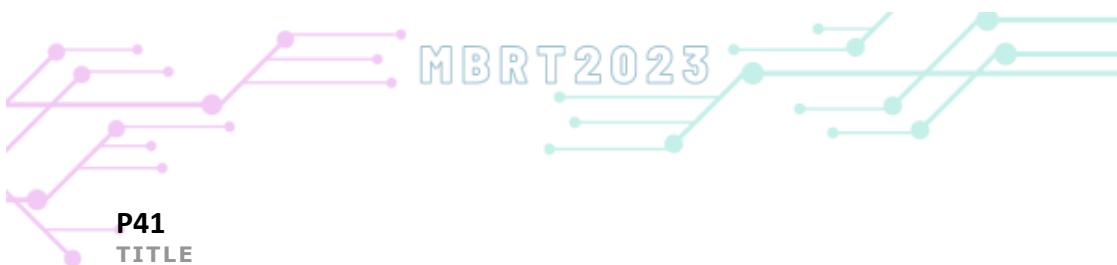
BACKGROUND: Alternative splicing plays a pivotal role in cell differentiation and orchestrating cell-specific responses. This added layer of genetic variation contributes to the complexity observed in gene expression studies. Given the limitations of analysing context-specific isoforms using short-read sequencing platforms, the advent of long-read sequencing has expanded our ability to explore the complexities of alternative splicing.

METHODS: The rapid improvement of downstream tools for increased sequence accuracy, this endeavour included an investigation to evaluate the potential of ONT basecalling tools to enrich sequencing accuracy from prior studies. Fast5 files were transformed into Pod5 format and subjected to basecalling using ONT's latest basecaller, Dorado. Subsequently, the basecalled reads were aligned to the reference genome via MiniMap2. Isoform detection and quantification were performed using the Bambu tool. In addition, to investigate the implications of alternative splicing in disease, transcript usage profiles of IFN- β stimulated and unstimulated A549 cells were compared to IFN- β stimulated and unstimulated HT1376 cells.

RESULTS & DISCUSSION: A549 cells exhibit a distinctive pattern of co-expression involving both functional and non-functional RIG-I isoforms. This co-expression introduces competition for viral Pathogen-Associated Molecular Patterns (PAMPs), highlighting the intricate and cell-specific nature of the immune response.

CONCLUSIONS: This study advances the understanding of alternative splicing in cell-line-specific responses and underscores the potential to leverage lower-quality data for transcript usage studies.

ACKNOWLEDGEMENTS: NRF



P41

TITLE

FOXP3 and SARS-CoV-2 Nucleocapsid: A Clot to Uncover

AUTHORS

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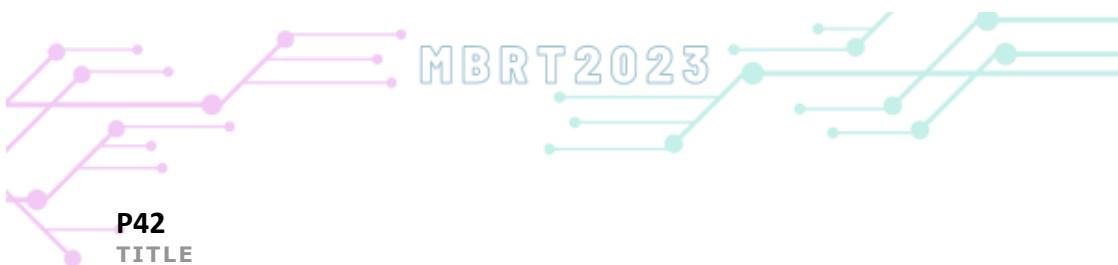
BACKGROUND: While the worst of the COVID-19 pandemic has passed, SARS-CoV-2 is predicted to become endemic. Therefore, it is crucial to untangle the mechanisms allowing the virus to wreak havoc throughout the body. During COVID-19, excessive intravascular clotting can lead to strokes, embolisms, and organ dysfunction; and may also contribute to long-COVID. This coagulopathy results from platelet overactivation. The transcription factor FOXP3 plays a vital role in platelet development, and FOXP3-deficient megakaryocytes produce platelets that resemble those seen during COVID-19. The SARS-CoV-2 nucleocapsid (NC) is a multifunctional protein with roles in viral assembly, protein expression, and suppression of anti-viral responses. Interestingly, NC is possibly capable of altering expression of host proteins by directly binding promoter elements in the nucleus. Therefore, NC may be interfering with FOXP3 function.

METHODS: SARS-CoV-2 NC was expressed in T7 E. coli and purified using affinity chromatography. Electrophoretic mobility shift assays (EMSA) were utilised using various DNA sequences to study NC DNA binding.

RESULTS & DISCUSSION: EMSA showed that NC forms non-specific interactions with diverse DNA sequences, as expected from a viral genome packaging protein. Most intriguingly, NC forms additional specific interactions with a FOXP3-specific DNA sequence from the p38 α MAPK promoter sequence. Given that dysregulation of p38 α can lead to platelet overactivation, this may explain the aberrant clotting seen during COVID-19.

CONCLUSIONS: SARS-CoV-2 NC may directly compete with FOXP3 through both non-specific and specific DNA interactions; leading to dysregulation of p38 α expression and subsequent platelet dysfunction. This opens the door to potential therapeutic approaches aimed at treating the coagulopathy seen during COVID-19.

ACKNOWLEDGEMENTS: Funding: Wits Post-graduate Merit Award and Medical Research Council.

**P42****TITLE**

Cassava Mosaic Disease recovery is associated with phytohormones, South African cassava mosaic virus titre, and viral effector-proteins

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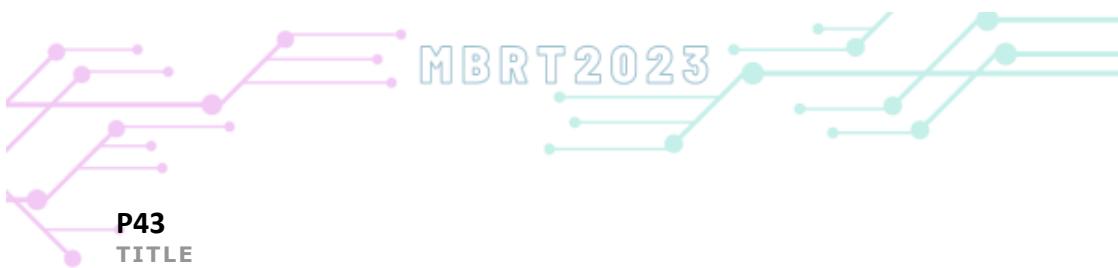
BACKGROUND: Manihot esculenta Crantz (cassava) is a perennial tuber crop that serves as a food source for people and feed for animals. Concerningly, cassava is susceptible to infection by South African cassava mosaic virus, a bipartite geminivirus whose multifunctional proteins aid in infection and suppression host immune responses, which are coordinated by phytohormones. While cassava cultivars show differing responses to SACMV infection (susceptibility, tolerance, or resistance), cassava mosaic disease (CMD) results in significant losses of cassava yields, posing a threat to food security.

METHODS: Cassava plantlets (susceptible T200 and tolerant TME3) were agro-infected with SACMV. Apical leaves were harvested during the early, systemic, and late infection stages. Viral load determination was done via qPCR on extracted DNA. Extracted RNA was used for RT-qPCR for phytohormone biosynthesis and response genes, and SACMV viral genes.

RESULTS & DISCUSSION: Our results show that a decrease in viral titre can be associated with a decrease in symptom severity in cassava during SACMV infection. There is also differential gene expression of the biosynthesis and response genes related to jasmonic acid, ethylene, and abscisic acid across early, systemic, and late infection in T200 and TME3. Furthermore, there is also differential expression of the viral genes AC2, AC4, BC1 and BV1, which have been shown to target hormone-modulated immune pathways.

CONCLUSIONS: In conclusion, these results illustrate the differences between the susceptibility and tolerant responses to CMD. Ethylene plays a role in susceptibility while jasmonic acid and abscisic acid play a role in tolerance, all under the influence of viral proteins.

ACKNOWLEDGEMENTS: The Wits University Postgraduate Merit Award AND The National Research Foundation.

**P43****TITLE**

Structural characterization and crystallization of the TBR1 DNA binding domain in the absence and presence of DNA and FOXP2.

AUTHORS

Hartman, R., and Fanucchi, S.

AFFILIATIONS

MCB and PSFRU

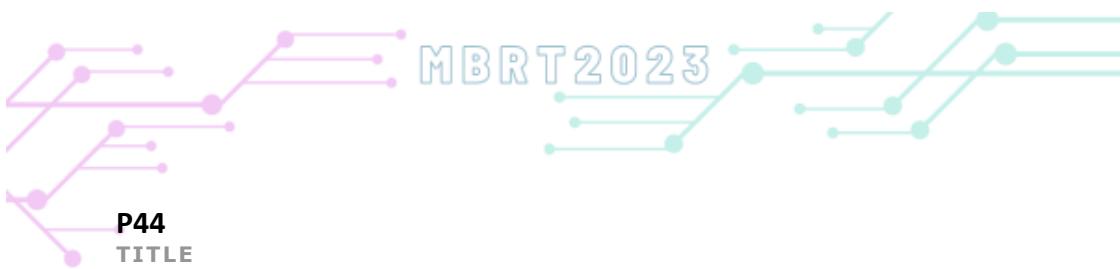
BACKGROUND: Transcription factor activity is regulated through neuronal stimulation, which is important in the formation of neural circuits and mature synapses; as well as the development of cognitive processes such as language. T-box brain transcription factor 1 (TBR1) is a neuron-specific transcription factor that is required for forebrain development. TBR1 has been identified through recent studies as a master regulator of transcriptional pathways related to Autism Spectrum Disorder. FOXP2 is another transcription factor which has been implicated in language and speech disorders. The main aim of this project is to resolve the crystal structure of the TBR1 DNA binding domain (DBD). The study will investigate the structure of TBR1 in the presence and absence of palindromic DNA and also in the presence and absence of its binding partner, FOXP2 Forkhead domain (FHD). Through the structures and some additional techniques, the aim is to better understand the mechanism of TBR1 DBD interactions.

METHODS: Both proteins were successfully expressed using T7 Escherichia coli cells and purified using immobilized metal affinity chromatography as well as size exclusion chromatography. Secondary and tertiary structure analysis was performed using circular dichroism spectroscopy and intrinsic tryptophan fluorescence respectively. Functional studies were performed using electrophoretic mobility shift assays.

RESULTS & DISCUSSION: TBR1 DBD and FOXP2 FHD demonstrated a characteristic β -sheet and α -helical secondary structure respectively. Tertiary structure analysis of TBR1 DBD and FOXP2 FHD showed successful three-dimensional folding.

CONCLUSIONS: The expressed constructs were determined to be active. The palindromic DNA – TBR1 DBD complex has yielded micro-crystal formation and is currently undergoing optimization.

ACKNOWLEDGEMENTS: A big thank you to my supervisor Dr. Sylvia Fanucchi for giving me the support and guidance needed throughout my academic journey.



P44

TITLE

Using ChIP-Seq and Gene Expression Microarray data to explore transcriptional dysregulation of PXDN in cardiovascular diseases

AUTHORS

Shiven Naidoo, Professor Demetra Mavri-Damelin, Doctor Nikki Gentle

AFFILIATIONS

PMA, NRF

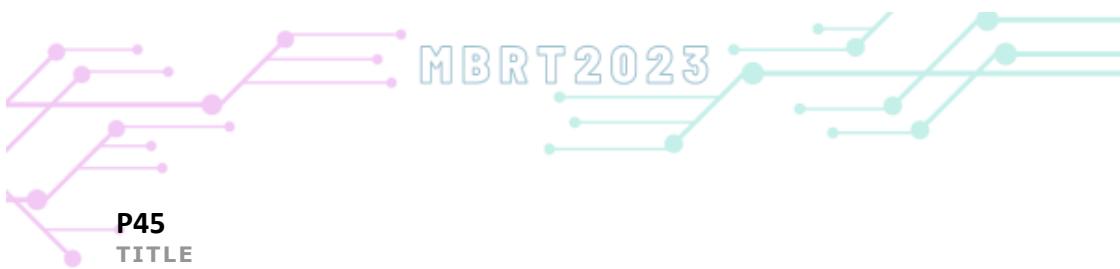
BACKGROUND: PXDN encodes peroxidasin, a peroxidase family protein involved in vital cell pathways. Dysregulated PXDN expression can modulate the pathogenesis of some cardiovascular diseases. However, the transcriptional regulators of PXDN in the cardiovascular system are unknown. This study mined public transcriptomic and epigenomic databases for insights into the regulation of PXDN in cardiovascular diseases using bioinformatics tools.

METHODS: First, transcription factor (TF) binding data for over 400 ChIP-seq datasets were collected from ChIP ATLAS and filtered to isolate TFs binding within 5kb of PXDN's transcription start site (TSS). Next, a custom bioinformatics pipeline was developed to analyse microarray data from the Gene Expression Omnibus (GEO), which was applied to investigate the correlation between cardiovascular diseases and the variation in expression levels of PXDN and its TFs.

RESULTS & DISCUSSION: We identified 19 TFs that bind to PXDN's promoter, many of which are involved in cardiovascular morphology and inflammatory response. Notably these include: BRD4, EP300, ERG, FLI1, KLF4, NOTCH1, RBPJ, RELA, TAL1, TCF21, and TEAD1. Differential gene expression analysis of microarray data revealed no significant change in PXDN expression nor its TFs in Tumour Necrosis Factor- α stimulated cell lines, cardiomyopathy and heart failure tissue samples, although considerable variation between tissue samples may have masked overall changes in expression.

CONCLUSIONS: This study makes three major contributions: firstly, this is the only study to have identified transcriptional regulators of PXDN in cardiovascular cells. Secondly, this study found no significant changes in mRNA expression levels for PXDN or its TFs for the conditions investigated. Lastly, this study developed a bioinformatics pipeline which can be used to mine GEO microarray datasets.

ACKNOWLEDGEMENTS: NRF and PMA for funding, friends and family for their support, supervisor (Prof Mavri-Damelin) and co-supervisor (Dr Nikki Gentle) for their guidance.



P45

TITLE

Unveiling the biochemical pathway between Type 2 Diabetes Mellitus and early Alzheimer's disease

AUTHORS

Shweta Tooray and Dr Eloise van der Merwe

AFFILIATIONS

NRF

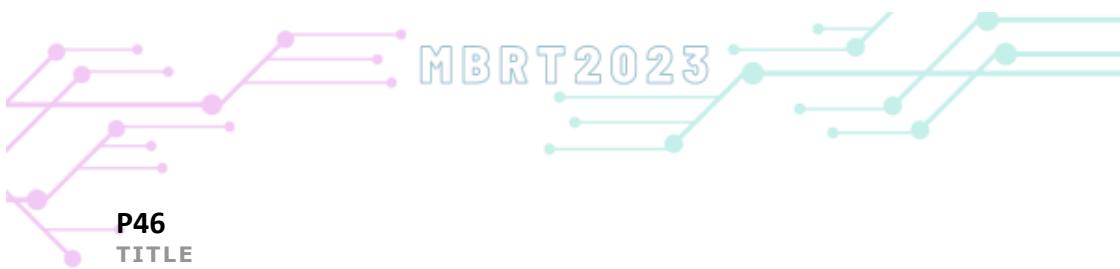
BACKGROUND: Alzheimer's disease (AD) is a neurodegenerative disease with no cure. AD has two predominant hallmarks, namely, extracellular senile plaques formed by the beta-amyloid (A β) peptide and intraneuronal neurofibrillary tangle formation (NFTs) caused by Tau hyperphosphorylation. These plaques and tangles progressively lead to the cognitive and behavioural impairments of AD. The upstream mechanisms and pathways causing these effects are not fully understood. Previous studies suggest persistent hyperglycaemia leads to damaging of biological systems by various mechanisms including oxidative stress caused by ROS formation and mitochondrial dysfunction, which are additionally present in AD.

METHODS: Due to this previously reported link, a hyperglycaemic AD cell model was created by culturing Human Embryonic Kidney (HEK) 293 cells in low glucose media and treating them with various combinations of glucose, A β 42 and a glucose lowering drug (GLD). A glucose concentration and time study was completed over ten days. Mitochondrial activity was then investigated using the Alamar blue assay. Mitochondrial function was assessed by measuring mitochondrial DNA content. Oxidative stress was analyzed. Thereafter, telomerase activity was measured using the qPCR TRAP assay.

RESULTS & DISCUSSION: The constant hyperglycaemic conditions lead to decreased glucose uptake, and various signs of cells stress were observed. The hyperglycaemic-AD conditions lead to increased mitochondrial activity. The hyperglycaemic conditions increased mitochondrial DNA content. Over time the hyperglycaemic-AD conditions increased ROS production whilst GLD prevented these spikes. The hyperglycaemic-AD conditions decreased telomerase activity.

CONCLUSIONS: Together these results suggest a link between AD and T2DM through A β 42 and ROS.

ACKNOWLEDGEMENTS: NRF



P46

TITLE

DNA damage response lncRNA's DDSR1, TUG1, PANDA, and ANRIL expression changes in response to neocarzinostatin in human oesophageal squamous carcinoma cell lines.

AUTHORS

Hlatshwayo, S and Jivan, R

AFFILIATIONS

Genetics and development biology in molecular and cell biology

BACKGROUND: Oesophageal cancer is the second most common causes of cancer related deaths in South Africa with incidences reported to be higher in males. Radiotherapy used concurrently with chemotherapy (chemoradiotherapy) remains the standard treatment for locally advanced oesophageal cancer. Neocarzinostatin (NCS), a radiomimetic drug, is used to induce DNA double strand breaks (DSBs). Long non-coding RNA's (lncRNAs) DDSR1, PANDA, ANRIL and, TUG1 have known functions in DNA damage repair pathways and cell cycle regulation. The aim of this study is to determine whether expression of these lncRNAs is altered in response to NCS and identify novel targets for cancer therapy.

METHODS: MTT assays were used to determine EC30 values for NCS, which were then used to treat OSCC cells for 8 hours. DSB formation was determined by γ H2AX expression assessed by western blot. Preliminary lncRNA expression was detected by PCR and levels are to be quantified by qPCR.

RESULTS & DISCUSSION: EC30 values were calculated using Graphpad Prism 8.0.1.244, HEK293 ($1,33 \pm 0,44$), WHCO1 ($0,72 \pm 0,04$), WHCO5 ($1,17 \pm 0,30$), and SNO ($1,13 \pm 0,17$). Preliminary assessment of lncRNA expression by RT-PCR indicate that lncRNAs TUG1, PANDA, DDSR1 and ANRIL are expressed in HEK293, WHCO1, WHCO5 and SNO cell lines. TUG1 and ANRIL expression levels were slightly elevated after NCS treatment in WHCO5 cells. DDSR1 levels did not appear to be altered in response to NCS in any cell line. RT-qPCR will be conducted to confirm this data. Additionally, western blot analysis of γ H2AX, P-p53(Ser20) and P-ATM (ser1981) will be performed.

CONCLUSIONS: Functional roles of lncRNAs TUG1 and ANRIL in response to NCS are worth further investigation.

ACKNOWLEDGEMENTS: This research project is funded by an NRF grant to the supervisor (TTK210217586902).

**P47****TITLE**

Exploring the Structure, Function and Stability of Glutathione Transferases Engineered from Intra- and Inter-class Consensus Sequences: How Forgiving is Nature?

AUTHORS

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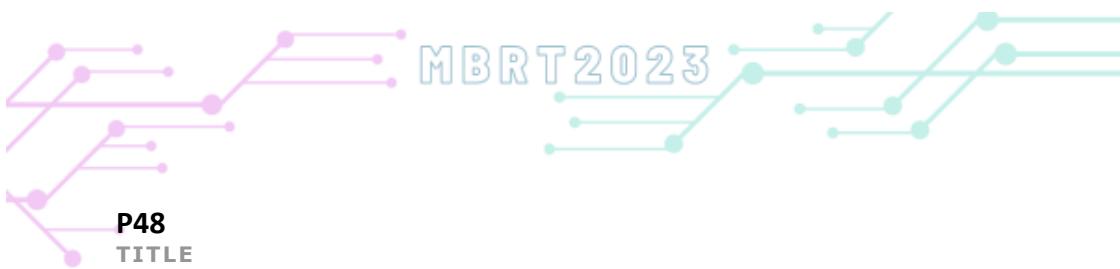
BACKGROUND: Glutathione-S Transferases (GSTs) are a superfamily of enzymes critically involved in detoxification. The GST family consists of numerous classes and subclasses, with the cytosolic GSTs being the focus of many studies. There are 8 cytosolic GST classes. Even though many GSTs possess unique secondary structural and active site characteristics, as well as a low sequence identity across the GST family, they exhibit significantly similar quaternary structures. The question then arises, what is the purpose of the many classes found in the GST family?

METHODS: •Over-expression and purification hGSTA1-1, M1-1, Alpha consensus, Mu consensus, Cytosolic GST consensus •Analysis of the secondary structure of each of the specified proteins using far-UV CD •Ligandin studies for each protein by assessing the fluorescence of ANS, in the presence and absence of GSH •Assess enzyme kinetics of the proteins using a GSH-CDNB conjugation activity assay.

RESULTS & DISCUSSION: Proteins were successfully purified. SDS-PAGE results indicated that the purity was greater than 95%. Secondary structural studies showed that there were no differences in the composition of all proteins, as they produced the characteristic spectrum for predominantly alpha helical proteins. Furthermore, the ANS fluorescence spectra showed a greater blueshift in the samples containing the consensus proteins, indicating it is likely that the consensus proteins have more exposed hydrophobic patches. Specific activity results indicated that the consensus proteins were less catalytically active than their natural counterparts.

CONCLUSIONS: The overall results indicate that divergent evolution of the GST family created a functionally diverse group of proteins while maintaining the thermodynamic and kinetic stability required for protein folding.

ACKNOWLEDGEMENTS: The National Research Foundation, Professor Yasien Sayed, Doctor Ikechukwu Achilonu.



P48

TITLE

Investigating the regulation of PXDN expression by the early growth response 1 (EGR1) transcription factor in the context of human fibrotic diseases

AUTHORS

Thokozile Makhanya and Demetra Mavri-Damelin

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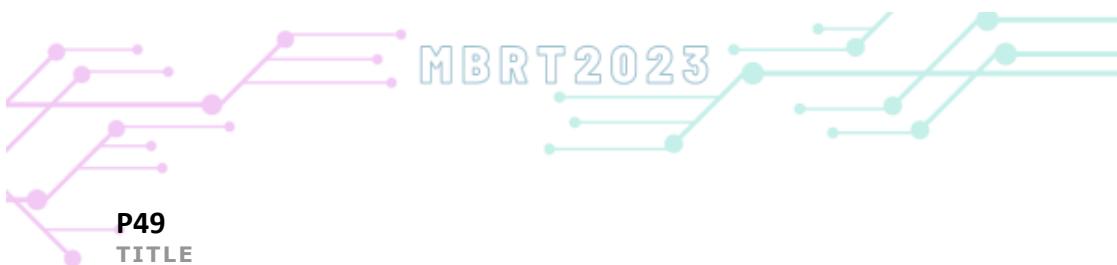
BACKGROUND: Peroxidasin (PXDN) is a haem-containing peroxidase that consolidates the extracellular matrix (ECM) by using hydrogen peroxide (H_2O_2) as a substrate to catalyse the formation of sulfilimine bonds between collagen IV protomers. Aberrant PXDN expression is linked to diseases that remodel the ECM such as cardiovascular and ocular diseases, cancer, and fibrosis. Fibrosis develops due to repetitive tissue injury, followed by aberrant wound healing that causes the excessive deposition of ECM proteins such as collagen into the injured tissue. In turn, crosslinking enzymes such as PXDN are upregulated, and the matrix becomes thick and heavily crosslinked.

METHODS: This study aims to elucidate whether early growth response 1 (EGR1), a zinc-finger transcription factor which regulates cell proliferation, differentiation, apoptosis, and a key pro-fibrotic protein, can drive PXDN expression. To induce a fibrotic response pathway, HEK293 cells were treated with TGF- β 1 and western blot was performed to determine if EGR1 and PXDN were expressed in human embryonic kidney 293 (HEK 293) cells, followed by immunofluorescence microscopy to evaluate the localisation of their expression and the amount. Chromatin immunoprecipitation (ChIP) paired with PCR was then done to determine whether the EGR1 transcription factor interacts with the PXDN promoter.

RESULTS & DISCUSSION: Western blot results indicated that both EGR1 and PXDN were expressed in the HEK293 cells. Immunofluorescence microscopy showed EGR1 is expressed in the nuclei whereas PXDN expression was in the ECM. The correlated total cell fluorescence (CTCF) analysis indicated a significant increase in the expression of both target proteins following TGF- β 1 treatment. ChIP-PCR showed that EGR1 binds to the PXDN promoter. PXDN is thus a TGF- β 1 responsive gene and its promoter has a transcription factor binding site that allows for the binding of EGR1. Future work includes performing the luciferase reporter assay to determine if EGR1 can activate or repress the expression of the PXDN gene.

CONCLUSIONS: In conclusion, we showed that EGR1 interacts with the PXDN promoter. This further establishes a role for PXDN in fibrosis and identifies it as a possible anti-fibrotic therapeutic target.

ACKNOWLEDGEMENTS: NRF for funding and Professor Demetra Mavri-Damelin for supervision.



P49

TITLE

Early epigenetic modulations guide the differentiation of monocytes into macrophages

AUTHORS

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BACKGROUND: Upon tissue injury or infection, monocytes are recruited to tissues where they can differentiate into macrophages, participating in tissue repair and defence against pathogens. This process is frequently investigated using in-vitro THP-1 cell lines differentiated by phorbol 12-myristate 13-acetate (PMA) treatment. A wide variety of PMA protocols has successfully differentiated THP-1 cells into macrophage-like cells with a treatment time of above 48 hours.

METHODS: In this study, we investigated the impact of 24-hour PMA treatment on chromatin accessibility and its influence on gene expression in THP-1 cells. We identified accessible chromatin regions using HMMRATAC with quality-filtered ATAC-seq libraries aligned via Bowtie2. Dynamic chromatin regions ($|L2FC| > 2$; FDR < 0.05), identified using DiffBind, were annotated using GENCODE and geneHancer, along with the histone markers documented in ChIP-atlas. Differentially expressed genes were identified using DEseq2 ($|L2FC| > 2$; p.adj < 0.05).

RESULTS & DISCUSSION: Our result revealed a limited correlation between dynamic chromatin regions and gene expression changes, with the exception of genes associated with inflammatory responses and cell adhesion displayed modulation in both mechanisms. Within which, key genes related to monocyte-to-macrophage differentiation and macrophage activation, such as CSF1, CSF1R and IL1 α / β , exhibited increased gene expression along with an opening of their cis-regulatory elements. Therefore, our findings suggest that 24-hour PMA treatment initiates the transformation of monocytic THP-1 cells into macrophage-like cells by regulating the accessibility of key factors in monocyte-to-macrophage differentiation.

CONCLUSIONS: These findings provide a multi-omic perspective on how early responses shape the differentiation process by influencing the epigenetic landscape of key genes.

ACKNOWLEDGEMENTS: Author of this research has been funded by NRF Postgraduate Scholarships from National Research Foundation (NRF).

**P50****TITLE**

Long non-coding RNA (lncRNA) PANDA as a potential target for combination chemotherapy in oesophageal squamous cell carcinoma (OSCC) cell lines.

AUTHORS

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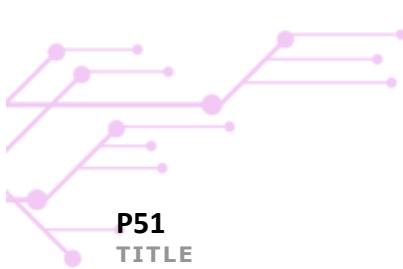
BACKGROUND: Oesophageal cancer is one of the leading causes of cancer death worldwide for which oesophageal squamous cell carcinoma (OSCC) is the major subtype in southern and eastern Africa. Cisplatin is a well-established drug used to treat multiple cancers including OSCC. Drug resistance is a major impediment for continued application. lncRNA P21 associated non-coding RNA DNA damaged activated RNA (PANDA) is known to function in cell cycle regulation in response to DNA damage and is upregulated in OSCC. We aim to determine lncRNA PANDA expression in South African derived OSCC cells and establish whether down-regulation of this lncRNA can be used to supplement cisplatin therapy.

METHODS: MTT assays were used to determine EC₅₀ concentrations of cisplatin in WHCO1, WHCO5 and SNO cells and HEK293 cells as a non-cancer control. RT-PCR was used to detect lncRNA PANDA in untreated and cisplatin treated cells. qRT-PCR will be used to quantify siRNA mediated knockdown of lncRNA PANDA. MTT assays with cisplatin and siRNA PANDA will then be used to determine the effect of lncRNA PANDA knockdown on cisplatin cytotoxicity.

RESULTS & DISCUSSION: EC₅₀ values for cisplatin were $22.32 \pm 4.23 \mu\text{M}$ in HEK293, $23.16 \pm 4.69 \mu\text{M}$ in WHCO1, $11.31 \pm 0.39 \mu\text{M}$ in WHCO5 and $11.03 \pm 3.11 \mu\text{M}$ in SNO. LncRNA PANDA expression was detected in all cell lines in the presence and absence of cisplatin. siRNA knockdown is currently being optimised prior to MTT assays for the final objective. The final objective will allow us to determine whether lncRNA PANDA knockdown can be combined with cisplatin for a more effective OSCC therapy.

CONCLUSIONS: This research has the ability to provide evidence that lncRNA PANDA could serve as a potential target for cancer treatment and overcome chemo-resistance against cisplatin in OSCC cell lines.

ACKNOWLEDGEMENTS: I would like to thank Dr Rupal Jivan for her continuous help, guidance and support as well as the University of the Witwatersrand for their funding throughout my MSc.

**P51****TITLE****PD-L1 in a South African Cohort of Endometrial Carcinomas****AUTHORS****¹Reubina Wadee and ¹Innocent Maposa****AFFILIATIONS**

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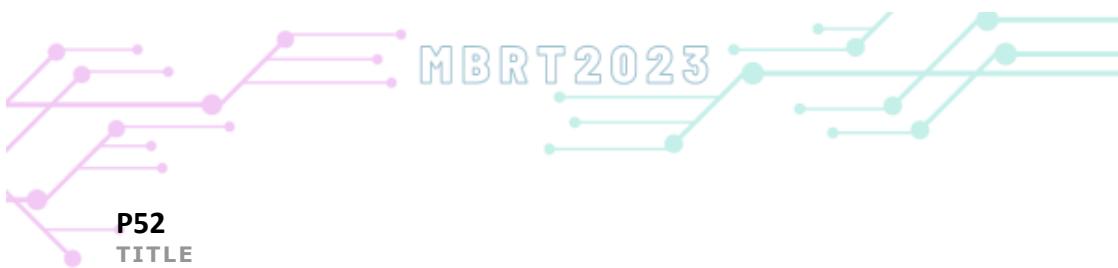
BACKGROUND: The Programmed Death-1(PD-1)/Programmed Death Ligand 1(PD-L1) pathway is the focus of numerous clinical trials on various tumours using immunotherapy. Endometrial tumours with Polymerase E gene mutations and microsatellite unstable neoplasms have demonstrated strong immune responses against mutationally associated neoantigens, with favourable results using immune checkpoint inhibitors. We aimed to assess PD-L1 on endometrioid endometrial carcinomas (EECs) in South Africa's state hospital sector, which, to the best of our knowledge, has not been investigated.

METHODS: We performed PD-L1 immunohistochemistry on 145 EECs and compared PD-L1 status to our data on cases that had previously undergone mismatch repair (MMR) immunohistochemistry, microsatellite instability assessment by polymerase chain reaction and methylation analysis.

RESULTS & DISCUSSION: PD-L1 was expressed in 13.1% of EECs, 7 of which showed MMR deficiency. There was MMR deficiency in 27% of PD-L1 negative cases ($p=0.37$). There were 47.4% microsatellite unstable PD-L1 positive cases while 52.6% of PD-L1 positive cases were microsatellite stable by PCR ($p=0.23$). Of the cases that underwent methylation testing and were PD-L1 positive, 2 were unmethylated and 8 cases were methylated ($p=0.54$). In contrast to a Jordanian study, on a population that has also not been extensively investigated, our study showed far lower PD-L1 expression ($p=0.0170$).

CONCLUSIONS: This study may provide the impetus for future possible immune therapies for endometrial cancer patients in the state sector of South Africa. Our study provides data from a developing country which adds to current global data, thus allowing for provision of a greater knowledge pool from which gynaecologic oncologists, medical oncologists, radiation oncologists and anatomical pathologists may draw upon, with the goal of improved patient care.

ACKNOWLEDGEMENTS: This research has been funded by the National Research Foundation (NRF) Thuthuka Grant (Grant Number: 138436).

**P52****TITLE**

Investigating the effects of cholesterol-depletion on pancreatic cancer and drug resistance in vitro and in vivo

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BACKGROUND: Pancreatic cancer (PC) ranks 7th in cancer-related deaths due to its resistance to chemotherapy. This study aims to identify the role of cholesterol in PC and to use a cyclodextrin to reduce cell cholesterol and evaluate its anticancer potential in PC treatment, both alone and in combination with clinically used chemotherapeutic drugs.

METHODS: To investigate this aim, several assays were performed. These include cell viability assays, immunofluorescence of cholesterol bodies (lipid rafts, lipid droplets, free cholesterol) in cells, in vivo study, gene, and protein expression analysis in PC tumours derived from the in vivo study treated with cyclodextrin (KS-01), gemcitabine (GEM) and 5-fluorouracil (5-FU).

RESULTS & DISCUSSION: In the presence of KS-01, chemosensitivity of PC cells increased by almost 50%, but no changes were seen in membrane lipid raft content whereas free cholesterol content was reduced. In vivo data provided valuable insights into the metastatic ability of PC and tumour growth in xenograft mice model.

CONCLUSIONS: Novel data from this project demonstrates the aggressiveness of PC and the high cholesterol content it depends on to survive which paves the way for future research in therapeutically targeting PC. The study highlights the intriguing role of cholesterol in chemoresistance and provides baseline data for future studies to exploring the potential role of cyclodextrins in PC treatment.

ACKNOWLEDGEMENTS: This research is supported by SIR grant (SAMRC). Student support offered by WITS Postgraduate Merit Award, Phillip Valentine Tobias Bursary.

P53**TITLE**

Computational modelling of Tunicamycin C interactions with potential protein targets: perspectives from inverse docking with molecular dynamic simulation

AUTHORS

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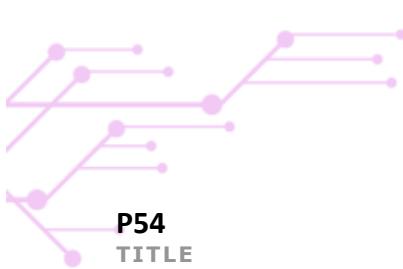
BACKGROUND: Aberrant protein glycosylation promotes colorectal cancer (CRC) progression, a leading cause of cancer mortality in South Africa. Previously, using *in silico* analyses, we identified thymidine kinase 1 (TK1) and protein kinase C (PrKC1) as potential binding targets for the protein glycosylation inhibitor, tunicamycin (Tu). TK1 synthesizes DNA precursors, while PrKC1 regulates protein activity through phosphorylation. The present study aimed to investigate the dynamic events prompting proximal binding of Tu within the binding pockets of TK1 and PrKC1, respectively.

METHODS: The Particle Mesh Ewald Molecular Dynamics (PMEMD) engine embedded in the AMBER18 suite was used to elucidate three-dimensional structural changes induced in these proteins after Tu binding and serial validation studies were used to identify active site residues that play an important role in this stabilized binding.

RESULTS & DISCUSSION: Tu binding caused conformational changes in the 3D structures of TK1 and PrKC1, inhibiting their activities. Time-based dynamics of Tu-protein interactions indicated a stable pattern that promoted an optimal interaction, resulting in maximal stabilization of Tu, within the hydrophobic pockets of TK1 and PrKC1. With serial validation studies, active site residues were noted as important in this stabilized binding. Binding energy calculations confirmed that Tu interacted extensively with TK1 and PrKC1; these being high-affinity binding events.

CONCLUSIONS: As reported here, a binding site associated hydrophobicity, fluctuation of active site residues, and alteration of bulk water surrounding the active sites are all key structural events that may block glycosylation of both TK1 and PrKC1, halting cancer cell proliferation and tumour progression.

ACKNOWLEDGEMENTS: We thank the National Research Foundation, the Wits/MRC Common Epithelial Cancer Research Centre (CECRC), and the Faculty of Health Sciences Research Committee (FRC) for funding this research.

**P54****TITLE**

Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans

AUTHORS

Bontle Masango^{1*}, Julia H. Goedecke^{2,3}, Michèle Ramsay⁴, Karl-Heinz Storbeck⁵, Lisa K. Mucklesfield², Tinashe Chikowore^{2,4}

AFFILIATIONS

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BACKGROUND: Elevated postprandial glucose (PPG), the amount of glucose in the blood after a meal, is a risk factor for type 2 diabetes (T2D). There is high inter-individual variability in PPG responses to identical meals. However, the precise genetic factors and metabolic phenotypes that account for the variation in PPG have not been determined in Black South Africans.

METHODS: This study included 794 participants from the Middle-aged Soweto Cohort (MASC). Postprandial glucose was calculated as the integrated area under the curve (iAUC) for glucose during the oral glucose tolerance test (OGTT) using the trapezoidal rule. Type 2 diabetes (T2D) polygenic risk score (PRS) was computed for each individual using PRSice. Principal component analysis was used to cluster 31 metabolic factors, stratified by sex. Multivariable linear regression was used to assess the proportion of variance in PPG accounted for by metabolic factors and PRS, while adjusting for selected covariates in men and women.

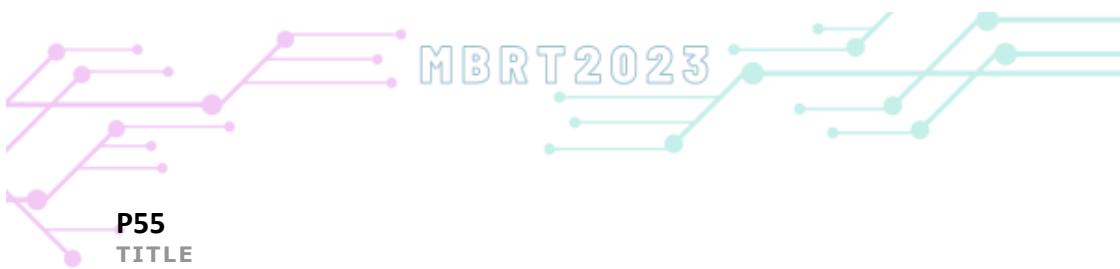
RESULTS & DISCUSSION: The T2D PRS did not contribute to the PPG variability in men or women. In men, the metabolic factors explained 10.6% of the variance in PPG, with principal component 1 (PC1) (peripheral fat), PC2 (liver enzymes and steroid hormones), and PC3 (lipids and peripheral fat) contributing significantly to PPG. In women, metabolic factors explained a similar amount of the variance in PPG (10.8%), with PC1 (central fat) and PC2 (lipids and liver enzymes) contributing significantly to PPG.

CONCLUSIONS: We identified metabolic factors that are associated with PPG variability in Black SA men and women. However, there are other unknown factors that contribute to the variability that still need to be identified.

ACKNOWLEDGEMENTS: The author would like to acknowledge and give her warmest thanks to the University of the Witwatersrand (WITS) and National Health Laboratory Service (N HLS) for allowing the authors to pursue the research study and the National Research Foundation (NRF) for funding the MSc (Med) Genomic Medicine degree. We are grateful to all MASC participants as well as DPHRU field staff.

Funding: The MASC study was funded by the South African Medical Research Council (SAMRC) with funds received from the South African National Department of Health, the UK MRC (via the Newton Fund), and GSK Africa Non-Communicable Disease Open Lab (via a supporting Grant project no: ES/N013891/1).

Supplemental funds were also received from the South African National Research Foundation (Grant no: UID:98561).

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P55

TITLE

Candidate genetic variation associated with the immune response to P2-VP8 Rotavirus vaccination in South African Infants

AUTHORS

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AFFILIATIONS

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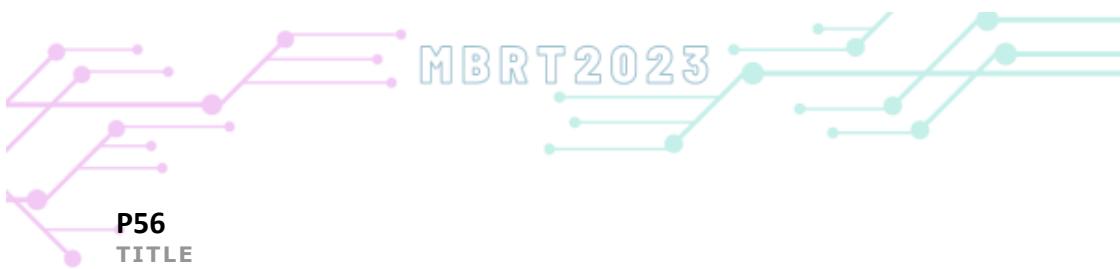
BACKGROUND: The annual death toll attributed to rotavirus disease in children under 5 has been curbed significantly since the implementation of oral vaccines in 2006 but remains at over 200 000 deaths. Non-response to rotavirus vaccination (less than a 4-fold increase in specific antibody titre) is more prevalent in lower income areas with higher child mortality rates, including South Africa. To combat this, new rotavirus vaccines are in development, including parenteral P2-VP8 rotavirus vaccines. As part of a larger study on multiple vaccines, we assessed the association between 95 candidate single nucleotide polymorphisms (SNPs) suspected to affect vaccine responses and IgA response to the P[8] serotype of P2-VP8 vaccination in 70 Black South African infants.

METHODS: Vaccine response data was available from previously published clinical trials. SNPs of interest were chosen by literature review across multiple vaccines. Blood samples were used for DNA extraction and SNPs were genotyped by MassARRAY. Statistical associations were performed in PLINK 1.07.

RESULTS & DISCUSSION: A multitude of SNPs significantly differed in frequency between our South African population and other global populations of the 1000 genomes project. Several SNPs were significantly associated with P2-VP8 rotavirus vaccine IgA 8 responses, including those related to the TLR, RIG-I, and interferon pathways, as well as Lewis phenotype determinant FUT3 and Secretor phenotype determinant FUT2.

CONCLUSIONS: This study works to increase the available knowledge on genetic variation within South Africa, as well as exploring genetic markers relevant to improving vaccine development in the South African populace.

ACKNOWLEDGEMENTS: • The University of the Witwatersrand and the National Research Foundation for bursary support. • This project was supported by grants from the African Leadership in Vaccinology Expertise (ALIVE) consortium and the Bill and Melinda Gates Foundation for genotyping and antibody assays, respectively.

**P56****TITLE**

Investigating the effects of antioxidants on the transcriptional activity of YY1 and FOXP3

AUTHORS

Alicia Joshua and Dr. S. Fanucchi

AFFILIATIONS

The School of Molecular and Cell Biology

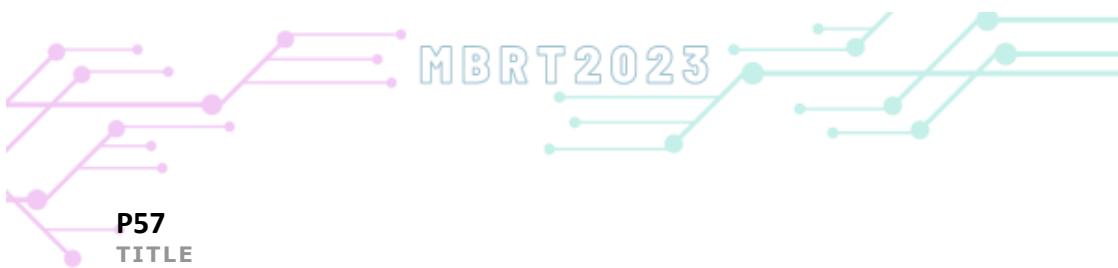
BACKGROUND: FOXP3 is a protein that belongs to the family of forkhead transcription factors. Unlike the other members of this family, FOXP3 is expressed in a subset of CD4+ Treg cells that has a suppressive role in the immune system. It also plays a role in the peripheral immune system as it regulates the development and functioning of Treg cells through transcriptional regulation. Although it has been suggested that intracellular metabolites and metabolic pathways can modulate the expression of FOXP3, the specific protein-vitamin interactions have not been identified.

METHODS: Molecular Docking, Circular Dichroism, Intrinsic Fluorescence and Fluorescence Anisotropy.

RESULTS & DISCUSSION: We examined the protein-vitamin interactions of four antioxidants, vitamin C, E, Silybin and Pyrroloquinoline quinone (PQQ). Molecular docking analysis has shown that Vitamin E, PQQ and Silybin display a strong binding energy towards FOXP3 FHD whereas vitamin C displays a weak binding energy towards the protein. Circular dichroism data has shown that vitamins C and E do not change the secondary structure of the protein whereas Silybin and PQQ have an apparent effect on the secondary structure. Intrinsic Fluorescence studies have shown that all the vitamins display a quenching effect when incubated with FOXP3 FHD.

CONCLUSIONS: These results demonstrate that antioxidants display an interaction with the FOXP3 FHD protein.

ACKNOWLEDGEMENTS: NRF for funding this project, my supervisor Dr. S. Fanucchi as well as my colleagues at the PSFRU.

**P57****TITLE****Autism Spectrum Disorders: Speaking through interactions****AUTHORS****Jessica Sian Brothwell, Dr. S. Fanucchi****AFFILIATIONS****Wits PMA, NRF**

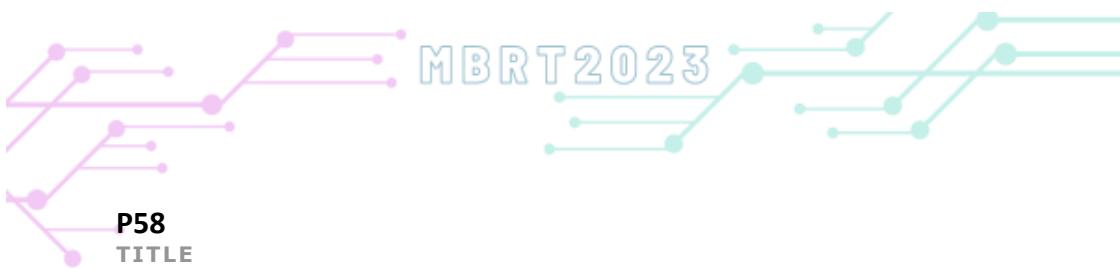
BACKGROUND: Transcription factors (TFs) are proteins that bind to specific sequences of DNA to regulate the rate of gene expression. Forkhead Box P2 (FOXP2), TBOX Brain Protein 1 (TBR1), and Paired Box 6 (PAX6) are TFs involved in developmental disorders of the brain such as autism spectrum disorder (ASD). The FOXP2, TBR1 and PAX6 proteins possess highly conserved DNA-binding domains; the forkhead domain (FHD), TBOX domain (TBOX) and paired domain (PD), respectively. In order to understand how these proteins interact with DNA, and one another, assessing their structural and functional characteristics is essential.

METHODS: The FOXP2 FHD, TBR1 TBOX and PAX6 PD proteins were expressed in 2 x YT media via IPTG induction and purified using immobilized metal affinity chromatography (IMAC). Their secondary structures were assessed using Far- UV circular dichroism (Far- UV CD) under physiological and denaturing conditions. The tertiary structures of the proteins were analyzed according to their intrinsic tryptophan fluorescence under physiological and denaturing conditions. The functionality of the proteins was determined using electrophoretic mobility shift assays (EMSA) and fluorescence anisotropy.

RESULTS & DISCUSSION: Both the FHD and PD were shown to be mainly alpha-helical in structure, whilst TBOX consisted mostly of beta-sheets. When denatured, their secondary structures became obsolete. Intrinsic tryptophan fluorescence of the FHD highlighted the non-polar environment of 2 tryptophan residues, with 1 tryptophan residue exposed to solvent. In addition, the PD displayed one tryptophan residue at the core of the protein. TBOX consists of 8 tryptophan residues, which were mainly contained in the hydrophobic core of the protein. Under denaturing conditions, tryptophan fluorescence displayed a bathochromic shift in wavelength for all proteins. This is indicative of exposure of the hydrophobic core to the polar environment. EMSAs revealed the functionality of the proteins by their ability to bind their respective target DNA sequences. Functional relationships between the proteins were established using fluorescence anisotropy. Here, the FHD and TBOX displayed binding to the PD with KDs of $4.8 \pm 0.9 \mu\text{M}$ and $4.1 \pm 0.4 \mu\text{M}$, respectively. Thus, these proteins bind one another with medium affinity.

CONCLUSIONS: The structural and functional characteristics of FOXP2 FHD, TBR1 TBOX and PAX6 PD are essential in identifying biomarkers for ASDs. It was determined that correctly folded and functional FOXP2 FHD and TBR1 TBOX independently bind to PAX6 PD with medium affinity. The results of this novel discovery may be the first step in understanding the complex nature of ASDs.

ACKNOWLEDGEMENTS: Dr. S. Fanucchi, Wits PMA, NRF, PSFRU.

**P58****TITLE**

Targeting Glutathione Transferases: A Computational Approach to Discovering Antischistosomal Porphyrin Compounds

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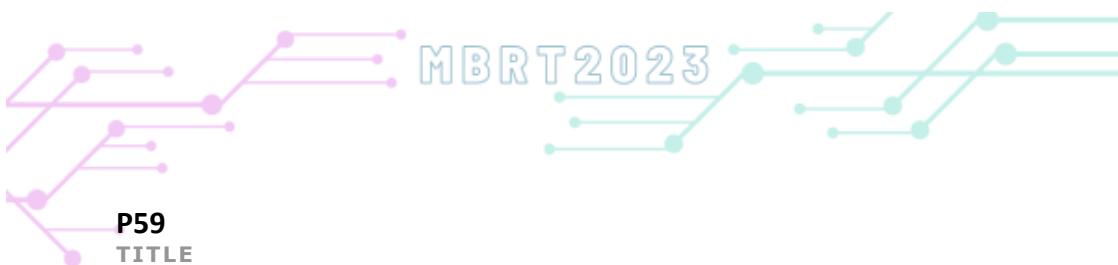
BACKGROUND: Schistosomiasis is a neglected tropical disease caused by parasitic trematodes, presenting an ongoing global health and veterinary challenge due to their acquired drug resistance. There is a pressing need for new, effective anthelmintic agents for both prevention and treatment. Natural compounds, such as porphyrins, have demonstrated the potential to inhibit crucial detoxification enzymes, known as glutathione transferases (GSTs), which play a role in evading host immune responses and counteracting drug therapy in these parasites.

METHODS: In this study, computational modeling was employed to screen 913 porphyrin IX-like compounds as potential GST inhibitors. Unlike traditional docking methods, we integrated a brief molecular dynamics (MD) simulation to determine the most stable protein structure conformation for high-throughput virtual screening.

RESULTS & DISCUSSION: Our results emphasize the critical importance of the starting point in this process. For the 26 kDa japonicum GST (PDB 6RWD), the top-scoring compounds were CID: 122690402 for the minimized structure and CID: 137797052 for the MD structure. In the case of the 28 kDa haematobium GST (PDB 1OE8), the leading compounds were CID: 70415734 and CID: 69301914, respectively, out of 461 filtered compounds. Furthermore, CID: 89195570 exhibited reasonable binding affinity across all four systems.

CONCLUSIONS: These findings provide valuable insights for both theoretical and empirical research, shedding light on the potential of porphyrins as agents optimized and developed for combating helminthic infections, particularly schistosomiasis.

ACKNOWLEDGEMENTS: Dr Ikechukwu Achilonu for his supervision, members of PSFRU and Department of water and sanitation for funding.

**P59****TITLE**

Obtaining High Yield Recombinant Enterococcus faecium Nicotinate Nucleotide Adenylyltransferase for X-Ray Crystallography

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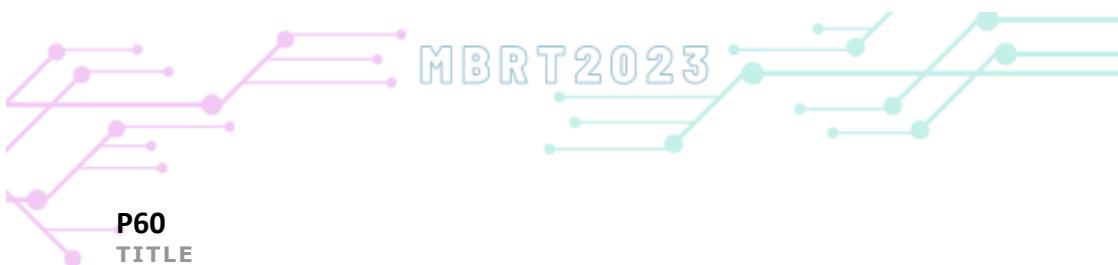
BACKGROUND: The enzyme Nicotinate Nucleotide Adenylyltransferase (NNAT) has been a significant research focus on druggable targets, given its indispensability in the biosynthesis of NAD+, which is crucial to the survival of bacterial pathogens. However, no information is available on the structure-function of *E. faecium* NNAT (EfNNAT). To provide this missing information, the availability of a highly purified EfNNAT is a significant step and a pipeline to accessing this knowledge. Hence, we established the expression and purification protocol for obtaining a high-yield recombinant EfNNAT and solved the enzyme's three-dimensional structure in this study.

METHODS: The EfNNAT sequence was codon-optimized, synthesized, and cloned into a pET-derived expression vector (pET-11a). Overexpression of the recombinant EfNNAT was carried out using the *E. coli* expression system, while a single-step Ni²⁺-IMAC purification method was used to isolate the protein. Finally, the three-dimensional structure of EfNNAT was determined by X-ray crystallography.

RESULTS & DISCUSSION: Approximately 101 mg of EfNNAT was obtained per 7.8 g of wet *E. coli* cells, estimated to be >98 % pure. Suggesting that the expression system and the purification technique were efficient. The high-resolution crystal structure of EfNNAT determined at 1.90 Å revealed the presence of ions occupying and interacting with conserved amino acid residues within the putative substrate binding site. Hence, providing insight into the probable substrate preference of EfNNAT.

CONCLUSIONS: Recombinant EfNNAT was successfully overexpressed and purified to homogeneity for the first time, thus opening the protein to further studies. With this first-ever three-dimensional structure of EfNNAT solved, structural evaluation and drug-based screening can now be achieved.

ACKNOWLEDGEMENTS: South African Medical Research Council (SA-MRC)
Protein Structure-Function Research Unit (PSFRU).

**P60****TITLE**

The expression and biophysical characterisation of Klebsiella pneumoniae adenyllyltransferase

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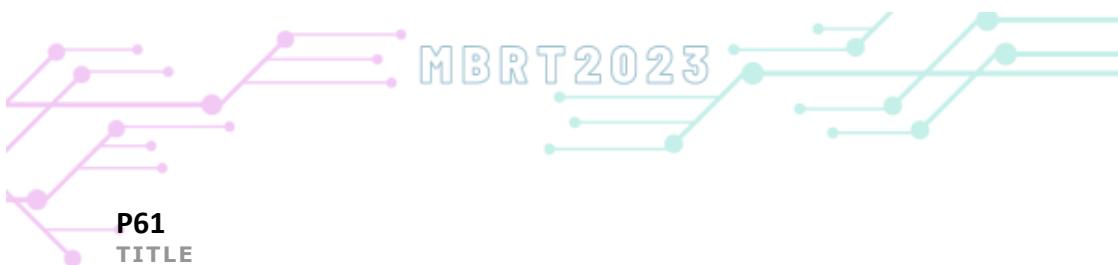
BACKGROUND: Nosocomial infections manifest 2 days following the admission of a patient to a healthcare institution. *Klebsiella pneumoniae* (*K. pneumoniae*) has become a critical pathogen posing serious concern globally due to the rising incidences of hypervirulent and carbapenem-resistant strains. Glutaredoxin is a redox protein that protects cells from oxidative stress as it associates with glutathione to reduce mixed disulfides. Protein adenyllyltransferase (PrAT) is a pseudokinase that has a proposed mechanism of transferring an AMP group from ATP to glutaredoxin. Inducing oxidative stress to the bacterium by inhibiting the activity of PrAT is a promising approach to combating its contribution to nosocomial infections.

METHODS: The pET-11a expression system and nickel affinity chromatography are used to express and purify KpPrAT, respectively. Structural analysis with Far-UV CD, for secondary structural content, and extrinsic fluorescence spectroscopy, for tertiary structural content. Additionally, the SYRPO-orange thermal shift assay for the analysis of the thermal stability of KpPrAT.

RESULTS & DISCUSSION: The pET expression system and nickel affinity chromatography were effective in expressing and purifying KpPrAT. Far-UV CD suggests that the protein is predominantly α -helical, even in the presence of Mg²⁺. Extrinsic fluorescence spectroscopy with ANS indicate that the presence of a hydrophobic pocket in the presence of ATP and Mg²⁺, while mant-ATP studies allude the potential nucleotide binding ability of KpPrAT and Mg²⁺ increases the thermal stability of the protein.

CONCLUSIONS: Conclusively, the presence of Mg²⁺ induces a conformation in KpPrAT that favours nucleotide binding.

ACKNOWLEDGEMENTS: My supervisor Dr. Achilonu, members of the PSFRU, and the Department of Water and Sanitation for funding me.

**P61****TITLE**

Molecular Docking and in vitro validation of Imiquimod as a potential Tankyrase 2 Inhibitor in Colorectal Cancer

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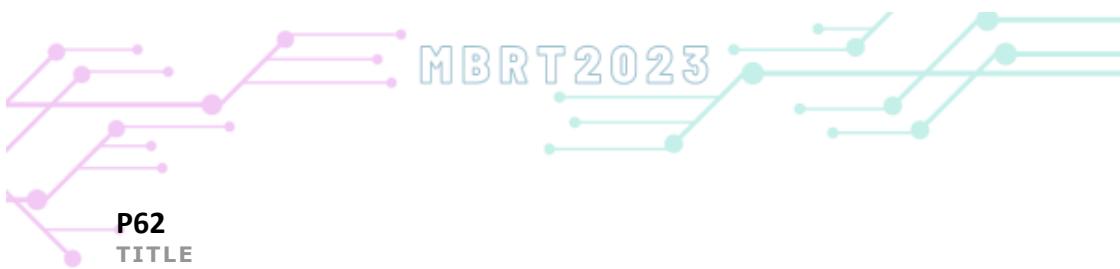
BACKGROUND: Colorectal cancer (CRC) is the third most diagnosed cancer in South Africa. Tankyrase (TNKS) is a positive regulator of the Wnt/β-catenin signaling pathway, which is upregulated during early colorectal carcinogenesis. TNKS inhibitors have shown potent activity against CRC and are currently being investigated in clinical trials. Using *in silico* investigation, we identified TNKS2 as a potential binding target for imiquimod, an immunomodulator. Although imiquimod has shown efficacy against many tumour types, its molecular mechanisms are still being investigated.

METHODS: Imiquimod (ChEBI36704) was screened using High-throughput Docking. This predicted Tankyrase 2 (PDB:3mhj) as the top binding target. Induced-fit docking (IFD) was performed on imiquimod and TNKS2 (Schrödinger). Reactome was used to conduct pathway-focused analysis of the top 35 predicted binding targets of imiquimod. Currently, beta catenin protein expression is being investigated following imiquimod treatment on CRC cell lines to validate the *in silico* predictions.

RESULTS & DISCUSSION: Induced-fit docking (IFD) reproduced a highly similar binding mode of imiquimod and the co-crystallized structure; with imiquimod having a slightly superior IFD score (-925.73, compared to -924.68). This finding suggests that Imiquimod may suppress WNT/Beta catenin signalling through inhibiting TNKS2. *In vitro* studies using CRC cell lines are currently being done to verify the efficacy of the predicted *in silico* results. We observed an enrichment of the RAS/RAF/MAPK pathway proteins which is highly deregulated in CRC and is known to cooperate with WNT signalling to promote the carcinogenesis of CRC.

CONCLUSIONS: To the best of our knowledge, this is the first study that proposes that Imiquimod may induce its anticancer activity through WNT/beta catenin inhibition. Since the WNT/beta catenin pathway is deregulated in CRC and several other cancers, this finding has profound implications in harnessing imiquimod's potential as an anticancer agent.

ACKNOWLEDGEMENTS: We acknowledge the University Research Council, University of the Witwatersrand for funding.

P62**TITLE**

Evaluation of the Xpert® Carba-R assay in detecting the blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP-1 gene sequences associated with carbapenem-non-susceptibility.

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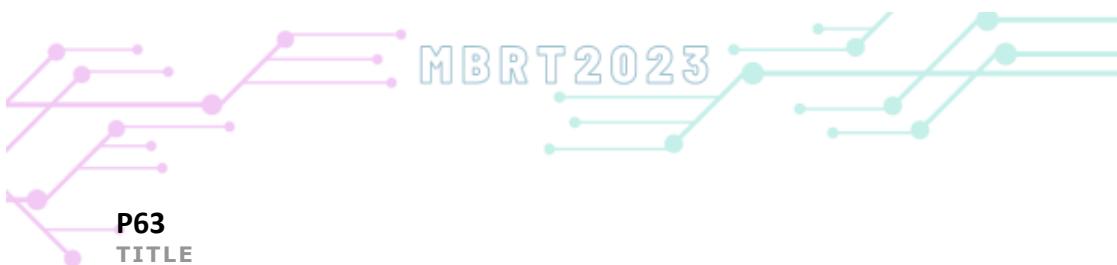
BACKGROUND: The prevalence of carbapenemase-resistant Enterobacteriales (CRE) continues to increase globally with a notable impact on patient management and public health. Carbapenemase-Producing Enterobacteriales (CPE) are especially problematic due to the carbapenemases being located on mobile genetic elements such as integrons, transposons, and plasmids, thus, have the potential for widespread transmission to other species and genera of bacteria. The Xpert Carba-R assay is a qualitative diagnostic test designed to rapidly detect the blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP-1 gene sequences associated with carbapenem-non-susceptibility. Therefore, this study aimed to evaluate the performance of the Xpert® Carba-R assay in detecting the blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP-1 carbapenemase genes from bacterial culture and rectal swabs using the Gene Xpert® Instrument System.

METHODS: The assay was tested on a collection of 95 well characterized Enterobacteriales. The bacterial cultures were processed and analysed following the manufacturer's instructions. The limit of detection for the blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP-1 carbapenemase genes as well as the sample stability up to 7 days was also evaluated.

RESULTS & DISCUSSION: The performance of the Xpert Carba-R was high with an accuracy of 99% for isolates cultured on blood agar and rectal swabs, respectively. The assay also had a sensitivity of 98% sensitivity and 100% specificity. Moreover, it was able to detect all the respective carbapenemases in double carbapenemase producers as well as carbapenemases in non-fermenter organisms. The rectal swabs inoculated with isolates positive for the five carbapenemase genes remained positive for up to 7 days at room temperature and the limit of detection ranged between 10E1CFU/ml- 10E2CFU/ml depending on the carbapenemase detected.

CONCLUSIONS: In conclusion, the Xpert Carba-R assay offers rapid and accurate identification of the five common carbapenemases and can be performed on pure bacterial culture as well as on rectal swabs with a time to results of approximately 50 minutes plus 5 minutes hands-on time per isolate.

ACKNOWLEDGEMENTS: The authors would like to thank the staff at the Infection Control Service Laboratory Unit and the Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses at the NICD for assisting us with the Enterobacteriales isolates used in this evaluation study.



P63

TITLE

Structural and functional studies of hemoglobin from the greater flamingo (*Phoenicopterus roseus*) through *in-silico* approach

AUTHORS

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BACKGROUND: The functional properties of avian haemoglobins (Hbs) are significantly closer to those of mammalian Hbs. Gaseous exchange takes place in avian species through specialised lungs that are smaller in size, more stable, and rigid in comparison to mammals. The greater flamingo (GF) is one of the largest species in the flamingo family in the world. The structure and function of avian Hbs are regulated by Inositol Pentaphosphate (IPP) and Adenosine Triphosphate (ATP).

METHODS: The amino acid sequence information of GF Hb was acquired from UniProt. BLAST was performed against the PDB followed by multiple sequence alignment. The graylag goose Hb structure was used as the template structure and two distinct structural forms of GF Hb (deoxy and oxy) were generated and simulated for 500 ns followed by docking studies using the regulators.

RESULTS & DISCUSSION: GF Hb showed the highest sequence identity of 82.3% (α -chain) and 96.6% (β -chain) with the graylag goose Hb. The MD simulation results were validated and analysed for both forms. The docking results of regulators were analysed based on their affinity scores and interactions followed by complex simulations. All these results are discussed in detail in the poster.

CONCLUSIONS: From the simulation, we concluded that the oxy form of GF Hb is more dynamic than the deoxy form. Both regulators exhibited greater affinity towards the GF oxy Hb compared to the GF deoxy Hb. Also, both ligands bind to the same allosteric site of the GF Hb in both forms, albeit with different binding modes.

ACKNOWLEDGEMENTS: The authors acknowledge Wits University and the NRF for financial support in purchasing a high-performance computer and the CHPC (CSIR) for providing the software used in this study.

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