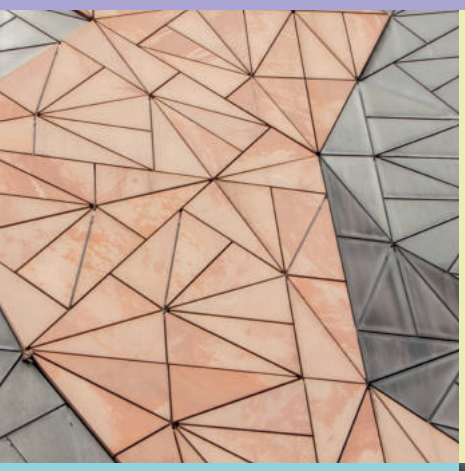
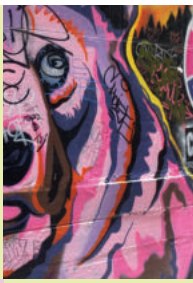
DELEGATE HANDBOOK



APVIC 2024

Asia-Pacific Vaccine and Immunotherapy Congress

14 - 17 May 2024
Marvel Stadium,
Melbourne Australia



APVIC is brought to you by

QIMR Berghofer Medical Research Institute

TRI TRANSLATIONAL RESEARCH INSTITUTE AUSTRALIA

MELBOURNE IMMUNOTHERAPY NETWORK

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DELEGATE INFORMATION

VENUE

Marvel Stadium
740 Bourke St, Docklands, VIC (ENTER THROUGH GATE 9)

P: (03) 8625 7700
https://www.marvelstadium.com.au/

CONFERENCE SECRETARIAT

ASN Events Pty Ltd
9/37 Smith Street
Fitzroy, VIC 3065, Australia

Contact person on-site:
Aileen Lozie | M: +61 479 096 720 | email: aileen.l@asnevents.net.au

REGISTRATION DESK

The registration desk will be open at the following times:

Tuesday, 14th May	1:00 PM – 6:00 PM
Wednesday 15th May	7:30 AM – 5:00 PM
Thursday 16th May	8:00 AM – 2:00 PM
Friday 17th May	8:00 AM – 2:00 PM

VISIT THE EXHIBITION FLOOR & WIN!

You will receive a Passport Game card when you pick up your name badge. You will need to visit at least 20 of the exhibitors on the floor and get their unique passport stamp on your Passport Game card. Once you have received 20 stamps, passports can then be deposited into a drop-box located at the registration desk.

Passports must be turned in by midday on Friday 17th May to be eligible for the prize lottery. Please be sure to put your name, email address and phone number on the card. All prize winners will be announced at the closing ceremony & awards session.

SPEAKER PRESENTATION INSTRUCTIONS

All conference sessions will take place in Victory Rooms C&D. Audio-visual equipment will be supplied and there will be a technician on site to assist with any enquires. Marvel Stadium have a 16:9 screen. **Please make sure your presentation is in 16:9 format**. Please bring your presentation to the AV technicians at the back of the session room at least 2 hours prior to your presentation. A remote mouse/laser pointer will be provided at the lectern.

POSTER PRESENTATION INSTRUCTIONS

Posters should be size A0, portrait orientation and can be attached to the poster boards with the provided Velcro. Additional Velcro supplies can be obtained from the registration desk. The poster boards are located in both the concourse and exhibition floor and are numbered according to your number in the program (please refer to the poster listing on page 23 – 28).

There will be 2 dedicated poster sessions:

- **Poster Session One:** Wednesday 15th May from 6:00pm – 7:30pm
- **Poster Session Two:** Thursday 16th May from 5:15pm – 6:45pm

You will be required to stand at your poster during your sessions for discussion. Poster presenters who have asked to be considered for an award will have their poster's scored during their poster session.

POSTER SET-UP AND REMOVAL

- All posters can be set up for the duration of the congress. You can begin setting up posters anytime from 2:30pm onwards and are required to take down your poster by the end of the lunch session on Friday 17th May.
- Any posters that are left on the boards after 3pm on Friday will be thrown out.

SOCIAL PROGRAM

Welcome Reception
(includes drinks & canapes)
Date: Tuesday 14 May
Time: 4:30 pm – 6:30pm
Location: Victory Rooms A&B (Expo Floor)

Poster Session 1
(includes drinks & canapes)
Date: Wednesday 15 May
Time: 6:00pm – 7:30pm
Location: Victory Rooms A&B (Expo Floor)

Poster Session 2
(includes drinks & canapes)
Date: Thursday 16 May
Time: 5:15pm – 6:45pm
Location: Victory Rooms A&B (Expo Floor)

All delegates are invited to attend the social functions and they are all included in your registration. If you would like to purchase an additional ticket for partners, friends, and children, please see the ASN staff at the registration desk.

EXHIBITORS

*floorplan subject to change onsite

EXHIBITOR LISTING

Booth 1 – Johnmorris Group
Booth 2 – Stemcell
Booth 3 – Sanofi
Booth 4 – Revvity
Booth 5 – United Bioresearch
Booth 6 - Miltenyi Biotec
Booth 7 – Somalogic
Booth 8 – Moderna
Booth 9 – MGI Australia
Booth 10 – Charles River
Booth 11 – Vector Builder
Booth 12 – Millenium Science
Booth 13 – Sino Biological
Booth 14 – Promega
Booth 15 – GenScript
Booth 16 – Integra Bioscience
Booth 17 – Integra Bioscience
Booth 18 – Thermofisher
Booth 19 – SGS Vitrology
Booth 20 – Mimotopes
Booth 21 – Solve Scientific
Booth 22 – Capsugel Australia (Lonza)
Booth 23 – Laftech
Booth 25 – Invirtro Technologies
Booth 26 – Twist Bioscience
Booth 28 – Chameleon Science
Booth 29 – Bioselect

INVITED SPEAKERS

INTERNATIONAL SPEAKERS

SYLVIE ALONSO
NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE

Dr Alonso obtained her PhD degree in Microbiology and Molecular Biology from the University Claude Bernard Lyon I (France), followed by two post-doctoral trainings at Pasteur Institute of Lille (France) and Cornell University (NY, USA). In 2004, she moved to Singapore and established her lab at the National University of Singapore, Department of Microbiology. Dr Alonso's research focuses on studying viral pathogenesis (specifically Enterovirus-A71 (HFMD) and Dengue virus), through the identification of viral determinants and host factors that drive viral fitness and virulence, and using unique symptomatic animal models that her lab has developed. Dr Alonso has also pursued her long-standing interest in vaccine, and in collaboration with Monash University, she has been developing a promising, versatile dendritic cell targeting vaccine platform.

AMY ANDERSON
NEWCASTLE UNIVERSITY, UNITED KINGDOM

Amy graduated with a BSc (Hons) in Medical Biochemistry from the University of Birmingham in the UK in 2000. Following this she then obtained her PhD in Immunogenetics from Imperial College London in 2005. Amy is currently a Senior Research Associate in the Musculoskeletal Research Group at Newcastle University. Her area of interest is understanding disease pathogenesis in rheumatoid arthritis for application to both biomarker discovery and the development of novel immunotherapies for tolerance induction. She is involved in numerous clinical studies, including BIO-FLARE which aims to understand the biological factors driving disease flare in rheumatoid arthritis, as well as AuToDeCRA2, a phase II clinical trial using autologous tolerogenic dendritic cells for the treatment of rheumatoid arthritis.

VALERIE CHEW
SINGHEALTH-DUKE NUS, SINGAPORE

Dr. Valerie Chew is a principal investigator in Translational Immunology Institute (TII) and an assistant professor in Duke-NUS Medical School, Singapore. Her research focuses primarily on understanding the complexity and diversity of the immune context of the tumor microenvironment and its influence on clinical outcome or response to therapy in patients with hepatocellular carcinoma (HCC). Her current work involves high- and multi-dimensional immunophenotyping and immunomonitoring of HCC microenvironment with cutting edge multiplex and single-cell technologies such as Time of Flight Mass Cytometry (CyTOF), single-cell RNA sequencing (scRNA seq) and spatial transcriptomics (ST). This powerful approach allows holistic profiling of immune landscapes and the identification of clinically relevant immune subsets or biomarkers predictive of tumour progression and response to therapy. Her work has gained recognition with multiple grant awards and several high impact publications.

IAN HERMANS
MALAGHAN INSTITUTE OF MEDICAL RESEARCH, NEW ZEALAND

Prof Ian Hermans is a Programme Leader at the Malaghan Institute of Medical Research in Wellington, New Zealand. A major focus of his work has been to investigate the role of unconventional T cells in priming of adaptive immune responses. After initiating this work with Prof Enzo Cerundolo at the University of Oxford, he returned to New Zealand on a Sir Charles Hercus Research Fellowship, where his team continue to develop approaches to harness unconventional T cells in vaccination and therapy. Recent work conducted in collaboration with Prof Gavin Painter at the Ferrier Research Institute and Prof William Heath at the Peter Doherty Institute has highlighted an important role for unconventional T cells in generating long-term T cell memory in the liver, which has been incorporated into the design of novel prophylactic and therapeutic vaccines for liver diseases.

GEOFF HILL
FRED HUTCHINSON CANCER CENTER, USA

Geoff Hill is a medical graduate of the University of Auckland and Hematologist, training in New Zealand and The Dana Farber Cancer Institute in Boston. He was PI of an immunology laboratory in Brisbane, Australia between 2001 and 2018 which focused on the interactions between cytokines, antigen presenting cells and T cell differentiation during transplantation. His laboratory developed a number of paradigms in the field that have instructed clinical practice over this period. Prof Hill moved to The Fred Hutchinson Cancer Center in Seattle in 2018 to take up the Jose Carreras/E. Donnall Thomas Endowed Chair for Cancer Research and Director roles for Hematopoietic Stem Cell transplantation and the Immunotherapy Integrated Research Center. He is also Senior Vice President and Division Director, Translational Science and Therapeutics at the Fred Hutch Cancer Center. Over the last 5 years his laboratory has developed new approaches to study aberrant and tumor-specific immune responses in tissue that have led to a number of new NIH R01, U01 and P01 funded preclinical and translational clinical studies.

PRASANNA JAGANNATHAN
STANFORD UNIVERSITY, USA

Dr. Jagannathan is an Assistant Professor of Medicine with a research program in human immunology of malaria and clinical trials of immune modulatory interventions. His group has been conducting detailed longitudinal cohort studies in order to study how repeated *Plasmodium* infections shapes both the innate immune and CD4+ T cell compartments in children using single cell immune profiling techniques. His group is also studying the impact of antimalarial chemoprevention on pediatric immune development, and the dynamics of malaria-specific immune responses following effective vector control measures. He is funded by the US National Institutes of Health and the Bill and Melinda Gates Foundation.

CRYSTAL MACKALL
STANFORD UNIVERSITY, USA

Crystal Mackall is the Ernest and Amelia Gallo Family Professor of Pediatrics and Medicine at Stanford University, the Founding Director of the Stanford Center for Cancer Cell Therapy, Director of the Parker Institute for Cancer Immunotherapy @ Stanford, and Associate Director of the Stanford Cancer Institute. During a career spanning more than three decades, she has led an internationally recognized translational research program focused on immune-oncology and engineered cell therapies, with a major focus on children's cancers. She is the recipient of numerous awards and is a member of the US National Academy of Medicine, a Fellow of the AACR Academy and the Academy of Immunooncology. She has co-founded 4 biotech companies, published over 250 manuscripts and filed more than 35 patents.

SEBASTIAN MAURER-STROH
UNIVERSITY OF SINGAPORE, SINGAPORE

Sebastian Maurer-Stroh studied theoretical biochemistry at the University of Vienna and wrote his master and PhD thesis at the Institute of Molecular Pathology (IMP). After FEBS and Marie Curie fellowships at the VIB-SWITCH lab in Brussels, he has been leading the sequence analytics portfolio in the A*STAR Bioinformatics Institute (BII) since 2007 and Infectious Disease Programme since 2010. He is the Executive Director of BII since January 2021. His computational team is well known for successes at the public-private interface in Singapore from Precision Medicine to Consumer Product and Food Safety and of course for his critical contributions to national and global viral pathogen surveillance through the GISAID data science initiative that has become the single most important source for virus outbreak data sharing and analysis in this pandemic powering public health responses globally.

AMIT SINGHAL
A*STAR INFECTIOUS DISEASES LABS (ID LABS), SINGAPORE

Dr. Amit Singhal is a Senior Principal Investigator at the A*STAR Infectious Diseases Labs (ID labs), Singapore. He chair's A*STAR's BSL3 biosafety committee and is a member of Singapore's Ministry of Health biosafety task force. His research focuses on understanding the mechanisms utilized by pathogens for evading host's immuno-metabolic signaling. The information gained from these investigations are being exploited for designing clinically relevant host-directed therapies. His current research is supported by grants from the NMRC-Singapore and NIH-USA.

CHENQI XU
CHINESE ACADEMY OF SCIENCES, CHINA

Chenqi Xu completed his PhD training in protein chemistry with Dr. Chengwu Chi in 2004 at Chinese Academy of Sciences. A part of his PhD work was done in University of Leuven and University of Hasselt in Belgium. He then received postdoctoral training in immunology with Dr. Kai Wucherpfennig at Dana Farber Cancer Institute, Harvard Medical School. In 2009, he moved back to China to start his independent program focusing on T cell signaling and metabolism. The Xu lab has developed cutting-edge biochemical and biophysical tools to study immunoreceptor signaling and translates the basic knowledge to the development of engineered T cell therapy. In addition, he is interested in the fundamental functions of membrane lipids in T cell biology and have demonstrated the application potential of cholesterol-based immunotherapy. His works have been published in Nature, Cell and other prestigious journals, and selected as Top 10 Science Breakthroughs in China. He have been recognized with prestigious awards, including Shanghai Natural Science Award, Science and Technology Award for Chinese Youth, Xplorer Prize, and Young Investigator Award of Chinese Biological Investigators Society.

QI ZHANG
TSINGHUA UNIVERSITY, CHINA

Dr. Qi Zhang is a research associate professor in School of Medicine, Tsinghua University. She graduated from Martin-Luther University, Halle-Wittenberg Germany with a Ph.D. of Biochemistry/Biotechnology. Her research focuses on pathogenesis, antibody drug and vaccine development in the field of emerging and re-emerging human viral pathogens such as SARS-CoV-1/2, Ebola virus and other major viral infectious diseases. Dr. Zhang's research aims to characterize protective antibody immunity in infected humans and rational design of effective vaccines and therapies against the viral infection. Together with Shenzhen 3rd People's Hospital and Brii Biosciences, Dr. Zhang in Prof. Linqi Zhang's team has developed an antibody combination therapy (amubarvimab/romlusevimab) that demonstrated 80% efficacy in reducing hospitalization and death among the high-risk population in a NIH-sponsored

multicenter, double-blind, randomized, placebo-controlled study. The combination therapy was the first approved by China National Medical Products Administration on December 9, 2021 to treat adults and pediatric COVID-19 patients. Dr. Zhang has also been actively collaborating with Walvax biotechnology in developing COVID-19 nasal vaccines. Her research results have been published in Nature, Immunology, Nature Immunology, Nature Communications and other top-tier journals, with more than 7700 citations.

NATIONAL SPEAKERS

SIMON BARRY
CARINA BIOTECH, SA

Professor Barry has over 25 years research experience in the cell and molecular biology of human T cells, and the manipulation of these cells to model gene function. He has also worked in the biotech sector in immune therapeutics, so has an ongoing interest in both basic and translational medical research. In basic research he has established world class Immunogenomics capability, including the application of multiple genome wide approaches to map the connectivity and accessibility of genes in human T cells**,** and he has applied this in both the immune tolerance and cancer immunology settings. With regards to autoimmunity in children Prof Barry is a chief investigator on the ENDIA birth cohort building a biobanks from birth to age 5 in children at risk from type 1 Diabetes. This $18 million research program is exploring the environmental determinants of type 1 diabetes, and is a parallel use of the immunogenomics proposed in this application. In 2021 he was awarded a $500K JDRF innovation grant to connect the genetic risk of T1D to the genes that are altered using the ENDIA longitudinal cohort to follow progression. Most recently he has co-founded a longitudinal COVID cohort study covering convalescents and newly infected people in South Australia. He is applying the immunophenotyping and immunogenomics expertise to the COVID cohort in the context of infection and vaccination, to molecular map robust durable immunity, whether by infection or vaccination or both.

In translational research he has been a theme leader of 2 Cooperative Research Centres (CRC for Biomarker Translation, $63M (2007-2013) and CRC for Cell Therapy Manufacturing $58M (2014-2019) he has developed a number of immunotherapy tools and technologies. In addition, his cancer immunotherapies program developing a novel pan cancer CAR-T cell therapy has now spun out into a $35M start-up; Carina Biotech. These research programs and funding successes have enabled him to build a research platform for applied immunology and immunogenomics. He is Director of a Phenomics Australia/NCRIS funded facility (Functional Genomics, SA) to enable genome wide unbiased functional genomics analysis by applying robotics based whole genome arrayed CrispR screening.

CALUDINE BONDER
UNIVERSITY OF SOUTH AUSTRALIA, SA

Professor Claudine Bonder (BSc(Hons), PhD) is the head of the Vascular Biology and Cell Trafficking Laboratory at the Centre for Cancer Biology in Adelaide, South Australia.

Having completed her undergraduate degree at the University of Adelaide and then a PhD at Flinders University, Claudine trained at the University of Calgary in Alberta, Canada before returning to Australia. She now has over 20 years' experience in advancing medical research with continued funding from competitive granting schemes such as the NHMRC, multiple CRCs and industry. Claudine has ~100 scientific publications, is an inventor on granted patents, mentors the next generation of scientists, liaises with consumer advocates, is scientific advisor to companies (Carina Biotech and TekCyte) and is the recipient of numerous awards.

The overarching focus of her research program is to better understand how blood vessels contribute to cancer progression such that better patient outcomes can be achieved.

MILES DAVENPORT
UNIVERSITY OF NEW SOUTH WALES, NSW

Miles Davenport leads the Infection Analytics Program at the Kirby Institute at UNSW Sydney. His team of applied mathematicians and evidence specialists incorporate statistical and computational approaches to understand infection and immunity. His research focus is on using meta-analysis and modelling to analyse host-pathogen interactions in infections including SARS-CoV-2, HIV, and malaria. He has a wide variety of clinical and experimental collaborations both within Australia and overseas and his work aims to integrate experimental and clinical data. He is a past-President of the Australasian Society for Immunology, past Section Editor at *Journal of Immunology*, and Senior Editor at *eLife*. His seminal work defining the correlates of protection from SARS-CoV-2 infection has helped inform vaccine policy for COVID-19 and understand immunity to viral variants.

RICCARDO DOLCETTI
PETER MACALLUM CANCER CENTRE, VIC

Prof. Riccardo Dolcetti is a clinician scientist with MD specializations in Oncology and Clinical Immunology with >20y experience in translational research. He is currently the Head of Clinical and Translational Immunotherapy at the Peter MacCallum Cancer Centre, Victorian Comprehensive cancer centre and Department of Microbiology and Immunology Oncology, University of Melbourne. The research of his lab is focused on the identification, validation and clinical exploitation of novel antigen-specific and cell-based immunotherapies and the development and clinical application of immune assays to monitor immune responses in cancer patients. He has a documented expertise in the field of infection-driven tumours, breast cancer and brain tumours. He recently co-developed a nanoparticle-based vaccination platform able to selectively deliver various types of antigens to the most potent antigen presenting cells *in vivo* and generate strong and specific immunity. He successfully administered multi-institutional research programs and projects and published >260 peer-reviewed papers with >10,000 citations. He had the privilege to be elected President of the Italian Society for Cancer Research (2012-13).

DALE GODFREY
UNIVERSITY OF MELBOURNE & PETER DOHERTY INSTITUTE, VIC

Professor Godfrey is an NHMRC Investigator Fellow, a Fellow of the Australian Academy for Health and Medical Sciences, Past President of the Australasian Society for Immunology, and founder and Past President of the Melbourne Immunotherapy Network. Godfrey's area of interest has for many years been in the field of unconventional T cells, spanning NKT cells, MAIT cells, group 1 CD1 restricted T cells, and gd T cells, including their development, function and therapeutic potential.

DAVID GOTTLIEB
UNIVERSITY OF SYDNEY & WESTMEAD HOSPITAL, NSW

David Gottlieb is Professor of Haematology at the University of Sydney. He trained in medicine and haematology in Sydney and undertook post-graduate studies in London and Milan. His major clinical research interests are in stem cell transplantation and the use of cell therapies for infection and tumour immunity. He is the Director of the Blood Transplant and Cell Therapies Program at Westmead Hospital in Sydney and leads the Westmead T Cell Therapies Group. He is past President of the Bone Marrow Transplant Society of Australia and New Zealand and the Haematology Society of Australia and New Zealand.

PAUL HERTZOG
HUDSON INSTITUTE FOR MEDICAL RESEARCH, VIC

Professor Paul Hertzog leads a multidisciplinary research team investigating the Regulation of Innate immune and Interferon Signalling at the Centre for Innate Immunity & Infectious Diseases, Hudson Institute. The program includes: structure-function of type I IFNs and receptors. IFN responses are examined by a systems biology approach. They discovered and are characterising the function of the unique type I IFN epsilon, a constitutive, epithelial regulator of mucosal immunity against infection and cancer in the female reproductive, gastrointestinal and respiratory tracts. His group made seminal discoveries about IFN's mechanism of action, published in Nature, Science, Cell. They use their molecular insights for clinical impact. He was awarded the prestigious Milstein Award for Research Excellence by the International Cytokine and Interferon Society. He is a keen supporter of open, collaborative science, proud to have co-founded the VIIN network and the Lorne I&I conference which he co-convened from 2010 until 2021.

NICK HUNTINGTON
oNKo-Innate, VIC

Professor Huntington is the founder and CSO of oNKo-Innate Pty Ltd, a Melbourne-based Immuno-oncology Biotech. He is an international opinion leader on natural killer (NK) cell biology and NK cell immunotherapy with notably contributions to: regulatory mechanisms of IL-15 signalling, identification of human and murine NK cell differentiation pathways and identification of multiple checkpoint in NK cell activation and tumour immunity. Professor Huntington has published over 130 research articles on NK cell biology and cancer immunotherapy and has been recognised internationally with fellowships from HFSP, Melanoma Research Alliance, Cancer Research Institute and NHMRC and was the winner of the AAS Jacques Miller Award and NFMRI John Dixson Hughes Medal. Professor Huntington heads the Cancer Immunotherapy laboratory at Monash University Biomedicine Discovery Institute.

MISTY JENKINS
WALTER & ELIZA HALL INSTITUTE FOR MEDICAL RESEARCH (WEHI), VIC

Misty Jenkins is a NHMRC fellow and laboratory head in the Immunology Division at Walter and Eliza Hall Institute for Medical Research. Misty leads the immunotherapy program within the Brain Cancer Centre and is dedicated to discovering novel immunotherapy targets for high grade gliomas in adults and children. Her research focusses on the development of novel chimeric antigen receptor T cells for brain cancer. Her group also uses cutting edge two-photon microscopy combined with mouse models of brain cancer to investigate the tumour microenvironment and uncover unique biology of brain tumours.

Prof Jenkins has a PhD in Immunology from The University of Melbourne, followed by postdoctoral positions at The Universities of Oxford, Cambridge and the Peter MacCallum Cancer Centre.

Prof Jenkins was awarded the L'Oreal for Women in Science Fellowship (2013), was Tall Poppy of the year (2015), was awarded the Top100 Women of Influence award (2016) and was inducted onto the Victorian Honour Roll of Women in 2020.

In addition to her research career, Prof Jenkins is experienced in governance and strategy as a company Director, co-chairs a Federal Health Medical Research Future Fund (MRFF) and is a passionate advocate for gender equity and Indigenous Health and education. She was awarded an Officer of the Order of Australia (AO) in 2023 for distinguished service to medical science in Immunology, the support of women in STEM, and to the Indigenous community.

GRANT MCARTHUR
UNIVERSITY OF MELBOURNE, VIC

Professor Grant McArthur is a Fellow of the Royal Australasian College of Physicians and holds a Ph.D. in Medical Biology. He is the Executive Director of the Victorian Comprehensive Cancer Centre Alliance (VCCC Alliance); inaugural Lorenzo Galli Chair of Melanoma and Skin Cancers at the University of Melbourne and is a Senior Principal Research Fellow (NHMRC). He is also Head of the Molecular Oncology Laboratory and a Senior Consultant Medical Oncologist at the Peter MacCallum Cancer Centre.

Professor McArthur was the inaugural winner of the Translational Research Award of the Foundation Nelia et Amadeo Barletta, has held the Sir Edward Dunlop Clinical Cancer Research Fellowship of the Cancer Council of Victoria, was awarded the inaugural Martin Lackmann medal for translational research, received the Medical Oncology Group of Australia's Novartis Oncology Cancer Achievement Award and has been the recipient of the prestigious Tom Reeve Award from the Clinical Oncology Society of Australia, and received a Monash University Distinguished Alumni Award.

He has been a national and international study co-chair of a number of clinical trials of targeted therapies. His research interests include melanoma, clinical trials of targeted therapeutics, discovery of novel drug targets in cancer, targeting oncogenes, immunological effect of targeted therapies, personalised medicine, cell cycle control, metabolism and protein synthesis in cancer.

VIVEK NARANBHAI
MONASH UNIVERSITY, VIC

Vivek Naranbhai (MBCHB, PhD, DPhil) is an Associate Professor and Head of the Laboratory of Translational Immunology at Monash University. He is a practicing oncologist (trained at the MGH-DFCI in Boston, USA) and has PhD and postdoctoral training in immunology virology and cancer biology. His lab works on the biology of antigen presentation and recognition.

He has over 100 career publications (Field Weighted Citation Impact score of 9.85, h-index 40) including Cell (4), Science (1), The Lancet (1), Nature Comms (2), Journal of Clinical Oncology (1), Cancer Cell (1) and Lancet Oncology (1).

Some of his key discoveries include inflammation as a driver of HIV infection, establishing the International Tuberculosis Host Genetics Consortium (ITHGC), defining the genetic regulation of gene expression in neutrophils, described how components of antigen presentation machinery mediate activating and inhibitory signalling and described several mechanisms by which genetic variation affects cancer immunotherapy outcomes. He also led many of the first COVID-19 sero-epidemiology studies, including the largest cohort study in patients with cancer, providing key insights into how neutralizing antibodies and T-cells mediate cross strain protection and effecting policy decisions on vaccine selection, timing, and development. He is involved in creation of early companies developing immunotherapeutics via roles with venture capital groups.

JANE OLIARO
THE UNIVERSITY OF MELBOURNE, VIC

A/Prof Jane Oliaro is a Group Leader in the *Cancer Immunology Program* and Chief Scientist for the *Centre of Excellence in Cellular Immunotherapy* Translation Laboratory at the Peter Mac. Jane completed a BSc (Hons) at Monash University in Melbourne, a PhD at Massey University in NZ, followed by postdoctoral training in France (INSERM) and Australia (Peter Mac). Jane's research program uses immunological assays, genetic screening and preclinical models to understand tumour immune evasion and resistance to immunotherapies such as checkpoint blockade and CAR-T cell therapy. She also leads a preclinical program focused on the development of novel cell-based immunotherapies for translation into pilot clinical trials through the *Centre of Excellence.*

JOSH OOI
MONASH UNIVERSITY, VIC

A/Prof. Ooi is Head of the Treg Therapies Group based at the Monash Health Translation Precinct, Monash University. The vision of his group is to translate their high-profile experimental work on regulatory T cells (Tregs) into clinical treatments for patients with autoimmune diseases. During his PhD and post-doctoral training, he demonstrated the importance of antigen-specific autoreactive T cells in causing autoimmune nephritis. He published his work in the prestigious journals *PNAS*, 2012; and *Nat Commun*, 2019; as well as >20 times in the top two nephrology journals *JASN* and *Kidney Int*. Importantly, he published a 1st author landmark paper in *Nature*, 2017, showing that targeted Tregs were key protectors of autoimmune disease. He has received numerous prizes for these works including the international Mosaic of Autoimmunity Award, Best Science Awards from both the Australian Immunology and Nephrology societies and, more recently, the Victoria Prize for Science and Innovation.

ISMAIL SEBINA
UNIVERSITY OF QUEENSLAND FRAZER INSTITUTE, QLD

Dr Ismail Sebina is a Research Fellow in Prof. Gabrielle Belz's laboratory at the University of Queensland's Frazer Institute. He holds a PhD in immunology from The University of Queensland (awarded in 2017) and a Master's degree in the Immunology of Infectious Diseases from the London School of Hygiene and Tropical Medicine (LSHTM; UK). He has contributed to discovery and translational immunological research in several laboratories across the USA, the UK, Uganda, and Australia, implementing studies in both preclinical mouse models and humans. He completed six years of rigorous postdoctoral training in immunology at the University of Washington (USA) and QIMR Berghofer Medical Research institute (QIMR Berghofer; Australia). He has demonstrated a strong record of publications in high-impact journals such as Immunity, Science Immunology, JCI and PNAS. Dr. Sebina's primary research focus revolves around uncovering mechanisms governing the development and maintenance of natural and vaccine-induced immunity to infections.

ANKUR SHARMA
CURTIN MEDICAL SCHOOL, WA

Ankur Sharma leads the Oncofetal Ecosystem Laboratory at the Harry Perkins Institute of Medical Research and Curtin University. He completed his PhD at the Indian Institute of Science, where he won the Best PhD Thesis award, and then completed postdoctoral training at the Genome Institute of Singapore. A trailblazer in single-cell genomics and spatial transcriptomics, Ankur is responsible for ground-breaking research that has given rise to the novel 'Oncofetal Ecosystems' field, with a foundational paper in *Cell*. This pioneering work has garnered widespread acceptance within the scientific community, making oncofetal cell profiling a routine task in the Liver Cancer Collaborative networks. The oncofetal ecosystem concept has received resounding endorsement from the scientific and clinical communities, demonstrated by the more-than-50 invitations Ankur has received to speak at prestigious scientific and clinical events. The impact of his research is already substantial, with the recent launch of the master observational trial TRACKERx (funded by the MRFF EMCR grant in 2022) and the investigator-initiated clinical trial PLANET2.0. Subsequent publications from Ankur in esteemed journals such as *Cell*, *Science*, *Immunity*, *Nature Cancer Review*, and *Nature Cancer* further illustrate the significance of this work. Ankur received funding from NHMRC Ideas Grant (CIA), MRFF EMCR grant (CIA), and CSL Centenary Fellowship.

MERU SHEEL
SYDNEY SCHOOL OF PUBLIC HEALTH & THE UNIVERSITY OF SYDNEY, NSW

Associate Professor Meru Sheel is an infectious diseases epidemiologist and vaccinologist, particularly interested in vaccines for epidemic control, and immunization amongst priority populations. Dr Sheel leads the Infectious Diseases, Immunisation and Emergencies (IDIE) team at the Sydney Infectious Diseases Institute and Sydney School of Public Health and the University of Sydney. Dr Sheel has been awarded ~AUD1.8million as a CI and is a co-investigator on grants worth AUD13million. Dr Sheel earned a PhD in life sciences from the Queensland Institute of Medical Research and the Queensland University of Technology working on new vaccines for bacterial pathogens - group A streptococcus. Dr Sheel's post-doctoral training was on parasite immunology with a focus on malaria and visceral leishmaniasis before transitioning into public health. Dr Sheel also holds an MPhil in Applied Epidemiology from the Australian National University.

Dr Sheel has extensive field experience of having worked in several dynamic and complex environments in the Asia-Pacific region including India, Cambodia, Samoa, American Samoa, Fiji, Lao PDR and Cambodia. Dr Sheel has also responded to international emergencies in Fiji, Dominica, Rohingya Crisis in Cox's Bazar Bangladesh, Tonga and Papua New Guinea. Dr Sheel also Co-Chairs the IA2030 SP7 on Research and Innovation and serves on the Data Use Working Group, and is a member of the WHO's Immunization and Vaccines Related Implementation Research Advisory Committee (IVIR-AC). In 2019, Meru was recognised as the Science and Medicine winner for 40 Under 40 Most Influential Asian-Australians, the 2020 ANU Vice Chancellors Awards for Impact and Engagement and 2023 finalist for Women's Agenda Leadership awards for Health. Meru's research interest including measles, COVID-19 and other vaccine-preventable but epidemic prone diseases and, operational and implementation research to drive evidence-based decision making.

CLARE SLANEY
PETER MACCALLUM CANCER CENTRE, VIC

Clare is a Senior Research Fellow at the Peter MacCallum Cancer Centre, Melbourne, Australia. Her current research interests are in understanding the interaction between the immune system and cancer, and in the use of immunotherapy to treat cancer. These interests include the use of genetically modified T cells (CAR T cells) to treat solid cancers. Clare has published over 40 papers in high-impact journals including first and last authorships in *Nature Medicine*, *PNAS, Cancer Research*, *Clinical Cancer Research* and *Cancer Discovery*. Her accomplishments have been acknowledged with a number of awards including the Seymour and Vivian Milstein Young Investigator Award for notable contributions to basic and clinical research in Switzerland, a Joseph Sambrook Award in Research Excellence, and the respected Mavis Robertson Award that is given each year to a female principal investigator considered to exhibit the greatest promise as a leader in breast cancer research in Australia. In 2021, a spinout company *Currus Biologics* was formed based on Clare's research that has attracted an $10 million serial A investment.

HELEN THOMAS
ST. VINCENT'S INSTITUTE, VIC

Professor Helen Thomas is head of the Immunology and Diabetes Unit at St Vincent's Institute in Melbourne. Her research is focused on prevention of type 1 diabetes. She aims to protect the insulin-producing cells in the pancreas from being destroyed by both the immune system and the stress that diabetes places on these cells. She has developed collaborations with industry to test immune suppressive drugs, in particular JAK inhibitors, in clinical trials for type 1 diabetes. Her work is also being applied to humans through the transplantation of islets isolated from organ donors by the Tom Mandel Islet Transplant Program in Victoria to reverse type 1 diabetes in severe cases.

RANJENY THOMAS
UNIVERSITY OF QUEENSLAND, QLD

Professor Thomas is Professor of Rheumatology at University of Queensland, Translational Research Institute, consultant rheumatologist at Princess Alexandra Hospital, and fellow of the Australian Academy of Health and Medical Sciences. She founded two Uniquest spin-off companies Dendright (2006-2021), and Liperate in the 2022 CUREator round. In the 2020 Queen's Birthday Honours, she was awarded member of the Order of Australia.

Her research seeks to understand autoimmune disease and restoration of immune tolerance. Through this work, she developed dendritic cell-based citrullinated antigen-specific immunotherapy in the first proof-of-concept trial in Rheumatoid Arthritis. She developed a liposome immunotherapy that targets dendritic cells to induce antigen-specific tolerance, opening new opportunities for the control and prevention of autoimmune disease. Dendright progressed a liposome-based tolerance strategy for rheumatoid arthritis to a phase I trial, and a trial of a liposome-based tolerance strategy for type 1 diabetes is planned for 2023. She has contributed major insights into immune tolerance mechanisms and interaction between microbiome and the immune system to trigger spondyloarthropathy.

INDUSTRY SHOWCASE SPEAKERS

CLAIRE BORG
MODERNA

Dr Borg is Associate Director of Scientific Leadership, Asia Pacific and Latin America, at Moderna. Claire joined the vaccines industry 14 years ago and has worked across a broad portfolio covering pediatric and adult vaccines.

Prior to her move to industry, Dr Borg was a research academic, completing her PhD in the Department of Medicine at Monash University where she investigated the genetic etiology of male infertility.

JAMES BABER
PFIZER

Dr James Baber is a Senior Director and Global Medical Monitor for Pfizer Vaccine Clinical Research and Development based in Sydney, Australia. He completed his medical degree (MBChB) from the Auckland School of Medicine in New Zealand, and MPH (specializing in infectious diseases epidemiology and control) from the University of New South Wales, Sydney, Australia.

PROGRAM

TUESDAY 14 MAY 2024

Welcome to Country
2:00 PM – 2:20 PM — Victory Rooms C&D

Congress Welcome
Chairs: Ashraful Haque & Lorraine O'Reilly
2:20 PM – 2:40 PM — Victory Rooms C&D

Session 1: Opening Session
Chair: Rajiv Khanna
2:40 PM – 4:30 PM — Victory Rooms C&D

Session sponsored by: CSL Seqirus

2:40PM	**Inaugural Lecture** **Speaker: Grant McArthur** The immunotherapy revolution in cancer: lessons from melanoma	*abs#1*
3:25PM	**Sylvie Alonso** A versatile and powerful dendritic cell-targeting platform to deliver vaccine antigen candidates against infectious diseases	*abs# 2*
3:55PM	**Miles Davenport** Using immune correlates to predict vaccine and treatment effectiveness	*abs# 3*

Welcome Reception
4:30 PM – 6:30 PM — Victory Rooms A&B

WEDNESDAY 15 MAY 2024

Myeloid Therapeutics Breakfast Workshop
8:15 AM – 9:15 PM — Medallion Sports Bar

Hosted and brought to you by: MYELOID Therapeutics, Inc.

(breakfast will be provided)

Session 2: Immunotherapy 1
Chairs: Riccardo Dolcetti
9:15 AM – 10:55 AM — Victory Rooms C&D

Session sponsored by: STANDARD BIOTOOLS, somalogic

9:15AM	**Nicholas Huntington** Next Generation Cytokine Therapies	*abs# 4*
9:45AM	**Jane Oliaro** Cellular Immunotherapy in Oncology: Current Status and Future Directions	*abs# 5*
10:10AM	**Simon Barry** Takin a novel CAR-T against LGR5 from R and D to the clinic	*abs# 6*
10:35AM	**Emily B Derrick** Investigating regulators of CXCL9 and CXCL10 expression to improve T cell infiltration and immunotherapy responses in solid tumors	*abs# 7*

10:45AM **Palak H Mehta**
Patient Age is a Distinct Variable that Impacts CAR T Cell Generation, and Age-Related Biomarkers Can Predict Manufacturing Outcomes *abs# 8*

Morning Tea & Networking
10:55AM – 11:25AM Victory Rooms A&B

Session 3: Autoimmunity
Chairs: Lorraine O'Reilly
11:25 AM – 1:10 PM Victory Rooms C&D

11:25AM **Amy Anderson**
Biomarkers of flare and drug-free remission in rheumatoid arthritis *abs# 9*

11:50AM **Ranjeny Thomas**
Prolonging remission in autoimmune disease with antigen-specific immunotherapy *abs# 10*

12:15PM **Kenneth Smith**

12:40PM **Helen Thomas**
The JAK inhibitor baricitnib preserves beta-cell function in patients with new-onset type 1 diabetes *abs# 12*

1:05PM **Claudia A Nold-Petry**
Monomeric IL-38 is a checkpoint inhibitor of the pattern recognition receptor-interferon axis *abs# 13*

Lunch Victory Rooms A&B
1:10PM – 2:10PM

Sponsored Workshop (GenScript)
1:10PM – 2:10PM Medallion Sports Bar
lunch will be available in both the Exhibition Floor and Medallion Sports Bar

Sponsored Workshop by: GenScript Make Research Easy

Session 4: Immuno-engineering
Chairs: Di Yu
2:10PM – 3:50PM Victory Rooms C&D

2:10PM **Chenqi Xu**
Rational design of E-CAR with self-condensation property *abs# 14*

2:40PM **Misty Jenkins**
Developing novel CART cell immunotherapies for brain cancer *abs# 15*

3:05PM **Clare Slaney**
Enhancing Chimeric antigen receptor T cells for solid cancers *abs# 16*

3:30PM **Paul Beavis**
CRISPR engineering of armored CAR T cells enables tumor-restricted payload delivery with enhanced safety and efficacy *abs# 17*

3:40PM **Vicky Qin**
Engineering a TGF-β switch receptor enhances CAR-T cell fitness and anti-tumor efficacy in immunosuppressive models *abs #18*

Afternoon Tea & Networking
3:50PM – 4:10PM Victory Rooms A&B

Session 5: Industry Showcase
Chairs: Ian Barr
4:10PM – 5:10PM Victory Rooms C&D

4:10PM **Lorena Preciado, Global Clinical Development Strategy Expert, Sanofi**
1 target, 3 innovative immunisation strategies: The case of RSV *abs# 19*

4:30PM **Katherine Young, Senior Medical Manager Vaccines – Vaccine and Biosecurity, CSL Seqirus**
Self-amplifying mRNA vaccines: Introducing a second-generation approach *abs# 20*

4:50PM **Claire Borg, Moderna**
Beyond COVID-19: Future potential for disease prevention and therapeutics with mRNA *abs# 21*

5:10PM **James Baber, Senior Director, Vaccine Clinical Research & Development, Pfizer**
Vaccine Technologies: Where are we now and what is the future? *abs# 22*

Lightning Talks 1
Chairs: Nicole Campbell
5:10PM – 5:55PM Victory Rooms C&D

5:30PM **Felicia Bongiovanni**
Modelling the antibody response to infectious diseases to inform vaccine development and intervention strategies *abs# 100*

5:33PM **George Robin Ambalathingal Thomas**
Allogenic T-cell immunotherapy for the treatment of Progressive Multifocal Leukoencephalopathy (PML) using a HLA-definated peptide platform *abs# 101*

5:36PM **Shane He**
mRNA-based engineering for flexible 'off-the-shelf' allogeneic T cell therapy *abs# 102*

5:39PM **Hannah Tompkins**
A streamlined end-to-end manufacturing pipeline for mRNA vaccines and therapeutics *abs# 103*

5:42PM **Shane Horsefield**
Structural Characterisation and Functional Mapping of Immunogenic Domains of Human Cytomegalovirus Glycoprotein B *abs# 104*

5:45PM **Daryl Lee**
A Dendritic Cell-targeting Approach to Deliver a Universal Influenza Vaccine Candidate to the Respiratory Mucosa *abs# 105*

5:48PM **Yu-Chen Enya Chen**
Adoptive TCR-T cell therapy for HPV-associated cancers *abs# 106*

5:51PM **Kevin J Selva**
Mix & Match: Impact of delayed intervals and repeated COVID-19 mRNA boosters on systemic and mucosal antibody responses *abs# 107*

Poster Session 1 & Drinks
6:00PM – 7:30PM Victory Rooms A&B

THURSDAY, 16 MAY 2024

Session 6: Vaccine Design & Delivery
Chair: Dale Godfrey

9:00AM – 11:00AM — Victory Rooms C&D

Session sponsored by: sanofi

9:00AM	**Ian Hermans** Vaccines tailored for inducing liver-resident memory T cells	*abs# 27*
9:30AM	**Sebastian Maurer-Stroh** Vaccine antigens for disease X and monitoring antigenic change efficiently	*abs# 28*
9:55AM	**Vivek Naranbhai** Mechanistic biomarkers of checkpoint inhibitor therapy efficacy and toxicity in cancer	*abs# 29*
10:15AM	**Meru Sheel** 50 years of EPI – what next?	*abs# 30*
10:35AM	**Ismail Sebina** Pre-clinical assessment of mRNA vaccine-induced immunity to Group A Streptococcus	*abs# 31*
10:50AM	**Branka Grubor-Bauk** T cell vaccines against Zika virus	*abs# 32*

Morning Tea & Networking
11:00AM – 11:30AM — Victory Rooms A&B

Session 7: Immunobiology
Chair: Gabrielle Belz
11:30AM – 1:00PM — Victory Rooms C&D

11:30AM	**Ankur Sharma** Oncofetal Ecosystem in HCC: Towards Spatial Precision Oncology	*abs# 33*
12:00PM	**Valerie Chew** Understanding immunobiology of hepatocellular carcinoma for biomarkers and therapeutic discovery	*abs# 34*
12:30AM	**Dale Godfrey** Butyrophilin-dependant phosphoantigen detection by human gamma-delta T cells	*abs# 35*
12:50PM	**Xuan dong** Mutations in intergenic region predominantly contribute to the tumor specific antigens in hypermutated colorectal cancer patients	*abs# 36*

Lunch — Victory Rooms A&B
1:00PM – 2:30PM

Sponsored Workshop (Sanofi)
1:00PM – 2:30PM — Medallion Sports Bar
lunch will be available in both the Exhibition Floor and Medallion Sports Bar

Sponsored Workshop by: sanofi

Session 8: Infectious Diseases
Chairs: Ashraful Haque
2:30PM – 4:20PM Victory Rooms C&D

Session sponsored by: moderna

2:30PM	**Prasanna Jagannathan** Immunological consequences of early life malaria exposure in children	*abs# 37*
3:00PM	**Amit Singhal** Host-directed Therapies for Respiratory Infections	*abs# 23*
3:30PM	**Qi Zhang** The Race between Human Antibody Responses and Coronavirus	*abs# 24*
4:00PM	**Logan Trim** The development of an effective vaccine to protect against female genital tract chlamydial infections	*abs# 25*
4:10PM	**C. Glenn Begley** Novel non-invasive vaccine delivery technology for the creation of safe and reliable mucosal immunity	*abs# 26*

Afternoon Tea & Networking
4:20PM – 4:50PM Victory Rooms A&B

Lightning Talks 2
Chair: Pouya Faridi
4:50PM – 5:15PM Victory Rooms C&D

4:50PM	**Jasmine Chuah** Characterisation of Novel Cytokine Interferon Epsilon in the Murine Peritoneal Cavity	*abs# 141*
4:53PM	**Tirta (Mario) Djajawi** Epigenetic-targeted CRISPR screens identify PRMT1 as a suppressor of MHC-I and anti-tumour immunity	*abs# 142*
4:56PM	**Yadanna Zaw** Expanding neutralising antibody breadth with a polyvalent SARS-CoV-2 mRNA vaccine expressing three linked-RBD domains from different variants	*abs# 143*
4:59PM	**Nicholas YZ Cheang** Targeting Clec9A on type-1 conventional dendritic cells to induce broad and durable systemic and mucosal immune responses against sarbecoviruses	*abs# 144*
5:02PM	**Imogen Bermingham** Micro-projection array patch delivery of live-attenuated Measles and Rubella vaccine in a Phase 1 clinical trial	*abs# 145*
5:05PM	**Marina Leiwe** Identification of regulators of the immune microenvironment in non-small cell lung cancer	*abs# 146*
5:08PM	**Vasso Apostolopoulos** Vaccines in The New Era: What have we learnt in the last 30 years?	*abs# 147*

Poster Session 2 & Drinks
5:15PM – 6:45PM Victory Rooms A&B

FRIDAY, 17 MAY 2024

Session 9: Immune-regulation
Chair: Moritz Eissmen
9:00AM – 10:40AM Victory Rooms C&D

9:00AM **Geoffrey Hill**
Blood Cancers and T cell exhaustion in the bone marrow *abs# 38*

9:30AM **Joshua Ooi**
Engineered antigen-specific TCR-Tregs to treat autoimmune disease *abs# 39*

9:55AM **Paul Hertzog**
Interferon epsilon: a novel regulator of mucosal immunity in infectious and inflammatory diseases in cancer *abs# 40*

10:20AM **Daniel T Utzchneider**
Stem-like potential of T cells in infection and cancer is regulated by ID3 and c-Kit *abs# 41*

Morning Tea and Networking
10:40 AM – 11:10 AM Victory Rooms A&B

Session 10: Immunotherapy 2
Chair: Nicholas Huntington
11:10 AM - 12:45 PM Victory Rooms C&D

Session sponsored by: VectorBuilder

11:10AM **Riccardo Dolcetti**
Novel conventional and unconventional target antigens for improved cancer vaccines *abs# 42*

11:35AM **Claudine Bonder**
Anti-tumour immunity: a co-ordinated approach *abs# 43*

12:00PM **David Gottlieb**
Using T lymphocytes for therapy: challenges and solutions *abs# 44*

12:25PM **Stephen J Blake**
Anti-CD40 is effective in immune cold colorectal cancer *abs# 45*

12:35PM **Cheok Weng Chan**
Leveraging DC activation to overcome tumour heterogeneity in CAR T cell therapy *abs# 46*

Lunch & Networking
12:45PM – 1:45PM Victory Rooms A&B

Session 11: Peter Doherty Oration
Chairs: Rajiv Khanna
1:45PM – 2:45PM Victory Rooms C&D

1:45PM **Crystal Mackall**
CAR T cells for Cancer *abs# 47*

Award, Prizes, Thanks & Closing Address
2:45PM – 3:00PM Victory Rooms C&D

POSTER LISTING

POSTER SESSION ONE – WEDNESDAY 15 MAY 2024

Leesa Lertsumitkul
Novel human EphA3-specific CAR T cells eliminate adult and paediatric high-grade gliomas. *abs# 108*

Thomas J Cole
Enhancing CAR T cells against solid tumours with novel gene-editing approaches *abs# 109*

Ashleigh Davey
Tuning chimeric antigen receptor (CAR)-T cell functions by manipulating receptor structure *abs# 110*

Avishy maliach
Crosslinking of Ly6a metabolically reprogram CD8 T cells for cancer immunotherapy *abs# 111*

Davide Moi
Overcoming immune checkpoint inhibitor resistance to improve melanoma therapy *abs# 112*

Tony Huang
Enhancing CAR T cell therapy in solid tumours with site-specific secretion of BiTEs *abs# 114*

Tara L Cassidy
Quality Control Testing – The Next Generation *abs# 115*

Anne Huber
Proof of principle that loss of mismatch repair protein reduces tumour burden in mouse model of gastric cancer *abs# 116*

Anran Lin
Targeting Mediator kinases in immune cells for immunotherapy in colorectal cancer *abs# 117*

ANDREA DI PIETRO
Novel Insights on CD8+ Tissue-Resident Memory T cells in lymph node metastases from melanoma patients in response to immune checkpoint inhibitors. *abs# 118*

Annemarie Laumaea
Dimeric IgA as an effective therapeutic strategy *abs# 119*

Li-Teng Ong
Epigenetic treatment modality and biomarker to improve anti-HER2 Immunotherapy in HER2+ breast cancer *abs# 120*

Reem ALHulais
Drug targeting of colorectal cancers including cancer stem cells *abs# 121*

Kah Min Yap
Identifying Optimal Tumour-specific Promoters for CRISPR Knock-in Generated Armoured CAR T Cells *abs# 122*

Yuxiao Wang
Chimeric Antigen Receptor Monocytes With Enhanced Anti-Tumor Activities By Harnessing Innate Immunity Pathways *abs# 123*

Shengbo Zhang
CAR-DC1: A novel next-generation dendritic cell therapy for targeting solid tumours *abs# 124*

Chad Poloni
Adoptive transfer of type 1 regulatory T cells prevents inflammation and gut damage in mouse model of colitis *abs# 125*

Kaveh Baghaei
The myeloid cell kinase HCK as a therapeutic target to improve immune checkpoint blockade in high-grade serous ovarian cancer *abs# 126*

Paulo Martins
"Off-the-shelf" CAR T-cell Therapy Targeting Ephrin Receptor A3 Expressed by Glioma Stem Cells and Tumour Vasculature *abs# 127*

Grace Huang
Uncovering Therapeutic Targets For HLA-dependent Immunotherapy Using Immunopeptidomics In Paediatric Ewing Sarcoma *abs# 128*

John JV Vandermeide
Examining epigenetic enzymes as a target in breast cancer metastasis and treatment resistance *abs# 129*

Chen Yang
RASAL3 is a potential negative regulator of CD8+ T cell in antitumor immune response *abs# 130*

Purva Trivedi
Macrophages in HCK knock out mice share a common gene signature across various cancers *abs# 131*

Erwin Tanuwidjaya
Exploring soluble HLA peptidome for cancer biomarkers through immunopeptidomics. *abs# 132*

Lauren Holz
mRNA vaccine against malaria tailored for liver-resident memory T cells *abs# 133*

Amania A. Sheikh
T-bet high expressing CD4 T cell progenitors seed TRM cells in the peripheral tissues *abs# 135*

Jo-Anne Chan
Deciphering the role of IgM in blocking human malaria transmission *abs# 136*

Shivali Savita Chinni
Age-related differences in mRNA vaccine adjuvancy and immunogenicity *abs# 137*

Ramya Ephraim
Identification of cancer pathways and biomarkers in mouse models of spontaneous chronic colitis: From inflammation to cancer *abs# 138*

Adam Thomas
Developing multi-antigen and multi-species mRNA vaccines against malaria *abs# 139*

Elham Moslemi
A precision approach to treatment enhancement by adjuvant Smac-mimetics in oral squamous cell carcinoma (OSCC). *abs# 140*

POSTER SESSION TWO – THURSDAY 16 MAY 2024

Yvonne Dube
Investigating role of non-VAR2CSA specific antibodies in protection from placental malaria *abs# 148*

HongHua Ding
Association of *Plasmodium falciparum* specific afucosylated IgG with placental malaria protection *abs# 149*

AKACHUKWU ONWUKA
Role of IgG antibodies in protection from placental malaria birth outcomes *abs# 150*

Elizabeth Aitken
Fc receptor binding of naturally acquired antibodies to placental binding *Plasmodium falciparum* infected erythrocytes *abs# 151*

Vivin R Kokuhennadige
Searching for broadly reactive monoclonal antibodies to placental malaria antigen, VAR2CSA *abs# 152*

Abolaji S Olagunju
Recombinant Listeria Monocytogenes Vaccine Anti-tumor Response is Independent of the Host Inflammatory Cell Death Machinery *abs# 153*

Renu Balyan
Next generation proteomic profiling of a pan-cancer cohort for the development of screening tools for cancer *abs# 154*

Christine Langer
Engineering of an intrinsically stable SARS CoV-2 soluble spike trimer. *abs# 155*

Jinmin Ma
Single-cell sequencing combined with artificial intelligence assists in COVID-19 vaccine antigen design and preclinical efficacy evaluation *abs# 156*

Tima Shamekhi
Contribution of long non-coding RNAs to the Paediatric Diffuse Midline Glioma immunopeptidome *abs# 157*

Geri Waldi Setiawan
Anti SARS COV 2 IgG levels a year after vaccination in healthcare workers at Prof Dr R D Kandou Hospital Manado *abs# 158*

Charlotte Dr Chen
In-depth characterisation of the tumour immune microenvironment in paediatric solid cancers – PFA ependymoma and osteosarcoma *abs# 159*

Zoe Magill
Differential responses of mouse and human dendritic cells to clinical inhibitors used to treat melanoma *abs# 160*

Geraldine Nadya Putri
Exploiting the Clec9A targeting vaccine platform to develop a safe and effective DENV vaccine *abs# 161*

Razvan C Stan
Febrile temperatures differentially modulate the binding of antibodies to Staphylococcal Enterotoxin B (SEB) and to Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) *abs# 162*

Marsia Gustiananda
Analysis of T-cell responses to the epitopes derived from SARS-CoV-2 nucleocapsid protein presented by HLA Class II alleles in Indonesian population *abs# 163*

Takahiro Asatsuma
CCR5-Mediated Monocyte Interaction and IL-2-Signaling Orchestrate T helper 1 cell differentiation in vivo. *abs# 164*

Shoaib Anwaar
Targeting of Regulatory T cells: A therapeutic paradigm for preventing Cutaneous Squamous Cell Carcinoma *abs# 165*

Amy Body
Humoral and cellular responses to SARS-CoV-2 vaccination in Australian adults with cancer. *abs# 166*

Kezia Christilla Singgih
Is BTN3A1 a regulator of αβ T cell responses? *abs# 167*

Mingyan Fang
Age-related dynamics and spectral characteristics of the TCRβ repertoire in healthy children: implications for immune aging *abs# 168*

Mingyan Fang
Single-cell sequencing combined with artificial intelligence assists in COVID-19 vaccine antigen design and preclinical efficacy evaluation *abs# 169*

Ashley M Firth
Interferon regulatory factors 1 and 2 regulate expression of programmed cell death-ligand 1 in dendritic cells *abs# 170*

Daniel Getts
In vivo Programming of Immune Cells Using mRNA-LNP Chimeric Antigen Receptors *abs# 171*

Harry Stannard
Trends in viral replication and lung pathogenesis of influenza A(H1N1)pdm09 viruses from 2009 to 2022 in the ferret model. *abs# 172*

Yingcheng Wu
Cancer Neutrophil Encyclopedia: A Deep Dive into Antigen-Presenting Warriors *abs# 173*

Sophie C Hamann
PVM infection after bone marrow transplantation leads to increased mortality which is associated with impaired viral antigen-specific T cell responses and IL-6-mediated pathogenic Th17 differentiation in the lung *abs# 174*

Taniya Ahuja
Inhibition of nuclear ACE2 translocation guards against SARS-CoV-2 replication and lung injury via epigenetic imprinting *abs# 175*

Nikita Deshpande
Characterisation of viral interactions between Influenza, SARS-CoV-2 and other circulating respiratory viruses *abs# 176*

Malet Aban
Immunity to zoonotic influenza viruses in individuals vaccinated with seasonal influenza vaccines *abs# 177*

Amir Shamshirian
Bhlhe40 Deficiency in $CD8^+$ T Cells Attenuates Alloreactive Immunopathology and Modulates Cytokine Responses in Graft-versus-Host Disease *abs# 178*

Sacha Zinn
Glycoengineering of Antigens for Focusing The Immune Response *abs# 179*

Aimee Talbot
Enhancing thermal stability of live virus vaccine using metal organic frameworks *abs# 180*

Gayathri M
Circulating CXCR#= CCR4+ and regulatory high-risk HLA class II-restricted islet-specific T cell phenotypes predict poor clinical outcome in children with T1D. *abs# 181*

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ABSTRACTS

1

The immunotherapy revolution in cancer: lessons from melanoma

Grant McArthur[1]

1. VCCC Alliance, Melbourne, ACT, Australia

Abstract not available

2

A versatile and powerful dendritic cell-targeting platform to deliver vaccine antigen candidates against infectious diseases.

Sylvie Alonso[1]

1. National University of Singapore, Singapore, SINGAPORE

Abstract not available

3

Using immune correlates to predict vaccine and treatment effectiveness

Miles Davenport[1]

1. UNSW Australia, Kensington, NSW, Australia

Abstract not available

4

Next Generation Cytokine Therapies

Nicholas Huntington[1]

1. oNKo-Innate, Moonee Ponds, VIC, Australia

Abstract not available

5

Cellular Immunotherapy in Oncology: Current Status and Future Directions

Jane Oliaro[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Abstract not available

6

taking a novel CAR-T against LGR5 from R and D to the clinic

Simon Barry[1]

1. Robinson Research Institute, North Adelaide, SA, Australia

Abstract not available

7

Investigating regulators of CXCL9 and CXCL10 expression to improve T cell infiltration and immunotherapy responses in solid tumors.

Emily B Derrick[1, 2], Imran G House[1, 2], Junyun Lai[1, 2], Phillip K Darcy[1, 2], Paul A Beavis[1, 2]

1. Peter MacCallum Cancer Centre, Melbourne, VICTORIA, Australia

2. The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC, Australia

Immune checkpoint blockade (ICB) has revolutionised the treatment of numerous cancer types, including melanoma and non-small cell lung carcinoma. ICB targets immune-inhibitory molecules on the surface of T cells, unleashing their anti-tumour potential. Despite ICB's success, a high frequency of patients fail to respond to this therapy. A key limiting factor to ICB responses is the number of T cells that infiltrate the tumour microenvironment. T cell infiltration in the context of ICB has been shown to be dependent on chemoattractant molecules CXCL9 and CXCL10. The expression of these chemokines is also predictive of a positive response to ICB across multiple cancer types, highlighting their importance in ICB efficacy (*Litchfield et al, Cell 2021*). We have previously demonstrated that these chemokines are predominantly produced by intratumoral macrophages (*House et al. Clinical Cancer Research 2020*). Therefore, we aimed to identify genes that we could target to enhance CXCL9/10 production in macrophages as a strategy to improve T cell infiltration and ICB responses in solid tumors. To this end, we performed CRISPR/Cas9 screening on a macrophage cell line to identify novel regulators of CXCL9 and CXCL10. To screen for secreted factors, we utilised a CRISPR-HDR technique we have previously validated (*House, Derrick et al. Cell Reports 2023*) to generate a macrophage cell line that expressed GFP and BFP as a bona fide readout of CXCL9/10 production. This screen identified PTPN2 as a key negative regulator of both CXCL9 and CXCL10 production. With PTPN2 inhibitors currently in clinical trials (*Baumgertner et al. Nature 2023*), we sought to define how PTPN2 depletion in macrophages might play a role in anti-tumor immunity. PTPN2 deletion enhanced CXCL9/10 production by primary murine/human macrophages *in vitro.* Myeloid-specific depletion of PTPN2 *in vivo* improved CXCL9 expression in both intratumoral macrophages and dendritic cells, and improved T cell infiltration when combined with ICB in a murine breast cancer model. Importantly, T cell infiltration was improved without elevating T_{reg} numbers This work has uncovered a novel role for PTPN2 inhibition through the elevation of chemokine production by macrophages. It also provides further rationale to combine PTPN2 inhibitors with ICB for improved responses to immunotherapy.

1. Litchfield, K., Reading, J.L., Puttick, C., Thakkar, K., Abbosh, C., Bentham, R., Watkins, T.B., Rosenthal, R., Biswas, D., and Rowan, A. (2021). Meta-analysis of tumor-and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. Cell 184, 596-614. e514
2. House, I.G., Savas, P., Lai, J., Chen, A.X., Oliver, A.J., Teo, Z.L., Todd, K.L., Henderson, M.A., Giuffrida, L., and Petley, E.V. (2020). Macrophage-derived CXCL9 and CXCL10 are required for antitumor immune responses following immune checkpoint blockade. Clinical Cancer Research 26, 487-504.
3. House, I.G., Derrick, E.B., Sek, K., Chen, A.X.Y., Li, J., Lai, J., Todd, K.L., Munoz, I., Michie, J., Chan, C.W., et al. (2023). CRISPR-Cas9 screening identifies an IRF1-SOCS1-mediated negative feedback loop that limits CXCL9 expression and antitumor immunity. Cell Rep 42, 113014. 10.1016/j.celrep.2023.113014
4. Baumgartner, C.K., Ebrahimi-Nik, H., Iracheta-Vellve, A., Hamel, K.M., Olander, K.E., Davis, T.G.R., McGuire, K.A., Halvorsen, G.T., Avila, O.I., Patel, C.H., et al. (2023). The PTPN2/PTPN1 inhibitor ABBV-CLS-484 unleashes potent anti-tumour immunity. Nature 622, 850-862. 10.1038/s41586-023-06575-7.

8

Patient Age is a Distinct Variable that Impacts CAR T Cell Generation, and Age-Related Biomarkers Can Predict Manufacturing Outcomes

Palak H Mehta[1], Shivali S Chinni[1], Patrick Leung[1], Aaron J Harrison[2], Hannah Hughes-Parry[3, 4], Misty R Jenkins[3, 5, 4], Michael H Kershaw[2], Anthony Jaworowski[1, 7, 6], Clare Y Slaney[2], Rachel M Koldej[8, 9], David S Ritchie[8, 9], Kylie M Quinn[1, 6]

1. School of Health and Biomedical Sciences , Royal Melbourne University Of Technology, Melbourne, VIC, Australia

2. Immune Innovation Laboratory , Peter MacCallum Cancer Foundation, Melbourne, VIC, Australia

3. Immunology Division, Walter and Eliza Hall Institute , Melbourne, VIC, Australia

4. Department of Medical Biology , University of Melbourne, Melbourne, VIC, Australia

5. Institute of Molecular Science, La Trobe University, Melbourne, VIC, Australia

6. Department of Biochemistry, Biomedicine Discovery Institute, Monash University , Melbourne, VIC, Australia

7. Burnet Institute , Melbourne, VIC, Australia

8. Department of Medicine, University of Melbourne , Melbourne, VIC, Australia

9. Australian Cancer Research Foundation Translational Laboratory , Royal Melbourne Hospital , Melbourne, VIC, Australia

Chimeric antigen receptor (CAR) T cell therapy (CTT) is a ground-breaking, T cell-based treatment for haematological cancers. It is well known that advanced age undermines T cell activation and function, which could compromise CAR T cell generation and function, but age has not been extensively examined as an independent variable for CTT. Our study therefore aims to dissect the impact of age *vs* malignancy on CAR T cell generation and test whether age-related markers can predict CAR T cell product characteristics. To examine this, PBMCs from younger (20-30 years old (yo)) and older (60+ yo) healthy donors, and multiple myeloma (MM) patients (60+ yo) were used to generate CAR T cells, with the evaluation of yield, phenotype, and function. Matching PBMCs were also assessed for age-related markers relating to T cell differentiation, senescence, exhaustion and metabolic dysfunction. Correlative analyses were performed with markers to predict specific CAR T cell product features. Both older and MM donors had more differentiated T cells, and both yielded fewer but more differentiated, more cytotoxic CAR T cells expressing more IFNg, CD107a and granzyme B than young donors. Older and MM donors did not differ in expression of

differentiation or senescence markers or in PD-1 expression, but LAG3 expression and metabolic deficits were specifically increased in MM donors. Markers of T cell differentiation were the most predictive for CAR T cell yield, phenotype and cytotoxicity, although a marker of senescence (CD57) was most predictive for cytokine production. Our findings underscore that patient age is a major variable that shapes CAR T cell yield, phenotype and cytotoxicity, and we hypothesise that it is likely to impact CTT efficacy. Age-related markers, particularly markers of T cell differentiation, are predictive of CAR T cell generation outcomes and could support the design of age-optimised CTT treatment plans.

9

Biomarkers of flare and drug-free remission in rheumatoid arthritis

Amy Anderson[1]

1. Newcastle University, Newcastle Upon Tyne, TYNE AND WEAR, United Kingdom

Our knowledge of immune-mediated inflammatory disease (IMID) aetiology and pathogenesis has improved greatly over recent years, however, very little is known of the factors that trigger disease relapses (flares), converting diseases from inactive to active states. The underpinning mechanism(s) of flare have been difficult to study because they occur unpredictably. Within both the BioRRA and BIO-FLARE studies, we have focussed on rheumatoid arthritis (RA) to establish a highly relevant human model that generates a 'synchronised' population of RA patients in clinical remission, approximately 50% of whom relapse within 6 months following treatment cessation, the other 50% maintaining drug-free remission. Using this experimental medicine approach we have conducted an in-depth analysis of circulating immune cell subsets and their mediators, as well as detailed investigation of the joint synovium, to dissect the molecular and cellular factors driving disease flare. Through this work we have identified key pathways and cellular mediators associated with RA disease flare, as well as those involved in drug-free remission. Our ultimate goal is to develop biomarkers that will underpin relapse prevention by appropriately targeted therapeutics, and targeted treatment withdrawal in patients most likely to maintain a drug-free remission. The knowledge generated in these studies may also provide clues to the transition from pre-RA to RA at disease onset, as well as provide potentially relevant understanding of other relapsing and remitting IMIDs, such as inflammatory bowel disease.

10

Prolonging remission in autoimmune disease with antigen-specific immunotherapy

Ranjeny Thomas[1]

1. University of Queensland, Woolloongabba, QLD, Australia

Abstract not available

11

Title coming soon

Kenneth Smith[1]

1. Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

Abstract not available

12

The JAK inhibitor baricitinib preserves beta-cell function in patients with new-onset type 1 diabetes

Helen Thomas[1]

1. St Vincent's Institute, Fitzroy, VIC, Australia

Abstract not available

13

Monomeric IL-38 is a checkpoint inhibitor of the pattern recognition receptor-interferon axis

Ina Rudloff[2, 1], Steven X Cho[2, 1], Sarah A Jones[3], Jan Schroeder[4], Naiara Bediaga[5], Joshua Ooi[3], Matt Johansen[6], Philip Hansbro[6], Jose Polo[7, 5], James Whisstock[7], Eric Morand[3], Michelle Tate[1], Andrew Ellisdon[7], Marcel F Nold[1, 8], Claudia A Nold-Petry[1, 8]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Department of Pediatrics, Monash University, Melbourne, VIC, Australia

3. Centre for Inflammatory Diseases, Monash University, Melbourne , Australia

4. Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University , Melbourne, VIC, Australia

5. Adelaide Centre for Epigenetics, School of Biomedicine, University of Adelaide, Adelaide, SA, Australia

6. Centre for Inflammation, Centenary Institute and University of Technology Sydney, Centenary Institute and University of Technology Sydney, Sydney, NSW, Australia

7. Department of Physiology, Biomedicine Discovery Institute, Monash University, Clayton , VIC, Australia

8. Department of Paediatrics, Monash University, Melbourne, VIC, Australia

Type I and type III interferons (IFNs) play a key role in host defense on the one hand, but on the other hand they can contribute to the pathogenesis of auto-immune and auto-inflammatory diseases. For example, type I and III IFNs are major drivers of interferonopathies, a group of debilitating diseases predominantly affecting young women and causing substantial long-term, multi-organ morbidity and high mortality. Examples include systemic lupus erythematosus (SLE), dermatomyositis, systemic sclerosis and Sjoegren's syndrome. Although type I and III IFNs can be induced in multiple ways, activation by pattern recognition receptors (PRRs) is the central pathogenetic pathway in interferonopathies. Therefore, targeted therapies to regulate this PRR-IFN axis are highly sought-after.

Here, we identify interleukin (IL)-38, an IL-1 family member, as the first known endogenous inhibitor of this PRR-IFN axis. In pre-clinical models of SLE such as deficiency of the kinase Lyn, knockout of IL-38 augmented IFN-driven inflammation, including up to 8-fold increases in the IFN-signature genes *Irf7*, *Rsad2* and *Oas3* and in the SLE-related pro-inflammatory cytokines IL-6, IL-23, CCL2 and IFNβ itself. IL-38-deficiency also aggravated SLE-like disease activity, including kidney damage. Accordingly, injection of recombinant IL-38 ameliorated inflammation induced by imiquimod, an agonist of the SLE-relevant PRR Toll-like receptor (TLR)7, including reductions in IFNα, IFNβ, IFNγ, IFNλ and IL-6 by up to 98%.

To decipher signaling mechanisms, we performed experiments in multiple cell lines deficient in pathway intermediates of the PRR-IFN axis. Furthermore, we found that IL-38 can form a domain-swapped homodimer – a property that is novel in the IL-1 cytokine family and rare in cytokine biology overall. Such dimerization attenuated IL-38 activity, while a naturally occurring single nucleotide polymorphism prevented dimerization, thereby increasing IL-38 bioactivity.

In summary, our study identifies monomeric IL-38 as a promising therapeutic with a novel and differentiated mechanism-of-action, brightening the outlook for millions of patients worldwide suffering from diseases with pathological IFN involvement such as SLE and other interferonopathies.

14

Rational design of E-CAR with self-condensation property

Chengi Xu[1]

1. Shanghai Institute of Biochemistry and Cell Biology,Center for Excellence in Molecular Cell Science Chinese, Academy of Sciences, SHANGHAI, China

Abstract not available

15

Developing novel CART cell immunotherapies for brain cancer

Misty Jenkins[1]

1. Walter and Eliza Hall Institute, Parkville, VICTORIA, Australia

Abstract not available

16

Enhancing Chimeric antigen receptor T cells for solid cancers

Rachel Xu[1, 2], Evan Pappas[1, 2], Joseph A Trapani[1, 2], Phillip K Darcy[1, 2], Michael H Kershaw[1, 2], Michael Dickinson[1, 2], Clare Y Slaney[1, 2]

1. Cancer Immunology Program, Peter MacCallum Cancer Centre, East Melbourne, Vic, Australia

2. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, 3000

Chimeric antigen receptor (CAR) T cell therapy has revolutionised the treatment paradigm of haematological cancers but remains largely ineffective in solid cancers. Understanding the difference in anti-cancer responses in blood cancers and solid cancers will contribute to the development of a valid CAR T cell therapy in solid cancers. In this study, we generated new CARs having the same backbone structure and directly compared these CAR T cell efficacies in blood and solid cancers.

The CD19-CAR and Her2-CAR T cells generated from the same donors demonstrated very similar profiles in their CD8: CD4 ratio, CAR transduction rate, T cell memory phenotype, exhaustion markers and the speed of expansion *in vitro*. Both CAR T cells are highly effective in killing their target cancer cells and secrete IFN-g *in vitro*. Consistent with other preclinical and clinical reports, in NSG mice, while CD19-CAR T cells were highly effective in suppressing $CD19^+$ NALM6 acute lymphoblastic leukemia (ALL) progression, Her2-CAR T cells failed to inhibit the growth of MDA-MB-468-$Her2^+$ breast cancers.

To dissect the tumour- and CAR-related factors in the *in vivo* models, we enforced MDA-MB-468-$Her2^+$ breast cancer cells to express CD19 and the $CD19^+$ NALM6 leukemia cells to express Her2. Our data showed that CD19-CAR T cells were efficient in suppressing all the $CD19^+$ cancers *in vivo* including both the blood and solid cancers, while Her2-CAR T cells failed to suppress all the $Her2^+$ cancers. Together, our study demonstrated that antigen selection is critical for CAR T cell treatment. Our current study focuses on developing novel strategies to force tumour cells to express CD19 *in vivo* and developing new CARs and reagents to redirect CD19-CAR T cells to kill $CD19^-$ cancers.

17

CRISPR engineering of armored CAR T cells enables tumor-restricted payload delivery with enhanced safety and efficacy

Paul Beavis[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

The efficacy of chimeric antigen receptor (CAR) T cell therapy in solid tumors is limited by immunosuppression and antigen heterogeneity. To overcome these barriers, “armored” CAR T cells, which secrete proinflammatory cytokines, have been developed. However, their clinical application has been limited due to toxicities related to peripheral expression of the armoring transgene. Here, we developed a novel CRISPR knock-in strategy that leverages the regulatory mechanisms of endogenous genes to drive transgene expression in a tumor-localized manner. By screening endogenous genes with tumor-restricted expression, the *NR4A2* and *RGS16* promoters were identified to support the delivery of cytokines such as IL-12 and IL-2 directly to the tumor site, leading to enhanced anti-tumor efficacy and long-term survival of mice in both syngeneic and xenogeneic models. This was concomitant with improved CAR T cell polyfunctionality, activation of endogenous anti-tumor immunity, a favorable safety profile, and was applicable using CAR T cells from patients.

18

Engineering a TGF-β switch receptor enhances CAR-T cell fitness and anti-tumor efficacy in immunosuppressive models

Vicky Qin[1, 2], **Niko Thio**[1], **Xiao Jing Ong**[1, 2], **Arthur Xuan Wang**[1, 2], **Paul Neeson**[1, 2], **Criselle DSouza**[1, 2], **Joe Zhu**[1, 2]

1. Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

2. Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria, Australia

Introduction

Chimeric antigen receptor T (CAR-T) cells have limited efficacy in advanced solid cancers. A critical hurdle to success is the immunosuppressive tumor microenvironment (TME) wherein CAR-T cell function is profoundly inhibited by transforming growth factor-beta (TGF-β). Current strategies to address this issue include systemic blockade of TGF-β signaling but toxic side effects can arise from imbalanced T-cell homeostasis.

Method

To address these challenges, we designed a novel chimeric switch receptor comprising a TGF-β-binding domain and a T-cell co-stimulatory domain to initiate CAR-T-cell activation upon TGF-β binding. The conformation of the switch receptor was evaluated by Förster resonance energy transfer (FRET) assays, and signal transduction assessed by RNAseq and mass spectrometry. *In vitro* chronic stimulation assay, mitochondria stress test, chromium release assay, AlphaLISA cytokine assay, and CTV proliferation assays were used to characterize the response of switch CAR-T cells. Their *in vivo* efficacy was assessed in TGF-β^{high} prostate and breast cancer models. Further characterization of switch CAR-T cells in the peripheral blood, spleen and tumor was performed in the MDA-MB468 orthotopic breast cancer model.

Result

Using FRET assays, we found only the switch receptor with a short intracellular sequence augmented ligand-dependent homodimers without disturbing the endogenous TGF-β-receptor complex. A switch receptor incorporating a 4-1BB signaling domain showed significantly enhanced cytotoxicity and TNF-α secretion in the presence of TGF-β. Switch CAR-T cells also demonstrated higher proliferative capacity in short-term and under chronic antigen stimulation with increased mitochondrial biogenesis in response to TGF-β. This superior function was induced by enhanced oxidative metabolism and MAPK signaling. Both switch and conventional CAR-T cells had equivalent levels of SMAD2-phosphorylation in response to TGF-β, indicating that switch CAR-T cells retained endogenous TGF-β signaling. Next, tumor-bearing mice treated with switch CAR-T cells showed significantly better tumor control, this was associated with decreased TGF-β and increased IFN-γ levels within the tumor. In a TGF-β^{high} breast cancer xenograft model, tumor-infiltrating switch CAR-T cells showed increased activation (CD69 and CD137 levels) and lower expression of immune inhibitor receptors. Importantly, switch CAR-T cells showed no evidence of activation at site outside the tumor (peripheral blood and spleen), indicating their potential as a safe therapeutic.

Conclusion

In conclusion, the novel switch receptor activated CAR-T cells in response to TGF-β, leading to improved CAR-T-cell fitness in a tumor-specific manner. Switch CAR-T cells also preserved endogenous TGF-β signaling and balanced T-cell homeostasis, indicating they will be safe to use in the clinic.

19

1 target, 3 innovative immunisation strategies: The case of RSV

Lorena Preciado[1]

1. Sanofi, Sydney, NSW, Australia

Abstract not available

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Self-amplifying mRNA vaccines: Introducing a second-generation approach

Katherine Young[1]

1. CSL Seqirus, Parkville, VIC, Australia

Abstract not available

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Beyond COVID-19: Future potential for disease prevention and therapeutics with mRNA

Claire Borg[1]

1. Moderna, Melbourne, VIC, Australia

Abstract not available

22

Vaccine Technologies: Where are we now and what is the future?

James Baber[1]

1. Pfizer, Sydney, NSW, Australia

Innovative approaches are needed to develop and optimize vaccines against diseases which continue to cause significant morbidity and mortality such as Respiratory Syncytial Virus (RSV), Influenza, COVID-19, Pneumococcal disease and Group B Streptococcus. Pfizer is addressing these medical needs by applying the right technology to the right pathogen. Vaccine development efforts are focused on three different technology platforms; protein subunit, mRNA and polysaccharide conjugate technology. RSVpreF vaccine is an example of how advances in protein subunit technology have facilitated development of an efficacious vaccine for both maternal and older adult indications after over fifty years of failed attempts. Future efforts aim to expand the potential of the mRNA platform in respiratory combination vaccines, and to enhance established conjugate technology to develop next generation pneumococcal vaccines.

23

Host-directed Therapies for Respiratory Infections

Amit Singhal[1]

*1. A*STAR Infectious Diseases Labs, Singapore*

Respiratory infectious diseases such as tuberculosis and COVID-19 are the leading cause of morbidity and mortality worldwide. It is known that immunopathological changes among the patients are complex and may associate with disease severity. We performed genome-wide epigenomic approaches, Histone acetylation (HWAS) and DNA methylation (WGBS), to investigate the heterogeneous epigenetic networks in longitudinal blood samples from TB and COVID-19 patients. In this talk I will discuss the data from these investigations which has resulted in the identification of novel immune factors of host defense, those can be targeted to design host-directed therapies.

24

The race between Human Antibody Responses and Coronavirus

Qi Zhang[1]

1. Tsinghua University, Beijing, BEIJING, China

Abstract not available

25

The development of an effective vaccine to protect against female genital tract chlamydial infections

Logan Trim[1], **Yanli Li**[2], **Taylor Poston**[2], **Jenna Girardi**[2], **Nilu Goonetilleke**[2], **Alison Carey**[1], **Jonathan Harris**[1], **Toni Darville**[2], **Kenneth Beagley**[1]

1. Queensland University of Technology, Brisbane, QLD, Australia

2. University of North Carolina, Chapel Hill, United States of America

Chlamydia trachomatis (CT) is the most common sexually transmitted bacterial infection worldwide and is responsible for a wide range of reproductive morbidities. Vaccines are the most promising prospect for reducing the incidence of infection and pathology. However, 70 years of CT vaccine research has yielded only one Phase 1 clinical trial. Here, we utilised a human-informed data driven vaccine design pipeline to develop an effective chlamydial vaccine. The primary correlate of protection against CT are T helper cells secreting interferon-γ. We identified novel immunoprevalent CT T cell antigens by screening immune cells from CT seropositive women for the secretion of interferon-γ by ELISPOT after antigen stimulation. Human-informed immunoprevalent antigens for further vaccine development were refined by screening *ex vivo* responses of *Chlamydia muridarum* (Cmu) infected mice which identified CPAF as the immunodominant antigen. In mice, CPAF-specific $CD4^+$ T cells are recruited to the female genital tract (FGT) during Cmu infection and secrete interferon-γ. Intranasal immunization with recombinant CPAF combined with select adjuvants elicited robust CPAF-specific Th1 responses. Upon challenge, immunized mice had significantly lower infectious burden and earlier clearance of infection compared to mock immunized controls. To our knowledge, this is the greatest reduction of infectious burden conferred by a recombinant subunit vaccine in this model. In summary, we have identified chlamydial antigens that are immunogenic in women which are also protective when used in a subunit vaccine in the mouse model of *Chlamydia* infection. Designing a vaccine informed by human immune responses offers the best chance for success in future clinical trials.

26

Novel non-invasive vaccine delivery technology for the creation of safe and reliable mucosal immunity.

C. Glenn Begley[1], **Nicolas H Voelcker**[2], **Sean M Langelier**[3], **Mark Unger**[1, 2]

1. muPharma, Malvern, VIC, Australia

2. Drug delivery, Disposition and Dynamics Department, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia

3. Melbourne Centre for Nanofabrication, Monash University, Clayton, VIC, Australia

Vaccines injected intra-muscularly (IM) or subcutaneously generate systemic immunity but do not create the strong mucosal immunity desirable for protection against pathogens that enter the body via mucosal surfaces (such as influenza, RSV and SARS-Cov-2).

Challenging pathogens at their portal of entry will likely increase the efficacy of existing vaccines, reduce disease transmission, and may protect against future pathogen variants.

Intra-nasal vaccinations have typically failed to generate strong and reliable mucosal or systemic responses.

The phase 1 clinical study of AstraZeneca's COVID-19 vaccine demonstrated it was successful when administered IM but failed when administered intranasally (1,2). University of Oxford's Prof Alexander Douglas led the team and, in describing the results, said "We urgently need more research to develop vaccines which can block transmission of respiratory pandemic viruses using delivery routes which are safe and practical at large scale" (2).

muPharma has created a non-invasive, ultrasound mediated, hand-held, vaccine delivery device for application to the lip mucosa which in three independent studies generated mucosal and systemic immunity.

A Peter Doherty Institute for Infection and Immunity study, using a Live Attenuated Influenza Virus vaccine delivered to the lip mucosa by the muPharma device in a mouse model of influenza, demonstrated mucosal and systemic cellular immunity against an influenza virus. Importantly, protective levels of mucosal cellular immunity was generated in the upper respiratory tract. Another study performed at the Australian National University demonstrated in a live mouse model generation of mucosal cellular immunity in the gut (in addition to systemic cellular immunity) using a HIV vector virus vaccine delivered to the lip mucosa by the muPharma device. Duke University has demonstrated in live mice models that muPharma device delivery of a vaccine to the lip mucosa elicited levels of IgG and IgA in the vagina and levels of IgG in the spleen far in excess of that created by the intranasal and sublingual route.

muPharma's delivery system will be discussed, and its safety and pre-clinical results presented.

muPharma's delivery method addresses Prof Douglas's challenge and raises the possibility that this approach could have clinical utility.

(1) Madhavan M et al. Tolerability and immunogenicity of an intranasally-administered adenovirus-vectored COVID-19 vaccine: an open label partially randomised ascending dose phase 1 study. *eBioMedicine* 85, 104298 (2022).
(2) Carvalho T. Intranasal COVID-19 vaccine fails to induce mucosal immunity. *Nature Medicine* **28**, 2439-2440 (2022)

27

Vaccines tailored for inducing liver-resident memory T cells

Ian Hermans[1]

1. Malaghan Institute of Medical Research, Wellington, New Zealand
Abstract not available

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Vaccine antigens for disease X and monitoring antigenic change efficiently

Sebastian Maurer-Stroh[1, 2, 3]

*1. A*STAR Bioinformatics Institute, Singapore*
2. DBS and YLLSOM, National University of Singapore, Singapore
3. GISAID Data Science Centre, Singapore
This talk will start discussing a typical computational workflow to identify suitable vaccine antigens for a disease X. We also propose a new data integration approach for monitoring influenza antigenic change efficiently. To achieve this, we stratify influenza surveillance sequences into closely-connected transmission clusters via genetic distance and analyze clusters for enrichment of epidemiological and geo-temporal data accessible through an interactive webtool for near-real-time surveillance/analysis also by non-computer-experts and without exposing potentially sensitive clinical data. FluCluster-AI enables real-time detection of emerging variants both in local and global context through connection with the GISAID platform and provides a privacy-preserving option to find correlations of any user-provided meta data labels with variants and specific mutations. We provide examples for automated analysis of HI titre data to pinpoint mutations driving influenza antigenic drift.

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Mechanistic biomarkers of checkpoint inhibitor therapy efficacy and toxicity in cancer

Vivek Naranbhai[1]

1. Monash University, Melbourne, VIC
Checkpoint inhibition to treat cancer is now widespread across cancer types and stages, but response rates remain suboptimal, and immune related adverse events are a major challenge. I will try to provide a sythensis of current biomarkers of cancer immunotherapy efficacy and toxicity from a clinician and a mechanistic scientists' perspective and highlight some vignette's of our contributions viz. HLA-A*03 as a poor predictor of response (Naranbhai et al, Lancet Oncology 2022), IL-7 genetic variants as predictors of IRAEs (Groha et al., Nat medicine 2022) and expression variation in the immunoproteasome system as predictors of response (Ravi et al., Nat Genetics 2023). The focus will be on focussing efforts towards what specifically is needed, in my opinion, to propel the field forward in validating and leveraging these and other biomarkers to improve the utility of checkpoint inhibitors for patients.

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50 years of EPI – what next?

Meru Sheel[1]

1. University of Sydney, Camperdown, NSW, Australia
This talk will present data on achievements of immunisation programs globally and present some of the key needs and emerging challenges for vaccine programs in the Asia-Pacific region.

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Pre-clinical assessment of mRNA vaccine-induced immunity to Group A Streptococcus

Ismail Sebina[1]

1. The University of Queensland, Woolloongabba, QLD, Australia
Abstract not available

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T cell vaccines against Zika virus

Branka Grubor-Bauk[1], Zelalem A Mekonnen[1], Makutiro G Masavuli[1], Arthur Eng Lip Yeow[1], Wilfred A.A Saron[2], Dawn Whelan[1], Ryan Santos[1], Andreas Suhrbier[3], Ashley L St. John[2], Eeric J Gowans[1]

1. Basil Hetzel Institute for Translational Health Research & University of Adelaide, Woodville, SA, Australia

2. Program in emerging Infectious Diseases, Duke-National University of Singapore, Singapore

3. Inflammation Biology, QIMR Berghofer, Brisbane, Queensland, Au

Development of ZIKV vaccines has focused on the induction of neutralising antibodies, despite concerns of antibody-dependent enhancement of flavivirus infections. This poses a serious concern and a challenge due to the high level of DENV seroprevalence in areas where ZIKV is circulating. Alternative vaccine strategies that utilise T cell immunity, should be considered and explored.

We have developed a DNA vaccine expressing secreted ZIKV NS1 protein and demonstrated that vaccine is highly immunogenic, inducing strong T cell responses and anti-NS1 antibodies, but that vaccine efficacy is T cell mediated, and dependant on efficient secretion of NS1. Here we present data that shows that NS1 DNA vaccine is immunogenic in the immunocompetent C57BL6 pregnancy model of ZIKV infection and protects fetuses from intrauterine growth restriction, microcephaly, and brain damage. We show that NS1 DNA vaccination of male IFNAR$^{-/-}$ mice protects mice against ZIKV-induced damage to the testes and prevents viral persistence in the testes. Adoptive transfer studies demonstrated that this protection is T cell mediated.

Considering that T cell- based vaccines do not provide sterilising immunity, inclusion of additional antigens in a ZIKV vaccine may provide enhanced protection and efficacy. This may be particularly important during infection in pregnancy, to reduce any possibility of vertical transmission of ZIKV from mother to fetus. Therefore, we comprehensively evaluated *in vivo* and in real time ZIKV-specific effector, early and late memory T cell responses after ZIKV infection. Complementing and supporting the published data from human studies we show that ZIKV NS3 and NS4 are the dominant antigenic targets of T cell responses post-infection. Thus, we next evaluated if the immunogenicity and efficacy of the NS1 DNA vaccine is enhanced by the inclusion of ZIKV NS4 and NS3 antigens.

ZIKV NS1 DNA vaccine has progressed to non-human primate studies in rhesus macaques with vaccine delivered intradermally using PharmaJet Tropis device. Early evaluation has shown that the vaccine is highly immunogenic and protective against ZIKV infection.

Taken together our results have important implications for the development of protective and safe T cell based ZIKV vaccines, that can abrogate the risk of antibody dependant enhancement of flavivirus disease.

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Oncofetal Ecosystem in HCC: Towards Spatial Precision Oncology

Ankur Sharma[1]

1. Harry Perkins Research Institute, Nedlands, WESTERN AUSTRALIA, Australia

Abstract not available

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Understanding immunobiology of hepatocellular carcinoma for biomarkers and therapeutic discovery

Valerie Chew[1]

1. SingHealth-DukeNUS, Singapore, SINGAPORE

Abstract not available

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Butyrophilin-dependent phosphoantigen detection by human gamma-delta T cells

Dale Godfrey[1]

1. University of Melbourne, Parkville, VIC, Australia

Abstract not available

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Mutations in intergenic region predominantly contribute to the tumor specific antigens in hypermutated colorectal cancer patients

Xuan Dong[1], Siqi Liu[1], Longqi Liu[1], Stephanie Sun[2]

1. BGI Research, BGI Shenzhen, Shenzhen, Guangdong, China

2. BGI Australia, Brisbane, QLD, Australia

The genomic and transcriptomic mutation landscapes in tumors are distinct. However, exploring tumor-specific antigens (TSAs) has been narrowly confined to exonic mutations, limiting antigen discovery, particularly in low tumor mutation burden (TMB) scenarios. Here, we unveil the broader spectrum of TSAs by employing an integrative proteogenomic strategy that synergizes whole-genome sequencing, transcriptomic analysis, and MHC class I immunoprecipitation coupled with mass spectrometry (IP-MS). Analyzing ten paired primary colorectal cancer (CRC) specimens with diverse TMBs, we discovered 171 unique TSAs with no shared epitopes across patients, revealing a dominant genomic origin (86.55%) for these antigens. Intriguingly, we discovered that a significant majority (84.79%) of TSAs emanate from non-coding regions, with intergenic mutations alone contributing to 34.50% of the TSA pool. These intergenic TSAs were notably more prevalent in hypermutated CRCs, presenting nearly 40-fold more antigens than their non-hypermutated counterparts. We further validated the immunogenicity of these novel intergenic TSAs, marking them as promising candidates for expanding the neoantigen repertoire in immunotherapy. Our findings challenge the current neoantigen discovery paradigm and propose a new, untapped reservoir of immune epitopes poised to revolutionize the selection of targets for cancer immunotherapy.

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Immunological consequences of early life malaria exposure in children

Prasanna Jagannathan[1]

1. Stanford University School of Medicine, San Francisco, CA, United States

Abstract not available

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Blood Cancers and T cell exhaustion in the bone marrow

Geoffrey Hill[1]

1. Fred Hutchinson Cancer Center, Seattle, WA, United States

Abstract not available

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Engineered antigen-specific TCR-Tregs to treat autoimmune disease

Joshua Ooi[1]

1. Monash University, Clayton, VIC, Australia

Antigen-specific Tregs are potent and specific suppressors of pathogenic autoreactivity. A/Prof. Ooi has developed a unique platform that combines the use of peptide binding assays, high-throughput single cell TCR sequencing and lentiviral Treg transduction to enable the generation of therapeutic antigen-specific TCR-Tregs. In this talk, they applied this platform to developing a treatment for SLE. In SLE, autoreactivity to the Smith (Sm) antigen leads to more severe disease manifestations including the development of lupus nephritis. Using the TCR-Treg platform, A/Prof. Ooi's team identified the precise Sm-derived autoepitope driving disease and generated Sm-specific TCR-Tregs (Sm-Tregs). They showed that these Sm-Tregs could suppress patient derived autoimmunity in in vitro assays and could treat disease in a novel humanised mouse model of lupus nephritis.

40

Interferon epsilon: a novel regulator of mucosal immunity in infectious and inflammatory diseases in cancer

Paul Hertzog[1]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

Abstract not available

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Stem-like potential of T cells in infection and cancer is regulated by ID3 and c-Kit

Daniel T Utzschneider[1], Amania Sheikh[1], Catarina Gago da Graça[1], Dane M Newman[2], Ricky W Johnstone[2], Axel Kallies[1]

1. The University of Melbourne and The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

2. Cancer Biology and Therapeutics, Peter MacCallum Cancer Centre, Melbourne

CD8+ T cell responses to chronic infection and cancer are sustained by stem-like precursors of exhausted T (Tpex) cells. It is, however, insufficiently understood how stemness and differentiation potential of these T cells are regulated. Here we demonstrate that across different types of infection the transcriptional regulator ID3 is the common factor that identifies stem-like T cells that are uniquely adapted to respond to chronic infection or cancer. Furthermore, we describe an essential developmental transition, regulated by ID3, from the most stem-like Tpex cells, characterized by expression of CD62L and the transcription factor MYB to Tpex cells *en route* to effector differentiation marked by expression of the tyrosin protein kinase c-Kit. Mechanistically, ID3 released suppression of proliferation and expression of c-Kit, which itself promoted T cell expansion and effector differentiation. Finally, we show that IL-1 family members such as IL-36b and IL-18 promoted the generation of ID3+ T cells that provided superior tumor control. Overall, we identify ID3 as a common denominator of stem-like T cells, which together with c-Kit constitutes a novel checkpoint that balances proliferation and stemness of T cells in infection and cancer.

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Novel conventional and unconventional target antigens for improved cancer vaccines

Riccardo Dolcetti[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

The clinical outcomes of immunotherapy for mismatch repair proficient (MMRp) - microsatellite stable (MSS) colorectal cancer (CRC) remains unsatisfactory. The development of more effective immunotherapies is hampered by limited knowledge of the appropriate target antigens, also considering the low burden of mutations generating neoantigens of these tumours. Alternative classes of MSS CRC antigens may provide actionable epitopes. We have characterized the immunopeptidome of 2 MMRp/MSS CRC cell lines derived from the primary tumor (SW480) and a metastatic lymph node (SW620) of same patient. Given the low mutational burden of MMRp/MSS, we focused on peptides belonging to alternative classes of HLA-A*02 epitopes, such as proteasomal cis-spliced peptides derived from tumour-associated antigens and linear epitopes derived from Human Endogenous Retroviruses (HERVs), which are frequently reactivated in CRC. Among >50,000 HLA-bound peptides identified by in depth immunopeptidomics analysis, 11 cis-spliced peptides and 5 HERV peptides were functionally validated as immunogenic. Epitope-specific T cells showed specific cytotoxic activity against SW480 and SW620 CRC cells and against unrelated HLA-A*-02$^+$ CRC cell lines. The immunogenicity of both groups of peptides was confirmed *in vivo* in humanised mice. Adoptively transferred HERV or cis-spliced epitope-specific T cells significantly inhibited the growth of SW620 cells *in vivo*. Moreover, Clec9A-targeting vaccines exploiting these epitopes showed a significant therapeutic efficacy in humanised mice bearing tumours induced by SW620 CRC cells. T cell responses specific for cis-spliced and HERV epitopes were detected in PBMCs from 3/4 MSS CRC HLA-A*02$^+$ patients but in none of 4 HLA-A*02$^-$ MSS CRC patients or 7 healthy donors (4 HLA-A*02$^+$ and 3 HLA-A*02$^-$). We have identified and validated a new class of immunogenic HLA-A*02 epitopes derived from alternative classes of MMRp/MSS CRC antigens. We also provide the proof of principle supporting their immunotherapeutic exploitation in off-the-shelf cancer vaccines to improve the management of these poorly immunogenic tumours.

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Anti-tumour immunity: a co-ordinated approach

Claudine Bonder[1], Michaelia P Cockshell[1], Michael Ortiz[1], Kay K Myo Min[1], Charlie Ffrench[1], Anahita Fouladzadeh[1], Emma J Thompson[1], My G Mahoney[2]

1. Centre for Cancer Biology, University of South Australia & SA Pathology, Adelaide, SA, Australia

2. Thomas Jefferson University, Philadelphia, PA, USA

Cancer progression is controlled, in part, by infiltrating leukocytes which can either actively kill the cancer cells or promote their survival. Our current understanding of leukocyte recruitment into tumours is largely limited to the adhesion molecules and chemokines expressed by conventional endothelial cell (EC)-lined blood vessels. However, there is increasing evidence that cancer cells themselves can form vascular structures (a process known as vasculogenic mimicry (VM)); but whether VM vessels actively participate in the recruitment of leukocytes remains largely unknown.

We have building evidence that the most aggressive and difficult to treat tumours are more likely to be VM-competent and that these cancer cells often express multiple adhesion molecules (e.g. CD44, ICAM-1 and JAMs), cytokines (e.g. IL-6, TNF) and chemokines (e.g. CXCL8, CXCL12) relevant for leukocyte recruitment. Microfluidic-based adhesion assays reveal that, similar to ECs, VM-competent cancer cells facilitate the rolling and adhesion of leukocytes, particularly monocytes. Moreover, we identify ICAM-1 to be a key participant in this process. Transwell assays further suggest that VM-competent cancer cells facilitate monocyte transmigration towards a chemotactic gradient. Immunostaining of patient tissue microarrays reveal that tumours with high VM content also contain higher numbers of leukocytes (including macrophages). We have also identified an important

transporter protein that selectively carries pro-cancerous proteins to the surface of the cell and into the secretome. Inhibiting this transporter has proven effective in combating mouse models of melanoma and pancreatic cancer.

Taken together, this body of work suggests an underappreciated role of VM vessels in solid tumours via their active participation in leukocyte recruitment and begins to identify key transporter proteins, adhesion molecules, cytokines and chemokines that facilitate this deadly process.

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Using T lymphocytes for therapy: challenges and solutions

David Gottlieb[1]

1. University of Sydney, Westmead Hospital, Wentworth, NSW, Australia

Abstract not available

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Anti-CD40 is effective in immune cold colorectal cancer

Stephen J Blake[1, 2], **Yee T Chern**[1, 2], **Susan Woods**[1, 3], **David Lynn**[1, 2]

1. SAHMRI, Adelaide, SOUTH AUSTRALIA, Australia

2. College of Medicine and Public Health, Flinders University, Bedford Park, South Australia, Australia

3. School of Medicine, Adelaide University, Adelaide, South Australia, Australia

Colorectal cancer (CRC) has a devastating toll, with more than 17,000 Australians diagnosed and >5,000 deaths annually. PD1/L1 immune checkpoint blockade (ICB) have provided hope to CRC patients, as they are able to induce tumour reduction in ~33% of patients with microsatellite instable (MSI) CRC. Unfortunately, the vast majority (~95%) of advanced CRC is microsatellite stable (MSS), which is almost completely unresponsive to anti-PD1/L1 treatment. We have developed a novel orthotopic preclinical model of MSS-CRC by introducing common CRC mutations; $KRAS_{G12D}$, AKP and P53 deletions into murine colonic organoids via CRISPR-Cas9 targeting. Following colonoscope injection into the distal colon, these tumour organoids accurately recapitulate the MSS-CRC disease seen in patients, with immune effector cells excluded from a tumour microenvironment dominated by suppressive macrophages, neutrophils, and fibroblasts. Consistent with observations in patients, our model is resistant to anti-PD1 and anti-CTLA4 treatments. Excitingly then, we have found that the immune agonist antibody anti-CD40 drives a robust infiltration of activated effector immune cells into CRC tumours, including a 20-fold increase in $CD8^+$ T-cells. Anti-CD40 monotherapy had significant anti-tumour efficacy against established CRC tumours, inducing tumour shrinkage and significantly extending survival. Efficacy of anti-CD40 was dependent on $CD8^+$ T-cells while depletion of $CD4^+$ T-cells improved anti-CD40 efficacy, likely via effects on regulatory T-cells. Spatial transcriptomic and metabolomic analysis determined that anti-CD40 induced tumour intrinsic activation of innate and adaptive immune cells, including the upregulation of multiple suppressive pathways including PDL1-PD1 and CD28-CTLA4, suggesting we may observe synergy with immune checkpoint blockade. Despite these observations, we found no increase in efficacy when combining anti-CD40 with either anti-PD1 or CTLA4 and further evaluation with other combinations is currently underway. In summary, we have developed a novel mouse model of MSS CRC that may more closely model the outcomes of immunotherapies observed in human disease than the commonly used 2D tumour models of CRC, such as CT-26 or MC38 cells. We propose this model will be useful as a “second round” preclinical model to identify the most potent, clinically applicable immunotherapy combinations to take into clinical trials.

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Leveraging DC activation to overcome tumour heterogeneity in CAR T cell therapy

Cheok Weng Chan[1, 2], **Junyun Lai**[1, 2], **Paul A Beavis**[1, 2], **Ilia Voskoboinik**[1, 2], **Phillip K Darcy**[1, 2]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

2. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia

Adoptive cell transfer (ACT) therapy using patient-derived T cells genetically engineered to express a chimeric antigen receptor (CAR) is highly effective in B cell malignancies and is now FDA-approved. However, tumour heterogeneity remains a major challenge in treating solid tumours due to the relapse of tumours negative for CAR-targeted antigen. Previously we demonstrated that CAR T cells engineered to secrete (DC) growth factor Fms-like tyrosine kinase 3 ligand (Flt3L) promote host anti-tumour immunity to effectively eradicate heterogenous tumours by expanding intratumoural conventional type 1 dendritic cells (cDC1) (Lai et al. 2020 Nat Immunol). cDC1 is well-reported to be a critical mediator in activation of anti-tumour cytotoxic responses. A key aspect of this study was that despite the increased population of intratumoral cDC1s, cDC1s required a second activatory stimulus to elicit anti-tumour immunity. Here, we explored the possibility of leveraging the CD40 axis to promote cDC1 function and synergise with Flt3L-secreting ACT therapy. Engagement of upregulated CD40 on antigen-experienced DC with its ligand CD40L, which is traditionally expressed on activated T cells, induces DC activation. The effect of activation was observed via the upregulation of co-stimulatory molecules, CD80 and CD86, indicating DC maturation and expansion of tumour-antigen specific T cells upon anti-CD40 activation of Flt3L-expanded cDC1 in vivo. To incorporate the CD40 axis activation in adoptive cell therapy, successful engineering of CAR T cells was conducted using a novel construct to induce high CD40L expression constitutively. Improved DC maturation was observed upon CD40 engagement following adoptive transfer of CD40L-expressing T cells, correlated with improved therapeutic efficacy. Based on our results of the synergistic effect between Flt3L and CD40L on cDC1, we are poised to further incorporate this with Flt3L-secreting CAR T cell to achieve cDC1 expansion and activation upon ACT.

Our study has demonstrated an enhanced efficacy of CAR T cell treatment in solid cancers by harnessing the endogenous immune responses against tumours.

1. 1. Lai, J., Mardiana, S., House, I. G., Sek, K., Henderson, M. A., Giuffrida, L., ... & Beavis, P. A. (2020). Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. Nature immunology, 21(8), 914-926.

47

CAR T cells for Cancer

Crystal Mackall[1]

1. Stanford University, Stanford, CALIFORNIA, United States

Abstract not available

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Modelling the antibody response to infectious diseases to inform vaccine development and intervention strategies.

Felicia Bongiovanni[1, 2], **Lauren Smith**[1, 2], **Eamon Conway**[1, 2], **Jodie McVernon**[3, 4], **Ivo Mueller**[1, 2]

1. Walter and Eliza Hall Institute of Medical Research, Melbourne

2. Department of Medical Biology, The University of Melbourne, Melbourne

3. Victorian Infectious Diseases Reference Laboratory Epidemiology Unit, The Peter Doherty Institute for Infection and Immunity, Melbourne

4. Department of Infectious Diseases, The Peter Doherty Institute for Infection and Immunity, Melbourne

Humoral immunity to infectious diseases is important for protection against foreign attacks and clinical disease. Quantification of the antibody response is often measured through serological assays, and from this, seroprotection and seroconversion are determined by examining the changes in antibody titres in individuals over time. However, this approach lacks insight into the mechanisms underlying antibody kinetics and does not have the predictive power to infer how different conditions can alter antibody titres, leaving a gap in our understanding of factors that can influence antibody responses.

Mathematical models provide a powerful tool to simulate and predict antibody kinetics to better understand the dynamics of antibody production, decay, and longevity of the response. In this talk, I present the use of an established antibody kinetics model (White et al., 2014) to assess the longevity of the antibody response and serological exposure markers of protection against SARS-CoV-2 and *Plasmodium vivax* malaria. Using comprehensive clinical data, I have estimated the half-life of the IgG response to key COVID antigens by accounting for the biphasic decay of both antibodies and plasma cells and used it to infer time of infection or vaccination. I have also applied the model to data collected from *P. vivax* infected individuals in Thailand to determine optimal antigens for a new screening approach (Longley et al., 2020). Additionally, I have inferred the unknown time of primary infection for individuals in the Thai data to determine whether they were likely to be carrying dormant parasites in their liver at the time of the study, which cannot currently be clinically diagnosed.

Overall, this work will emphasise how critical mathematical modelling is in understanding the duration of protection given by the antibody response and the mechanisms behind antibody production and decay. This is important for vaccine development in helping inform the selection of antigens and the optimal timing to give booster doses, and treatment strategies from estimating the time of infection of an individual.

1. Longley, R. J., White, M. T., Takashima, E., Brewster, J., Morita, M., Harbers, M., Obadia, T., Robinson, L. J., Matsuura, F., Liu, Z. S. J., Li-Wai-Suen, C. S. N., Tham, W.-H., Healer, J., Huon, C., Chitnis, C. E., Nguitragool, W., Monteiro, W., Proietti, C., Doolan, D. L., … Mueller, I. (2020). Development and validation of serological markers for detecting recent Plasmodium vivax infection. Nature Medicine, 26(5), Article 5. https://doi.org/10.1038/s41591-020-0841-4
2. White, M. T., Griffin, J. T., Akpogheneta, O., Conway, D. J., Koram, K. A., Riley, E. M., & Ghani, A. C. (2014). Dynamics of the Antibody Response to Plasmodium falciparum Infection in African Children. The Journal of Infectious Diseases, 210(7), 1115–1122. https://doi.org/10.1093/infdis/jiu219

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Allogeneic T-cell immunotherapy for the treatment of Progressive Multifocal Leukoencephalopathy (PML) using a HLA-defined peptide platform

George Robin Ambalathingal Thomas[1], **Leone Beagley**[1], **Shannon Best**[1], **Archana Panikkar**[1], **Rajiv Khanna**[1]

1. Tumor Immunology, QIMR Berghofer medical research Institute, Brisbane, QLD, Australia

BACKGROUND:

PML is a demyelinating disease of the central nervous system caused by JC polyomavirus (JCPyV) occurring in immunocompromised patients that is usually progressive and often fatal. There are no currently available treatment options and high unmet medical need. Previous data has indicated that JCPyV- or BK polyomavirus-specific T cell products could be a promising treatment strategy for PML. We aimed to optimize and develop a novel allogeneic T cell-based immunotherapy, CE-VST01-JC ,which consists of JCPyV-specific T cells expanded using a targeted, highly curated mixture of JCPyV-specific platform derived from LT, ST, VP1, VP2 and VP3 antigens of JCPyV covering 35 class I and class II HLA alleles.

DESIGN/METHODS:

CE-VST01-JC drug product was manufactured by expanding JCPyV-specific T cells from healthy donors using the HLA-defined peptide mix for 17 days in culture media supplemented with IL-2 in GRex bioreactors. CS-VST01-JC T cell product was further extensively assessed for JCPyV-specificity, allogenicity, functional and phenotypic characterization using flow cytometry.

RESULTS:

CE-VST01-JC immunotherapy was manufactured by stimulating antigen-specific T cells with a panel of HLA class I and class II-restricted peptide epitopes. These T cells displayed polyfunctional profile with co-expression of IFN γ, TNF and IL-2. Phenotypic characterization of these T cells also showed enrichment of stem-cell like memory T cells (CD95, CD62L and CXCR3 co-expression) along with effector memory cells. Further, allogenicity assessment against 115 HLA-alleles showed no alloreactive T cells in CE-VST01-JC immunotherapy.

CONCLUSIONS:

We have developed a robust manufacturing process for CE-VST01-JC adoptive T cell therapy which includes highly potent JCPyV-specific T cells with stem-cell like memory T cells, with no detectable off-target reactivity. Clinical safety and efficacy of CE-VST01-JC will be evaluated with a large global study, entitled: 'ASCEND-JC: A Multi-center, Randomized, Double-blind, Phase 2 Study, Evaluating JCPyV-specific T cell therapy for the Treatment of PML' (NCT#05541549) .

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mRNA-based engineering for flexible 'off-the-shelf' allogeneic T cell therapy

Shan He[1], Qi Chen[1], Joey Ming Er Lim[1], Shou Kit Hang[1], Adeline Chia[1], Anthony Tanoto Tan[1], Antonio Bertoletti[1]

1. Program in Emerging Infectious Diseases, Duke-NUS Medical Shool, Singapore

Autologous T-cell therapies against cancer face disadvantages in terms of production cost, logistics, and compromised T-cell fitness. The use of allogeneic T cells can partially bypass these limitations but it brings new challenges involving host-versus-graft (HvG) and graft-versus-host (GvH) reactions.

Our aim is to develop a strategy to bypass these two obstacles by leveraging on the substantial flexibility of mRNA electroporation technology in engineering allogeneic mRNA TCR-T cells for multiple infusions into patients.

Instead of reducing allogeneic T cell immunogenicity through irreversible genetic approaches, we propose to prevent HvG reactions by transiently suppressing the host's immune system with a finite treatment of immunosuppression (Tacrolimus). At the same time, the functionality of these allogeneic T cells will be preserved through the conferment of transient Tacrolimus resistance by introducing a modified version of calcineurin B into T cells through mRNA electroporation. Our *in vitro* data show that Tacrolimus can effectively suppress the HvG reactions with minimal impact on the functionality of the Tacrolimus-resistant engineered allogeneic T cells.

To minimize the risk of GvH disease, we utilized different cytokine cocktails to expand our T cells into populations with reduced GvH reactions. We showed that expanding T cells with the addition of IL-4 and IL-7 resulted in allogeneic T cells with significantly lower GvH disease potential than conventional IL-2 expanded T cells, while maintaining both tumoricidal efficacy and electroporation efficiency.

Taken together, our preliminary results showed that mRNA electroporation and T cell expansion procedures can be utilized in combination to develop a safe and effective 'off-the-shelf' allogeneic TCR-T cell therapy product.

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A streamlined end-to-end manufacturing pipeline for mRNA vaccines and therapeutics

Hannah Tompkins[1], Abhishek Kulkarni[1], Helen Gunter[1], Natasha Chaudhary[1], Sky Seth[1], Seth Cheetham[1], Tim Mercer[1]

1. BASE facility, St Lucia, QUEENSLAND, Australia

The trailblazing success of the COVID-19 vaccine has accelerated the development of mRNA vaccines and therapies. Due to the wide applicability of this technology, there is now demand for rapid, high quality mRNA products within the scientific community. However, current approaches to manufacturing mRNA vaccines from plasmid DNA templates are laborious, error prone and can result in bacterial contaminants. We have developed a streamlined and reliable end-to-end method of manufacturing mRNA and LNP products.

Instead of using plasmid DNA as the initial raw material for mRNA production, our process employs a synthetic "gBlock" DNA template. The template is amplified using PCR instead of bacterial fermentation, significantly decreasing template production time, achieving purified product within a day rather than a week. Our synthetic DNA approach also effectively eliminates endotoxin contamination and avoids truncation of the polyA tail, a common error that arises in plasmid amplification. Furthermore, mRNA

synthesised from synthetic templates has consistently higher cellular expression than plasmid-derived mRNA, likely due to the increased integrity and purity.

This mRNA production is easily scalable from 100ug for small *in vitro* studies to 10mg for preclinical studies. We have optimised encapsulation of mRNA in several different LNP formulations for delivery to *in vivo* systems. mRNA products undergo a series of analyses including automated gel electrophoresis, high performance liquid chromatography (HPLC), and endotoxin analysis to ensure specificity and purity of the final product. To improve the resolution and sensitivity of mRNA vaccine quality control, we developed a long-read (Oxford Nanopore Technologies (ONT)) sequencing test (VAX-seq) to measure key quality features of our DNA templates Our sequencing workflow allows complete coverage of the length of the mRNA sequence, including the polyA tail, allowing for a quantitative and sensitive measure of mRNA identity and integrity.

The stability and efficacy of our mRNA and LNP products have been established through extensive transfection and expression analysis in various mammalian cell lines. We have shown that our mRNA retains its high integrity following repeated freeze thaw cycling and long-term storage for up to a year.

Overall, our streamlined end-to-end manufacturing pipeline and state-of-the-art analytics allows us to provide high quality mRNA and LNP products quickly and reliably for preclinical evaluation. Through manufacturing hundreds of diverse mRNA vaccines and therapies we developed innovative manufacturing solutions, while enabling the unprecedented growth of the Australian mRNA sector.

1. Gunter, H.M., Idrisoglu, S., Singh, S. et al. mRNA vaccine quality analysis using RNA sequencing. Nat Commun 14, 5663 (2023). https://doi.org/10.1038/s41467-023-41354-y

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Structural Characterisation and Functional Mapping of Immunogenic Domains of Human Cytomegalovirus Glycoprotein B

Shane Horsefield[1], Vijayendra Dasari[1], Lou Brillault[2, 3], Thomas Ve[4], Bostjan Kobe[2, 5, 6], Rajiv Khanna[1]

1. QIMR Berghofer, Herston, QLD, Australia

2. Australian Infectious Diseases Research Center, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

3. Centre for Microscopy and Microanalysis, The University of Queensland, Brisbane, Queensland, Australia

4. Institute for Glycomics, Griffith University, Southport, Queensland, Australia

5. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Aus

6. Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia

Human cytomegalovirus (CMV) is associated with significant disease burden, particularly in congenital infections, the immunocompromised and transplant recipients. Currently there is no licenced vaccine for human CMV and the development of an effective vaccine has been recognised as highest priority by the US National Institute of Medicine. Previous studies with a subunit vaccine based CMV-encoded glycoprotein B (gB) in combination with MF59 adjuvant showed up to 50% efficacy in preventing acquisition of the viral infection. To further enhance potential efficacy of this vaccine, we have developed a novel bivalent vaccine formulation that includes an engineered recombinant gB protein, polyepitope protein and TLR9 agonist (CpG1018). Our preclinical mouse models demonstrate this formulation generates robust CMV-specific neutralising antibodies and $CD4^+$ and $CD8^+$ T cell responses.

Utilising cryogenic electron microscopy (CryoEM), in combination with cutting-edge AI algorithms and functional immune analysis, we have resolved the structure of engineered recombinant gB protein to a resolution of 3.9 Å. CryoEM structure of this protein revealed a trimeric molecule resembling a post-fusion conformation, with exposed surface in domain I (DI), which includes fusion loops, critical for viral entry. These fusion loops are concealed by the membrane proximal region (MPR) in previously published structures of pre-fusion and post-fusion forms of gB. As our engineered gB protein lacks MPR and transmembrane domain, these fusion loops are no longer concealed and allows more efficient immune recognition. Indeed, a fine mapping of gB-specific immune response showed broad recognition of immunogenic epitopes recognized by B cells, $CD4^+$ and $CD8^+$ T cells from HLA transgenic mice immunised CMV vaccine and healthy seropositive individuals.

Together these observations provides insight into the molecular mechanisms underpinning the protective responses generated by bivalent CMV vaccine formulation based on a trimeric post-fusion gB protein.

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A Dendritic Cell-targeting Approach to Deliver a Universal Influenza Vaccine Candidate to the Respiratory Mucosa

Daryl Lee[1], Nicholas Cheang[1], Kirsteen Tullett[2], Peck Tan[2], Mireille Lahoud[2], Sylvie Alonso[1]

1. National University of Singapore, Singapore, SINGAPORE

2. Biochemistry & Molecular Biology, Monash University, Melbourne, Victoria, Australia

Influenza (flu) is a highly infectious acute respiratory disease that poses a huge economic burden to all societies across the world and is responsible for hundreds of thousand cases of severe illness and deaths every year. Current seasonal vaccines comprise

of trivalent or quadrivalent formulations that are based on prediction of circulating influenza A and B strains in the Northern and Southern hemispheres. This vaccination strategy has shown limited effectiveness especially when there is a mismatch between predicted and circulating strains. There is hence an urgent need to develop a universal flu vaccine that could provide basal protection to supplement seasonal flu annual vaccination, and in outbreak/pandemic situations caused by zoonotic influenza viruses.

The 23-amino acid ectodomain of the M2 viral protein (M2e) has been extensively studied as a promising “universal” flu antigen vaccine candidate owing to its highly conserved amino acid sequence across all the IAV subtypes. However, its inherent low immunogenicity has represented a major bottleneck in the development of M2e-based vaccines, with disappointing results in clinical trials. To overcome M2e’s weak immunogenicity, we have employed a powerful dendritic cell (DC) -targeting strategy to deliver M2e to a specific DC subset termed conventional Dendritic Cells 1 (cDC1), which excels in antigen uptake, processing, and cross-presentation. The vaccine construct consists of an anti-Clec9A monoclonal antibody where three copies of M2e have been genetically fused in tandem at the C-terminus of each heavy chain. Clec9A is a C-type lectin receptor that is exclusively expressed on cDC1. Here, we have explored the pulmonary route of immunization with the rationale that inducing a strong immunity at the primary site of infection (respiratory tract) is expected to confer strong protection and prevent both infection and transmission of IAV.

We show that a prime-boost regimen with 2µg only of the Clec9A-M2e construct (adjuvanted with poly I:C) induced very high systemic M2e-specific IgG titres that afforded full protection against lethal H1N1/PR8 challenge in mice, with minimal body weight loss. We also employed an antibody-dependent cell cytotoxicity (ADCC) reporter assay to demonstrate the functionality of immune sera collected up to 2 months post-booster shot. The Clec9A-M2e prime-boost immunization also generated significant M2e-specific T cell responses both systemically (spleen) and locally (lungs). Taken together, these results support that the Clec9A-targeting strategy represents a promising vaccine delivery platform able to overcome the weak immunogenicity of M2e and induce strong immune responses upon respiratory immunization.

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Adoptive TCR-T cell therapy for HPV-associated cancers

Yu-Chen Enya Chen[1], Kunal Bhatt[1], Jacqueline Burrows[1], Sweera Rehan[1], Corey Smith[1], Rajiv Khanna[1]

1. QIMR Berghofer, Herston, QLD, Australia

Human papillomaviruses (HPVs) are a family of double strand DNA viruses comprising more than 150 types. Selective infection of cutaneous or mucosal epithelia is a classic feature of HPV and their replication is closely linked to the differentiation and malignant transformation of the epithelium. HPV-16 and HPV-18 account for the majority of cancers of the cervix, anus, vagina, vulva, penis, tongue, thorax and tonsil. Adoptive immunotherapy with transgenic T cell receptor (TCR) T cells has emerged as a potential therapeutic strategy for human cancers. However, clinical translation of this therapy has achieved limited success in HPV-associated recurrent or metastatic disease while targeting HPV16-E6 or E7 antigens. Proteome-wide analysis of HPV-specific T cell responses in HPV-positive oropharyngeal cancer (OPC) patients revealed that T cell responses from these patients were not constrained to the E6 and E7 antigens; they also recognized E1, E2, E4, E5, and L1 proteins as dominant targets. These observations provided a ground-breaking insight into future development of cellular immunotherapies for HPV-associated cancers. We have now isolated multiple HLA class I and class II-restricted HPV-specific TCRs specific for E2, E5, E6 and E7 antigens and expressed multiple TCRs in Jurkat cells and human peripheral blood mononuclear cells. We have validated these TCRs using both in vitro and in vivo functional assays. Human T cells transduced with HPV-specific TCRs efficiently recognize HPV epitope sensitized target cells while the HPV-positive tumour cell recognition correlated with the expression of HPV-encoded antigens. Furthermore, adoptive transfer of HPV-specific TCR transduced T cells also blocked the outgrowth of HPV-positive tumours in NOD-Rag1^{null} IL2rg^{null} (NRG) mice. More importantly, TCR-transduced T cells were detected in peripheral blood and HPV-positive tumours in NRG mice following adoptive immunotherapy.

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Mix & Match: Impact of delayed intervals and repeated COVID-19 mRNA boosters on systemic and mucosal antibody responses.

Kevin J Selva[1], Wen Shi Lee[1], Jennifer Audsley[2], Mai C Trieu[3], Arnold Reynaldi[4], Jo Peterson[5], Helen E Kent[1], Julie Nyugen[1], Thakshila Haripriya Amarasena[1], Robyn Esterbauer[1], Ebene R Haycroft[1], Pradhipa Ramanathan[1], Miles P Davenport[4], Timothy E Schlub[6], Joseph Sasadeusz[5], Adam K Wheatley[1], Jennifer A Juno[1], Amy W Chung[1], Stephen J Kent[1, 7]

1. Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, Uni of Melbourne, Melbourne, VICTORIA, Australia

2. Department of Infectious Diseases, Peter Doherty Institute for Infection and Immunity, Uni of Melbourne and Royal Melbourne Hospital, Melbourne, VICTORIA, Australia

3. Department of Clinical Science, Influenza Centre, Uni of Bergen, Bergen, Norway

4. Kirby Institute, Uni of New South Wales, Kensington, NEW SOUTH WALES, Australia

5. Victorian Infectious Diseases Service, Royal Melbourne Hospital, Melbourne, VICTORIA, Australia

6. Sydney School of Public Health, Faculty of Medicine and Health, Uni of Sydney, Sydney, NEW SOUTH WALES, Australia

7. Melbourne Sexual Health Centre, Department of Infectious Diseases,, Alfred Hospital and Central Clinical School, Monash Uni, Melbourne, VICTORIA, Australia

Updated COVID-19 mRNA boosters have been effective at boosting humoral responses against circulating and emerging variants. However, it remains unclear if delaying dosing intervals for COVID-19 boosters could further improve antibody responses (magnitude, neutralisation), such as that previously observed with the primary COVID-19 vaccine regimes.

Furthermore, little is known about how repeated mRNA boosters would impact IgG subclass switching (from IgG1 to IgG4), particularly in individuals who have received non-mRNA primary COVID-19 vaccines (Vaxzevria; AstraZeneca).

Here, we conducted a randomized controlled trial (Nov 2022 – Aug 2023) and recruited participants to either receive the Moderna BA.1 bivalent mRNA booster upon recruitment (immediate arm) or 3 months following recruitment (delayed arm). Follow-up visits were completed for 48 participants (24 per arm), with paired saliva and plasma samples collected following each visit (Immediate: Day 0, 7, 14, 28, 84, 168; Delayed: 3 months prior, Day 0, 7, 14, 28, 84). Furthermore, 40% of the cohort had received 2 x Vaxzevria as their primary vaccine and were receiving the BA.1 bivalent booster as their second mRNA booster.

Neutralisation profiles between the immediate and delayed arms were similar in both plasma (against ancestral SARS-CoV-2; Omicron BA.1, XBB.1.5) and saliva (against ancestral). The magnitude and half-life of plasma and salivary antibody responses (total IgG and IgA) against ancestral and Omicron variants (BA.1, XBB.1.5) were also comparable across both immediate and delayed arms.

Prior to their second mRNA booster, ancestral IgG4 responses were significantly elevated (110-fold increase; $p<0.001$) in individuals with primary mRNA vaccines (2 x mRNA + 1 x mRNA booster) as compared to individuals with primary Vaxzevria vaccines (2 x Vaxzevria + 1 x mRNA booster). This difference in IgG4 responses between primary mRNA and Vaxzevria cohorts shrunk 2 weeks after the BA.1 bivalent mRNA booster (6-fold increase; $p<0.01$), as individuals with primary Vaxzevria vaccines developed strong IgG4 responses against ancestral SARS-CoV2 (38-fold increase, pre- vs post-booster; $p<0.001$) and Omicron variants (BA.1, 28-fold increase; $p<0.001$) (XBB.1.5, 5-fold increase; $p<0.001$).

Our findings suggest that while COVID-19 mRNA boosters do enhance protective antibodies, delayed boosting does not provide much benefit to antibody responses. Furthermore, we note that the second mRNA booster significantly increased IgG4 responses in individuals who received the primary Vaxzevria vaccines. Future work should be done to better understand the impact of elevated IgG4 following repeated mRNA boosters.

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Novel human EphA3-specific CAR T cells eliminate adult and paediatric high-grade gliomas.

Leesa Lertsumitkul[1, 2], Melinda Iliopoulos[1], Kathy A Watson[1], Ryan S Cross[1], Misty R Jenkins[1, 3, 2]

1. Walter and Eliza Hall Institute of Medical Research (WEHI), Parkville, VIC, Australia

2. Department of Medical Biology, The University of Melbourne, Melbourne, Victoria, Australia

3. Department of Biochemistry and Chemistry, La Trobe Institute for Molecular Science, Melbourne, VIC, Australia

High grade gliomas such as glioblastoma (GBM) and diffuse midline gliomas (DMG) are the most fatal and aggressive forms of brain cancer, and existing treatments have limited efficacy. Chimeric Antigen Receptor (CAR) T cell therapies are a promising approach to treat brain cancer due to their targeted nature and ability to cross the blood brain barrier. A major challenge for translating this therapy into the clinic is the limited number of antigens that are actively being explored. Our group have employed cell surface proteomics to map the surface proteome of adult and paediatric high grade glioma patient samples to identify novel CAR T cell targets. Ephrin tyrosine kinase A3 (EphA3) protein belongs to a family of Ephrin proteins that are highly expressed in brain cancer. EphA3 was a predominant protein identified in our primary patient datasets, further confirming existing evidence of EphA3 overexpression in GBM patient samples and cell lines. We therefore pursued EphA3 as a promising immunotherapy target.

This study aims to investigate an EphA3 CAR T cell targeted therapy for adult and paediatric high grade gliomas. A second generation EphA3-targeted CAR was designed and transduced into primary human T cells. EphA3-targeted $CD8^+$ CAR T cells were effective in killing GBM and DIPG cell lines in an antigen specific manner *in vitro.* These findings were subsequently translated into an intracranial orthotopic preclinical model of luciferase-labelled GBM and DIPG tumour bearing NSG mice. We generated human EphA3-targeted CAR T cells from three independent human donors and treated GBM and DIPG-tumour bearing mice. Mice were imaged weekly to monitor tumour burden. Excitingly, EphA3-targeted CAR T cells demonstrated potent anti-tumour efficacy, completely reducing the tumour burden within 4 weeks post treatment. We then performed rechallenge experiments in tumour-cleared mice to test for remaining functional CAR T cells. Mice exhibited full clearance of the second intracranially implanted tumours and sustained complete response up to 6 months post treatment.

Future work will incorporate this EphA3-targeted CAR T cell therapy into other brain cancer models and combination approaches for translation into promising novel immunotherapies, to help brain cancer patients and their families.

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Enhancing CAR T cells against solid tumours with novel gene-editing approaches

Thomas J Cole[1], Phillip K Darcy[1], Paul A Beavis[1], Kevin Sek[1]

1. Peter MacCallum Cancer Centre, Melbourne

CAR T cell therapy has been incredibly successful in treating haematological malignancies but has faced additional hurdles in the context of solid tumours. One such challenge is immunosuppression by secreted metabolites such as adenosine at the tumour site. We have demonstrated that CRISPR deletion of the A2A adenosine receptor could armour CAR T cells against the TME leading to greater therapeutic efficacies in solid tumours. Alternatively, engineering CAR T cells to express the adenosine A1 receptor (A1R) at the tumour site energises CAR T cells by driving enhanced cytokine production and T cell effector differentiation. Utilizing CRISPR homology-directed repair, we were able to achieve expression of the A1R in CAR T cells following antigen stimulation at the tumour site only. This has been demonstrated in multiple CAR T cell models that tumour-specific A1R expression enhances CAR T cell effector function and tumour control *in vivo*. Weighted gene co-expression network analysis (WGCNA), ATAC-seq and scRNA-seq point towards the role of several transcription factors involved downstream in A1R CAR T cell signalling. Specifically, we identified IRF8 as a key mediator of effector differentiation and cytokine production. Given that the effector phenotype of A1R CAR T cells increases cytokine production, there is scope to test this gene-editing strategy in syngeneic immunocompetent mouse models with an intact immune system. Furthermore, this approach has the potential to synergise with

checkpoint blockade as prolonged A1R signalling also drives exhaustion and upregulation of checkpoint receptors in CAR T cells. Leveraging the tumour-site specific expression of A1R, we can combine this approach with other strategies that promote memory and stem-like characteristics in CAR T cells leading to greater expansion and optimal CAR T cell differentiation upon antigen encounter.

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Tuning chimeric antigen receptor (CAR)-T cell functions by manipulating receptor structure

Ashleigh Davey[1, 2], Lincoln Smith[1], Emma Petley[1, 2], Melissa Call[1, 2], Matthew Call[1, 2]

1. Structural Biology Division, Walter and Eliza Hall Institute, Parkville

2. Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia

Chimeric antigen receptor (CAR) T cell therapy is a type of cancer immunotherapy that 'trains' a patient's own immune system to eradicate their cancer. CAR T cell therapy has attained up to 80% complete remission rates in B-cell leukemia and lymphomas, but the broader use of CAR T cell therapy as a first-line treatment remains limited by life-threatening side effects primarily caused by the excessive release of inflammatory cytokines, known as cytokine release syndrome (CRS). The ability to maximise anti-tumour efficacy whilst minimising the risk of inflammatory toxicities is therefore integral to the continued improvement of CAR T cell therapies. We aimed to investigate the currently ill-defined relationship between CAR structure and potency, with the intention of leveraging these findings to predictably control CAR activity.

In collaboration with computational modelling experts, we have developed *de novo* designed transmembrane (TM) domain sequences capable of controlling the oligomeric state of receptors. We subsequently designed CD19 targeted CARs containing these novel TM sequences, termed 'structurally programmed' CARs (proCARs), and validated their effector function in comparison to an otherwise identical CD28TM containing clinical product, axicabtagene ciloleucel (Yescarta®). Using our panel of proCARs we have demonstrated that cytokine secretion in response to target antigen is significantly reduced by all proCARs in comparison to CARs possessing a conventional CD28 TM domain, and a positive linear correlation of proCAR oligomeric state with inflammatory cytokine secretion is evident. All new CAR designs are effective in controlling B-ALL tumour growth *in* vivo similar to CD28TM CARs, with ProCAR-3 and ProCAR-4 showing significantly enhanced efficacy in comparison.

These findings present an opportunity to deliberately control the safety and potency of CAR T cell therapies by controlling for CAR oligomeric state through the TM domain. Furthermore, the absence of systematic optimisation of CAR TM sequences in the field, combined with the simple modularity of our TM designs, presents a compelling case for easy translation of our proCAR TMs into diverse clinical CAR-T cell designs.

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Crosslinking of Ly6a metabolically reprogram CD8 T cells for cancer immunotherapy

Avishy maliach[1], Yaron Carmi[2], carmit levy[1]

1. Department of Human Genetics and Biochemistry, , Tel Aviv University, Tel Aviv, Israel

2. Department of Pathology, Tel Aviv University, Tel Aviv, Israel

T-cell inhibitory mechanisms prevent autoimmune reactions. While cancer immunotherapy aims to remove these inhibitory signals, chronic UVB exposure attenuates autoimmunity through promotion of unknown immune suppression mechanisms. We here show that mice with subcutaneous melanomas were unresponsive to checkpoint blockade therapy following chronic UV irradiation. This no responsiveness was due to the suppression of skin-draining lymph node T-cells' killing ability. Using mass cytometry analysis and single cell RNA sequencing, we uncovered a skin-specific UV-induced suppression of T-cells marked by upregulation of Ly6a. Ly6a^{high} T-cells are subtype of exhausted T cells that induced by the chronic inhibitory effect of type-1 interferon following UV exposure. Out of the UV context, we found enrichment of exposed Ly6a^{high} T-cells in the tumor microenvironment and demonstrate that Ly6a crosslinking enhances T-cell anti-tumoral cytotoxic activity, change T cells proteomic profile and reprograms their mitochondrial metabolism. Remarkably, in vivo treatment with anti-Ly6a antibody significantly inhibited tumor growth in mice resistant to anti-PD1 therapy. Appling our findings in humans could lead to a new immunotherapy treatment for patients with resistance to existing treatments.

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Overcoming immune checkpoint inhibitor resistance to improve melanoma therapy

Davide Moi[1], Sheena Daignault[1], Bijun Zeng[1, 2], Rituparna Bhatt[1, 3], Robert ju[1], Gency Gunasingh[1], Liam Griffiths[1], Melina Haritopoulou-Sinanidou[1], Brian Gabrielli[3], Glen Boyle[4], Loredana Spoerri[1], Roberta Mazzieri[2], Riccardo Dolcetti[1, 3], Nikolas Haass[1]

1. University of Queensland Frazer Institute, The University of Queensland Frazer Institute, Brisbane, QLD, Australia

2. Sir Peter MacCallum Department of Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

3. Mater Research Institute, Brisbane, QLD, Australia

4. Cancer Drug Mechanisms Group, the Cancer Program at QIMR Berghofer, Brisbane, QLD, Australia

Despite the unprecedented success of immune checkpoint inhibitors (ICI) in cancer therapy, one major unresolved dilemma is treatment resistance. **Immunogenic cell death (ICD)** constitutes a prominent pathway for the activation of anti-cancer immunity,

which in turn determines the long-term success of anticancer therapies. Only a few agents can elicit *bona fide* ICD, including the **proteasome inhibitor bortezomib**, as demonstrated in malignant myeloma and mantle cell lymphoma, but not yet in melanoma. We show that bortezomib causes ICD *in vitro* through the induction of endoplasmic reticulum stress followed by autophagy, apoptosis and translocation/secretion of damage-associated molecular patterns (DAMPs). Moreover, vaccination with bortezomib-treated apoptotic melanoma cells induced tumour immunogenicity *in vivo*. ***Intralesional injection of bortezomib synergized with subsequent systemic treatment with ICI***. Re-challenge demonstrated long-term protection through bortezomib combined with ICI. Polyfunctional T cell assays revealed that intralesional bortezomib injection generates a tumour-specific T cell response and was able to control secondary untreated tumours (***abscopal effect***). Importantly, ***ICI resistance was reverted by bortezomib-induced immunogenicity***.

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Immunopeptidomics Analysis of HLA-Bound Peptides Reveals Plasticity in Cancer Antigen Presentation

Pouya Faridi[1, 2]

1. Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Medicine, School of Clinical Sciences, Monash University, Melbourne, VIC, Australia

The plasticity of cancer antigen presentation by human leukocyte antigen (HLA) molecules is a crucial aspect of tumor immunology with profound implications for cancer immunotherapy. Leveraging immunopeptidomics, we conducted a comprehensive analysis to elucidate the dynamic landscape of HLA-bound peptides across distinct cancer types, including melanoma, triple-negative breast cancer (TNBC), diffuse intrinsic pontine glioma (DIPG), and Ewing sarcoma.

Utilizing high-throughput mass spectrometry techniques, we characterized the repertoire of HLA-bound peptides derived from cancer antigens within each malignancy. Our investigation revealed a remarkable variability in the HLA-bound peptide repertoire, indicative of diverse mechanisms underlying cancer antigen presentation. Notably, proteasomal splicing events accounted for 5-10% of peptides, shedding light on a previously overlooked avenue of peptide generation in cancer cells. Additionally, approximately 2% of peptides originated from non-coding genomic regions, suggesting the involvement of non-coding RNA in modulating the cancer immunopeptidome.

Moreover, our study unveiled the impact of the tumor microenvironment on HLA-bound peptide presentation. Specifically, inflammation-induced alterations in proteasome subunit expression were found to facilitate the expression of immunogenic neoantigens, underscoring the intricate interplay between tumor biology and immune recognition. Furthermore, we observed induction of HLA-II expression in certain tumours, highlighting another layer of complexity in tumour-immune interactions.

Overall, our immunopeptidomics analysis provides deep insights into the complex and dynamic nature of HLA-bound peptides in cancer antigen presentation across melanoma, TNBC, DIPG, and Ewing sarcoma. These findings hold significant implications for the development of tailored immunotherapeutic strategies aimed at exploiting the distinct immunopeptidomic landscapes of diverse malignancies.

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Enhancing CAR T cell therapy in solid tumours with site-specific secretion of BiTEs

Tony Huang[1], **Thang Hoang**[1], **DatMinh Nguyen**[1], **Joel Lee**[1], **Kah Min Yap**[1], **Amanda Chen**[1], **Cheng-I Wang**[2], **Phillip Darcy**[1], **Paul Beavis**[1]

1. Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

2. Agency for Science, Technology and Research , Singapore

Chimeric-antigen Receptor (CAR) T cells and bi-specific T cell engagers (BiTEs) are forms of immune therapies that harness the power of T cells to fight against cancer. However, despite the clinical success of these approaches in blood cancers, tumour antigen heterogeneity remains a major challenge in treating solid tumours.

Using CRISPR/Cas9 combined with AAV6-mediated Homology-directed repair (HDR) technology, we engineered HER2 (human epidermal growth factor receptor 2)-targeting CAR T cells to secret an anti-mEpCAM (mouse epithelial cellular adhesion molecule)/CD3 BiTE under the control of the NR4A2 promoter. These CAR T cells (1) exhibited superior tumour suppression with increased activation of endogenous T cells compared to control CAR T cells without BiTE, and (2) had a five-times improved safety profile compared to CAR T cells with a constitutive expression of BiTE.

In conclusion, a new generation of CAR T cells armed with BiTE which targets a second antigen was developed using CRISPR/HDR technology. This could not only prompt a tumour-localised secreting of BiTEs/nanobodies of desire but also allow the usage of BiTEs/nanobodies that were too toxic when given systemically.

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Quality Control Testing – The Next Generation

Tara L Cassidy[1]

1. Charles River Laboratories, Croydon, VIC, Australia

Quality control (QC) testing of biologicals, vaccines, and cell and gene therapy products are paramount. Traditional approaches for necessary QC steps can be limited in their approach. Assays can be low in sensitivity, time consuming, labour intensive, and costly. In addition, many approaches are primer dependent, hypothesis driven, low in resolution, and only provide a positive or

negative result. Next-Generation Sequencing (NGS) has the power to revolutionise QC testing techniques. NGS can detect all sequences in the sample and is not limited by primers or prior knowledge of the sample sequence. In cases of contamination detection, NGS enables the detection of agents that may be missed by conventional methods. NGS is rapid, cost effective, high in resolution and sensitivity, and enables identification of agents against chosen databases. NGS is compliant with the 3R initiatives, replace, reduce, refine and has the power to change the way we perform QC steps.

In this presentation we will walk you through NGS for quality control testing, provide an update on the regulatory framework relating to NGS, and share specific case studies where NGS has been used successfully for QC testing.

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Proof of principle that loss of mismatch repair protein reduces tumour burden in mouse model of gastric cancer

Anne Huber[1], Christine Dijkstra[1], Vicki Whitehall[2], Matthias Ernst[1], Moritz Eissmann[1]

1. Olivia Newton-John Cancer Research Institute, Melbourne/Yallambie, VICTORIA, Australia

2. QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

Aim

Gastric cancer (GC) remains the third leading cause of cancer-related death worldwide. Unfortunately, only a subset of GC patients, characterised by tumours with high microsatellite instability (MSI), responds to immune checkpoint (ICI) therapy. In the context of GC, the mechanisms of how MSI improves ICI therapy responses remain poorly understood. Here we are undertaking a proof of principle study to demonstrate in novel GC mouse models that loss of mismatch repair protein MLH1 confers MSI phenotype and impairs tumour growth via altered anti-tumour immune responses.

Methods

To study the functional and mechanistic effects of loss of MLH1 protein, we established MLH1-deficient (*Kras-, Pi3kca-, Tp53*-mutant) murine tumour organoids via CRISPR/Cas9 technology. These organoids were subcutaneously allografted into immunocompetent C57BL/6 mice to study tumour progression, immune surveillance, and responses to immunotherapy *in vivo*.

Results

MSI testing confirmed that MLH1-proficient parental organoids are MSI low, whereas MLH1-deficient organoids are MSI high. MLH1-deficient tumours were considerably smaller compared to MLH1-proficient tumours. MLH1-deficient tumours showed a significantly higher mutational burden, predicted neoantigen load, and number of infiltrating CD8+ T cells. Additionally, MLH1-deficient tumours were similar in size to MLH1-proficient tumours when allografted into $Rag1^{-/-}$ mice. Interestingly, CD8- and CD4-depleting experiments showed an involvement of CD4+ T cells in the impairment of MLH1-deficient tumour growth. Treatment with anti-PD-1 reduced tumour mass further.

Conclusion

Taken together, we provide evidence that loss of MLH1 leads to high MSI in gastric tumours, reduces tumour growth and increases response to ICI therapy. Our findings encourage further studies to investigate the mechanisms of impaired tumour growth after MLH1 loss in GC and may provide insights leading to improve ICI therapy responses for GC patients.

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Targeting Mediator kinases in immune cells for immunotherapy in colorectal cancer

Anran Lin[2, 1], Marius Dannappel [2, 1], Dane Newman[3, 4], Catherine Carmichael[2, 1], Ricky Johnstone[3, 4], Ron Firestein[2, 1]

1. Centre for Cancer Research, Hudson Institute of Medical Research, Melbourne, VIC, Australia

2. Molecular and Translational Science, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, VIC, Australia

3. Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Melbourne, VIC, Australia

4. Translational Hematology Program, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Over the past decade, immunotherapy has revolutionised cancer treatment, showing promising outcomes in lymphoma and melanoma. However, its effectiveness remains limited in various solid tumors like colorectal cancer (CRC), the 2nd leading cause of cancer mortality worldwide. The primary challenges in applying immunotherapies to CRC include the immunosuppressive tumour microenvironment (TME) and T cell exhaustion. Efforts are focused on enhancing immune cell activity to achieve more effective immunotherapy outcomes in CRC.

The Mediator complex is a multi-subunit protein module that integrates to regulate gene expression. It exerts its function partially via the Mediator kinase (MK) module, including cyclin-dependent kinase 8 (CDK8, paralog CDK19). CDK8/19 regulates RNA Polymerase II (Pol II) transcription by reversible association with the mediator complex, and phosphorylation effects on Pol II and gene-specific transcription factors (TF). A previous study (Freitas KA et al., Science, 2022) found that inhibiting MK in CAR-T cells improves their anti-tumour function and cytokine production, by increasing TF activity at enhancer regions. Therefore, we hypothesise that MK can be targeted to enhance T-cell effector function in a systematic model of CRC.

To address this, we established a tamoxifen-inducible CDK8 and CDK19 double knockout (DKO) mouse model using the Cre-loxP system. Using flow cytometry and single-cell RNA sequencing, we observed widespread changes in immune cell populations in the full-body CDK8/19 DKO model. Our preliminary results indicate that CDK8/19 deletion results in an increased innate immune cell population, suggesting the association of CDK8/19 with the inflammatory response *in vivo*. Moreover, we observed a reduced proportion of naïve T cells and an enhanced proportion of CD8 central memory and CD4 effector memory T cells in both spleen and lymph nodes. Similar findings of increased effector-like T cells were also observed by differentiating isolated

naïve T cells from the CDK8/19 KO mouse model *in vitro*. This demonstrates a critical role of MK in T cell differentiation. To test the potential of CDK8/19 as a target to enhance T cell function, we will also assess the immune function in our mouse model with targeted deletion of MK in CD8/CD4 T cells, both in the homeostasis state and the context of CRC.

Our study will expand understanding on the role of mediator kinase in the immune system, suggesting the potential to harness the function of CDK8/19 in immune cells for immunotherapy in solid tumours.

1. Freitas KA, Belk JA, Sotillo E, Quinn PJ, Ramello MC, Malipatlolla M, et al. Enhanced T cell effector activity by targeting the Mediator kinase module. Science. 2022;378(6620):eabn5647.

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Novel Insights on CD8+ Tissue-Resident Memory T cells in lymph node metastases from melanoma patients in response to immune checkpoint inhibitors.

ANDREA DI PIETRO[1], Lewis Au[1], Sean Macdonald[1], Patrick Crock[1], Niko Thio[1], Thu Ngoc Nguyen[1], Paul Neeson[1]

1. Peter MacCallum Cancer Center, Melbourne, VICTORIA, Australia

Within the spectrum of T-cell populations, tissue-resident memory T-cells (TRM) have emerged as key players in the immune control of melanoma, and their abundance is associated with a more favourable prognosis in response to immune checkpoint inhibitor (ICI) treatment. However, approximately half of the patients develop resistance to the treatment and, therefore, it is imperative to assess the role of TRM in this subgroup to refine therapeutic strategies. In this context, we hypothesised that TRM at the tumour site show features of exhaustion, resulting in the loss of a functional response and contributing to melanoma immune escape.

To characterise features associated with resistance, we conducted a comprehensive immune-profiling of T-cells in fresh surgical or formalin-fixed paraffin-embedded samples of lymph node metastases obtained from untreated, ICI-resistant, and ICI-responder patients with stage III/IV melanoma. Responder patients exhibited a higher expression of genes associated with a functional immune response. A curated gene module to analyse the TRM signature was applied among the patient groups, revealing a significantly higher z-score in ICI-responders. This was validated by multiplex immunofluorescence analysis which demonstrated an enrichment of both precursors (CD103+CD69+TCF1+) and terminally differentiated (CD103+CD69+TCF1-) CD8+ and CD4+ TRM within the tumour areas in the responder group, however, their proportion was significantly decreased in the resistant group.

While we validated the correlation between TRM abundance and the response to ICI, we further conducted single-cell RNA-Seq within the same patient cohort to assess whether CD8+ TRM exist in various differentiation states and functional polarisations based on the treatment status. Importantly, we observed that TRM were exhausted in the ICI-resistant tumours while effector/Tpex TRM were increased in ICI-responsive metastatic melanoma, displaying features of those seen in long term non-progressors on anti-PD1 treatment including expression of IFN-g and TNF. In addition, hyper-expanded TCR clones were enriched in CD8+ TRM clusters and were associated with ICI-responder and untreated groups, inferring an efficient response towards cancer cells. Interactome analyses were then performed to understand which cells could potentially support CD8+ TRM functions. We observed that CD8+ TRM interact with CD4+ TRM in the responder group, with the latter also showing cytotoxic features.

In conclusion, our study suggests that a pro-inflammatory microenvironment and specific cell-to-cell interactions play a crucial role in establishing functional TRM responses, contributing to a positive clinical outcome following ICI treatment. Conversely, the absence or diminished tumour responses by exhausted TRM were associated with ICI resistance or immune escape.

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Dimeric IgA as an effective therapeutic strategy

Annemarie Laumaea[1, 2], Charley McKenzie-Kludas[3], Laura D'Andrea[1], Hor Hor[1], Smitha Georgy[4], Peter Newman[1], Ewa Michalek[1], Kanta Subbarao[3, 5], David Anderson[1, 6], Jennifer Barnes[1], Heidi Drummer[1, 7, 8]

1. Burnet Diagnostics Initiative, Burnet Institute, Melbourne, Victoria, Australia

2. Immunology, Monash University, Clayton, Victoria, Australia

3. Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, VIC, Australia

4. Melbourne Veterinary School, , The University of Melbourne, Werribee, VIC, Australia

5. The Peter Doherty Institute for Infection and Immunity, WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, VIC, Australia

6. Walter and Eliza Hall Institute, Melbourne, VIC, Australia

7. Department of Microbiology, Monash University, Clayton, VIC, Australia

8. Department of Microbiology and Immunology, The University of Melbourne, Melbourne, VIC, Australia

There exists a paucity of effective immunotherapies targeting the mucosa that block entry of pathogens that invade through these sites. Immunoglobulin A (IgA), the second most abundant Ig class in circulation, after IgG, is an important antibody at the mucosa and can exist as monomeric and dimeric forms (dIgA) that predominate in systemic circulation, or secretory IgA (SIgA) found within secretions. SIgA, a complex formed between dIgA and the secretory component of the polymeric Ig receptor (pIgR), primarily functions to immobilize and trap pathogens for various immune clearance mechanisms. Conversion of dIgA to SIgA confers dimeric IgA structural stability, protection from degradation within the harsh acidic environment at mucosal surfaces and

importantly prevents transmission of mucosal pathogens. However, IgA has been underutilized as a therapeutic due to difficulty with generating sufficient yields.

Using recombinant technology, sequences of a potent SARS-CoV-2 IgG antibody were re-engineered from IgG into IgAs (dIgA and SIgA) and Immunoglobulins were expressed in high yields in Expi293 cells. RBD-ELISAs were performed to confirm antigen binding. In addition, flow cytometry and fluorescence microscopy were used to assess recognition of cell surface expressed pIgR. Transcytosis assays using polarized intestinal Caco-2 epithelial cells, stably expressing pIgR, were performed to confirm transcytosis of dIgA in a transwell assay. Finally, pilot animal challenge studies were conducted in mice to ascertain the *in vivo* efficacy of dIgA. We demonstrate here recombinant dIgA's specificity for its cognate receptor, pIgR, and its subsequent transcytosis across an intestinal epithelial cell transwell model. Furthermore, conversion of IgG to dIgA/SIgA was shown to improve neutralization potency against different SARS-CoV-2 variants of concern. Importantly, preliminary mice challenge studies showed that recombinant dIgA delivered intravenously is able to significantly reduce virus titres in mice infected with SARS-CoV-2 compared to a control group, as well as reduce immune cell infiltration in the interstitial space of lungs from dIgA-treated animals, confirming protection and thus the feasibility of this approach.

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Epigenetic treatment modality and biomarker to improve anti-HER2 Immunotherapy in HER2+ breast cancer

Li-Teng Ong[1], Wee Chyan Lee[1], Shijun Ma[1], Qiang Yu[1]

1. Genome Institute of Singapore, Singapore, SINGAPORE

The relapse of breast cancer patients following anti-HER2 antibody therapies such as trastuzumab has been associated with residual disease progression due to resistance to therapy. We identify interferon-γ inducible protein 16 (IFI16)-dependent STING signaling as a crucial immune cascade of anti-HER2 trastuzumab responses in HER2+ breast cancer. Down-regulation of immune-regulated genes (IRG) signature is specifically associated with poor clinical outcomes of HER2+, but not other breast cancer subtypes. Among IRG, IFI16 is identified as a direct target of EZH2, the underexpression of which leads to deficient STING activation and downstream CXCL10/11 expression in response to trastuzumab treatment. Dual inhibition of EZH2 and histone deacetylase (HDAC) significantly activates IFI16-dependent immune responses to trastuzumab. Notably, a combination of our in-house histone methylation inhibitor with an HDAC inhibitor induces complete tumor eradication through increased CD8+ T cells infiltration and long-term T cell memory in a HER2+ breast cancer mouse model. Our findings demonstrate an epigenetic regulatory mechanism suppressing the expression of the IFI16-CXCL10/11 signaling pathway that provides a survival advantage to HER2+ breast cancer to confer resistance to trastuzumab treatment.

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Drug targeting of colorectal cancers including cancer stem cells

Reem ALHulais[1], Stephen Ralph[1]

1. School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia

Cancer stem cells (CSCs) are notoriously drug resistant and are well known for their ability to undergo self-renewal and differentiation into more mature cancer cells. To date, there has been a notable dearth of investigations regarding the exact role and functions of isolated populations of CSCs. This study developed methods for selectively enriching CSC populations for drug targeting. SW480 and CT26 parental wild-type (WT) colorectal cancer cells were transfected with a vector encoding the octamer-binding transcription factor 4 (OCT4) promoter site regulating expression of enhanced green fluorescent protein (GFP). The most highly positive OCT4-GFP cell population after extensive rounds of sorting (top ~1%–5%) could be further enriched by intermittent cycling involving alternating conditions of growth (between normoxia and anoxia). The cycled cell populations were examined by immunoblotting with OCT4 antibody and by immunofluorescent staining for levels of OCT4 protein as a marker for CSCs. Next, the CT26 WT and enriched CT26 OCT4 cells were injected intraperitoneally into BALB/c mice to compare their tumour-initiating capacity as CSCs. Celecoxib was also tested for repurposing and its ability tested for inhibiting the CT26 WT and CT26 OCT4-GFP cell lines from forming colorectal tumours *in vivo*. CT26 OCT4-GFP and SW40 OCT4-GFP showed significantly higher levels of OCT4 expression when compared with the WT cells based on Western blotting and immunofluorescence staining. The highly enriched CT26 OCT4-GFP CSC population produced significantly greater tumour numbers with larger tumour sizes than did the CT26 WT inoculated mice. However, colorectal tumours formed by either cell types were significantly decreased (~50%) in numbers and volumes by celecoxib treatment. Significant levels of red blood cells were present in the peritoneal cavities of mice with the untreated colorectal tumours but greatly inhibited peritoneal angiogenesis was noted in the celecoxib-treated mice. Using these model systems for study will ensure that the role of CSC-enriched populations in tumour growth and metastasis and their therapeutic targeting can now be effectively conducted. The evidence obtained here also supports the potential for celecoxib to be repurposed and used in chemosensitising colorectal cancer cells, thereby rendering them more susceptible to standard chemotherapies such as doxorubicin and 5-fluorouracil.

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Identifying Optimal Tumour-specific Promoters for CRISPR Knock-in Generated Armoured CAR T Cells

Kah Min Yap[1, 2], Amanda X Y Chen[1, 2], Imran G House[1, 2], Phillip K Darcy[1, 2], Paul A Beavis[1, 2]

1. Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

2. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC, Australia

The tremendous success of chimeric antigen receptor (CAR) T cell therapy in haematological malignancies has yet to be recapitulated in the solid tumour setting, owing to immunosuppressive tumour microenvironment, tumour heterogeneity and inefficient tumour trafficking. One promising attempt to overcoming these barriers includes "armouring" CAR T cells with a therapeutic transgene. We previously demonstrated that CAR T cells engineered to secrete dendritic cell growth factor Flt3L could effectively engage the host immunity, which is critical in overcoming antigen-negative relapse[1]. However, synthetic promoters have demonstrated insufficiencies in achieving site-specific transgene expression, which had caused systemic toxicities and ultimately termination of a trial that expressed IL-12 using an NFAT-responsive promoter[2]. The advent of CRISPR/Cas9 gene editing tool has enabled the precise engineering of CAR T cells for safety and efficacy enhancements. We previously showed that CRISPR knock-out (KO) of immunosuppressive gene $A_{2A}R$ enhanced CAR T cell function[3]. Now, we aim to exploit a CRISPR knock-in (KI) strategy that leverages all endogenous regulatory elements of target genes to restrict transgene expression to the tumour site. We performed genome-wide RNA-Sequencing on CAR T cells and identified 27 genes with tumour-specific expression as potential KI sites. As target gene expression is disrupted during KI, we first assessed the impact of each gene KO on CAR T cell function/phenotype. Subsequently, 7 genes that did not adversely impact function/phenotype following KO had the reporter gene GFP knocked in. *RGS16* and *NR4A2* emerged as novel promoters that upon KI elicit higher transgene expression in tumours and lower transgene expression at non-tumour sites relative to the prototypic PD-1 promoter. This enabled the generation of armoured CAR T cells that secrete proinflammatory cytokines such as IL-12 and IL-2 specifically at the tumour site, leading to enhanced safety and efficacy in both syngeneic and xenogeneic models that was concomitant with improved CAR T cell polyfunctionality and proliferative capacity as well as activation of the host anti-tumour immunity. Notably, we also showed that this CRISPR KI strategy was applicable using patient-derived CAR T cells, further demonstrating the clinical translatability of this approach.

1. Lai, J., Mardiana, S., House, I. G., Sek, K., Henderson, M. A., Giuffrida, L., . . . Beavis, P. A. (2020). Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. Nature Immunology, 21(8), 914-926. doi:10.1038/s41590-020-0676-7
2. Zhang, L., Morgan, R. A., Beane, J. D., Zheng, Z., Dudley, M. E., Kassim, S. H., . . . Rosenberg, S. A. (2015). Tumor-Infiltrating Lymphocytes Genetically Engineered with an Inducible Gene Encoding Interleukin-12 for the Immunotherapy of Metastatic Melanoma. Clinical Cancer Research, 21(10), 2278-2288. doi:10.1158/1078-0432.Ccr-14-2085
3. Giuffrida, L., Sek, K., Henderson, M. A., Lai, J., Chen, A. X. Y., Meyran, D., . . . Beavis, P. A. (2021). CRISPR/Cas9 mediated deletion of the adenosine A2A receptor enhances CAR T cell efficacy. Nat Commun, 12(1), 3236. doi:10.1038/s41467-021-23331-5

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Chimeric Antigen Receptor Monocytes With Enhanced Anti-Tumor Activities By Harnessing Innate Immunity Pathways

Neha Diwanji[1], Tyler Nicholson[1], Thomas Prod'homme[1], Bruce McCreedy[1], Yuxiao Wang[1], Daniel Getts[1]

1. Myeloid Therapeutics, Cambridge, MASSACHUSETTS, United States

Myeloid cells, including monocytes and macrophages are the primary orchestrators of immune responses and are found to accumulate abundantly in solid tumors. These cells express a wide range of innate immune sensors such as Toll-like receptors (TLRs), RIG-I and cGAS-STING. The activation of these innate immune pathways can remodel tumor microenvironment and initiate broad immune responses against tumor. Here we designed a new class of chimeric antigen receptors for monocytes that couple tumor recognition with multiple innate immune signaling domains. We screened a broad range of innate immunity signaling molecules and optimized a CAR that incorporates a domain of the TLR adaptor molecule TRIF. The TRIF-CAR targeting human HER2 upregulated the pro-inflammatory cytokines and chemokines including IP10, CCL5 and IFNalpha and enhanced tumor killing activity. Transcriptome-wide analysis revealed that the addition of TRIF to the CAR broadly upregulated genes associated with a pro-inflammatory response, T-cell co-stimulation as well as myeloid cell migration and trafficking. Immunosuppressive molecules such as TREM2 were downregulated. Our data show that the incorporation of a TRIF domain into a myeloid-specific CAR can enhance the innate anti-tumor activity of myeloid cells.

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CAR-DC1: A novel next-generation dendritic cell therapy for targeting solid tumours

Shengbo Zhang[1], Cindy Audiger[1], Amanda Woon[1], Shalin NaiK[1], Michael Chopin[2], Stephen Nutt[1]

1. Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia., Parkville, VIC, Australia

2. Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, VIC, Australia

Immunotherapy involves harnessing the patient's own immune system to fight cancer. One class of immunotherapy involves the engineering of patient T cells to express a Chimeric Antigen Receptor (CAR) that directs T cells to seek out and kill cancer cells. CAR-T products have been successful in treating some blood cancers, but their application in the setting of solid tumours has proven to be more problematic, due to issues of tumour diversity and poor T cell infiltration. Dendritic cells (DCs) represent an attractive alternative cellular immunotherapy due to their ability to take up tumour-associated antigens and promote polyclonal T-cell expansion, functional polarisation and tumour killing. Although monocyte-derived DCs have been extensively trialled as tumour "vaccines" with limited success, recent evidence has shown that it is type 1 conventional DCs (DC1s) that best promote anti-tumour immunity. In this research program, we aim to develop a novel cell therapy platform whereby DC1s are engineered with CAR constructs (CAR-DC1s) to precisely target solid tumours. More specifically, CAR-DC1s better recognise tumour cells and initiate potent and persistent anti-tumour responses. We identified a proprietary lead CAR-DC1 construct which displayed

superior antigen presentation capacity and evoked proinflammatory anti-tumour T cells responses. Thus we propose a potentially a first-in-class cell therapy platform to address the large unmet need of treating cancer patients unresponsive to current treatments, especially those with intractable solid cancers.

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Adoptive transfer of type 1 regulatory T cells prevents inflammation and gut damage in mouse model of colitis

Chad Poloni[1, 2], Andrew Sze[1], Laura Cook[2], Ted Steiner[1]

1. University of British Columbia, Vancouver, BC, Canada

2. University of Melbourne, Melbourne, VIC, Australia

Inflammatory bowel disease (IBD), an umbrella term for Crohn's disease and ulcerative colitis, affects almost 1 in 150 Australians. Current treatments cause side-effects and are unable to reverse tissue-remodeling and scarring. New therapeutics are urgently needed. Type 1 regulatory cells (Tr1 cells), a subset of $CD4^+$ regulatory T cells, have been shown to suppress inflammation and promote wound healing *in vitro.* We hypothesized that Tr1 cellular therapy could prevent gut inflammation and damage.

We tested our hypothesis in a DSS-induced colitis mouse model. First, $CD4^+$ T cells were isolated from $Thy1.1^+$ IL-10-GFP reporter mice and stimulated *ex vivo* for 48-hours with anti-CD3/CD28 antibodies and IL-27 with or without all-trans retinoic acid (ATRA), and the $IL\text{-}10^+$ Tr1 cells were isolated by cell sorting. We confirmed these Tr1 cells had significantly higher expression of the co-inhibitory marker ICOS than endogenous Tr1 cells, they retained high levels of IL-10 secretion, and those treated with ATRA had increased expression of the gut homing integrin α4β7. Next, 10-week-old C57BL/6J mice were sub-lethally irradiated and injected (I.V.) with $1x10^6$ Tr1 cells (N = 10) or PBS (controls; N = 11). Following a 7-day engraftment period, mice were administered drinking water containing 1.5-2% DSS for another 7-days. Mice were then given normal drinking water for 7-days, allowing for recovery, at which point blood, spleen, mesenteric lymph nodes (MLN), and colonic cross-sections were acquired for flow cytometric analysis and histology.

Mice receiving Tr1 cellular therapy retained significantly more weight at day 7 compared to controls ($p = 0.0163$) and had improved colon length, width, and histologic scores ($p = 0.0406$, 0.0234, 0.0229 respectively). Our most striking finding was that mice treated with ATRA-conditioned $ICOS^+$ Tr1 cells retained more weight than controls and had decreased levels of the inflammatory marker lipocalin-2 in stool samples. RNA-sequencing revealed ATRA-conditioned Tr1 cells upregulated *Icos, Il10, Ahr, and Maf* transcripts, all associated with functional Tr1 cells in both mice and human studies.

Our data show that Tr1 cellular therapy prevents weight loss and colonic damage in a DSS-induced murine model of colitis. Preliminary data suggests ATRA-induced $ICOS^+$ Tr1 cells are superior at preventing murine colitis and may be a suitable cellular therapy for IBD.

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The myeloid cell kinase HCK as a therapeutic target to improve immune checkpoint blockade in high-grade serous ovarian cancer

Kaveh Baghaei[1], Ashleigh Poh[1], Annalisa Carli[1], Matthias Ernst[1]

1. Cancer and Inflammation Laboratory , Olivia Newton John Cancer Research Institute, Melbourne, VIC, Australia

High-grade serous ovarian cancer (HGSOC) is the most common and lethal subtype of ovarian cancer and has shown limited response to immune checkpoint blockade (ICB), which is thought to arise from a myeloid cell-orchestrated immune-suppressive tumour microenvironment. We have recently shown that ablation of the cytosolic tyrosine kinase HCK, which is expressed in tumour-associated myeloid cells, converts immune-suppressive to immune-permissive tumour microenvironment and confers effectiveness of ICB therapy to otherwise unresponsive pancreatic cancer in mouse models.

Here, we test the hypothesis that HCK may also be a therapeutically targetable signaling node that limits ICB-response of HGSOC by using p53/Brca2 syngeneic cell model in immune competent hosts. We observe significantly reduction in ascites formation and tumour burden in host which genetically lack HCK expression, and this is associated with reduced tumour cell proliferation and enhanced recruitment and activation of CD8+ T- and NK- effector cells. These findings coincide with increased expression of immune-activating factors and decreased expression of immunosuppressive markers, alongside reduced proportions of immunosuppressive $CD206^+$ macrophages and myeloid derived suppressor cells. Importantly, in HCK-deficient hosts we find improved anti-PD1 responses of HSGSOC tumours and associated ascites accumulation. These observations can be partially replicated by therapeutic administration of the tool compound RK20449, with known activity against HCK and the related T-cell receptor-associated LCK kinase.

We are currently developing novel small molecule inhibitors with increased affinity to HCK over LCK using biochemical and cellular assay. For the latter, we have substituted the growth factor-dependency of BaF0/3 cells by selective expression of constitutive active isoforms of HCK either LCK, in order to use selective inhibition of proliferation for the functional assessment of specificity of candidate inhibitor molecules.

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"Off-the-shelf" CAR T-cell Therapy Targeting Ephrin Receptor A3 Expressed by Glioma Stem Cells and Tumour Vasculature

Paulo Martins[1], Rochelle D'Souza[2], Niclas Skarne[2], Lea Lekieffre[1], Shane Horsefield[1], Madusha Ranjankumar[1], Xiang Li[1], Thuy Lee[1], Fiona Smith[2], Corey Smith[3], Jacqueline Burrows[1], Bryan Day[2], Rajiv Khanna[1]

1. Tumour Immunology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

2. Sid Faithfull Brain Cancer Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

3. Translational and Human Immunology Laboratory , QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

Background: Adoptive T cell therapy targeting antigens expressed in glioblastoma multiforme (GBM) has emerged as potential therapeutic strategy to prevent or delay recurrence and prolong overall survival. Ephrin receptor A3 (EphA3), which is highly expressed on GBM; in particular, by the tumour vasculature and glioma stem cells is an ideal target for immune-based therapies.

Methods: We have designed an EphA3-taregting chimeric antigen receptor (CAR) using the single chain variable fragment of a novel monoclonal antibody and assessed its therapeutic potential against EphA3-expressing patient-derived glioma neurospheres, organoids and xenografted GBM tumours in immunodeficient mice.

Results: In vitro expanded allogeneic EphA3 CAR T cells form healthy individuals efficiently recognize and kill EphA3-positive GBM cells in vitro. Furthermore, these effector cells demonstrated curative efficacy in an orthotopic xenograft model of GBM. EphA3 CAR T cells were equally effective in targeting patient-derived neurospheres and infiltrate, desegregate, and kill GBM-derived organoids.

Conclusions: This study presents compelling evidence supporting the therapeutic potential of allogeneic EphA3-CAR T cell therapy against GBM. EphA3 is expressed in multiple tumour types, including colon, prostate, gastric, lung, kidney, and breast cancers. The ability to target EphA3-expressing cancer stem cells and the tumour vasculature in various tumours underscores the translational significance of EphA3-CAR T cell therapy in the quest for effective and targeted treatments.

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Uncovering Therapeutic Targets For HLA-dependent Immunotherapy Using Immunopeptidomics In Paediatric Ewing Sarcoma

Grace Huang[2, 1], Terry Lim[1, 4, 3], Tima Shamekhi[2, 1], Erwin Tanuwidjaya[2, 1], Jason Cain[1], Pouya Faridi[2, 1, 3]

1. Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, VIC

2. Department of Medicine, School of Clinical Sciences, Monash University, Clayton, VIC, Australia

3. Monash Proteomics and Metabolomics Platform, School of Clinical Sciences, Monash University, Clayton, VIC

4. Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Clayton, VIC

Ewing Sarcoma (ES) is the second most prevalent primary bone tumour in children, adolescents, and young adults with less than 30% to survive after five years if the tumour relapses or spreads to other parts of the body. Current treatment is rapidly losing effectiveness in treating the tumour. A potential novel therapy for ES is HLA-dependent T cell-based immunotherapy, which uses the body's immune system to target peptide antigens displayed on human leucocyte antigens (HLA) and destroy the tumour cells. In this study, we present an in-depth immunopeptidomics analysis to identify peptide antigens present on HLA class I in a cohort of ES cell lines.

We analysed the immunopeptidome of four ES cell lines under basal condition and 100IU/ml interferon-gamma (IFNγ) treatment for 72hr. Our optimised immunopeptidomics protocol allowed us to identify 35,132 peptides, matched against the human proteome database across all cell lines by processing 100 million cells per cell line and condition (n=1). We found that IFNγ treatment increased HLA class I peptide antigens by 4-fold, with over 78% of unique peptides in each cell line after treatment. Identified peptides were found to be sourced from 8657 proteins. Similarly, IFNγ also increased the diversity of sourced proteins and cancer antigens (CAs), with over 65% and 60% found after IFNγ treatment in each cell line, respectively. Moreover, 35 CAs were shared across our cohort. Our findings suggest that an antigen discovery approach using in-depth immunopeptidomics analysis and IFNγ stimulation can help identify potential peptide antigen targets for ES immunotherapy.

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Examining epigenetic enzymes as a target in breast cancer metastasis and treatment resistance

John JV Vandermeide[1]

1. QIMR berghofer, South Brisbane, QLD, Australia

Publish consent withheld

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RASAL3 is a potential negative regulator of CD8+ T cell in antitumor immune response

Chen Yang[1]

1. Shanghai cancer insititute, New Pudong Area, SHANGHAI, China

CD8+ T cell dysregulation is a major cause of the failure of immune checkpoint blockade (ICB) therapies. To identify the molecular determinants involved, we used an integrative data analysis approach that leveraged single cell transcriptome data from human tumor materials in the context of ICB treatment, and data from functional genetic screening in T cells assessing gene functions.

First, by integrating and in silico sorting of single-cell data from five cohorts comprising 97 ICB-treated patients, we extracted a total of 161,071 tumor-infiltrating CD8+ T cells and identified two subclusters of potential tumor-reactive T cells: terminal Tex and CD137+ Tex. We then used the Weighted VIPER approach to infer protein activities in these tumor-reactive cells and compared them between ICB-response and non-response groups. Finally, we conducted an intergrative analysis of the differential protein activity data and the data from 20 CRISPR-based genetic screens in T cells.

Our analysis identified several genes, such as PTPN22, MAP4K1, CD5, and DKGZ, known to contribute to T cell exhaustion or impair T cell functions. Among the same cluster of the hits, we identified RASAL3 as a potential modulator that negatively regulates CD8+ T cells in the ICB treatment. Currently, we are evaluating the effect of RASAL3 and other hits in models of T cell-based immunotherapies.

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Macrophages in HCK knock out mice share a common gene signature across various cancers

Purva Trivedi[1], Ashleigh Poh[1], Megan O'Brien[1], Matthias Ernst[1], Moritz Eissmann[1]

1. Olivia Newton John Cancer Research Institute, Melbourne, VIC, Australia

Hemopoietic Cell Kinase (HCK) belongs to the SRC family of non-receptor tyrosine kinase widely expressed in myeloid cells, most notably in macrophages. It is well established that macrophages play a role in creating an immunosuppressive, pro-tumoral and pro-angiogenic environment in cancers. Tumour associated macrophages (TAM) polarised to specific subtypes known as M2-like, alternative activated macrophages that are induced by T helper type 2 cells via interleukins such as IL4. HCK activity promotes polarisation of TAMs toward a tumour-promoting M2-like endotype thereby limiting infiltration and activation of immune effector cells and associated anti-tumour immune responses, including those elicited by immune checkpoint blockade.

We analysed scRNAseq from 1) Metastatic high grade ovarian cancer, and 2) Orthotopic lung cancer models established in either HCK Knock out (KO) and HCK wild type (WT) hosts. We looked at differential gene expression profiles in macrophages in these cancer models to seek for HCK-dependent, common gene signatures. Furthermore, we compared this dataset with bulk RNA sequencing of murine bone marrow-derived macrophages derived from HCK-KO and HCK-WT mice and stimulated in vitro by interleukin 4 (IL-4). Compared to immune cell subsets from HCK-WT host, in cells of HCK-KO hosts we observed consistent down-regulation of genes associated with pathways involved in cellular proliferation, receptor and integrin signalling and angiogenesis. By contrast, genes involved in regulation of T cell differentiation, cell population proliferation, intrinsic apoptotic signalling pathway, T cell mediated cytotoxicity, antigen processing via MHC class 1, humoral immune response, macrophage migration, type I and II interferon-mediated signalling pathway and negative regulation of apoptotic process were upregulated in cells of HCK-KO hosts. Importantly, there were significant differences in the extent of differential gene expression between the TAMs in these models and in vitro polarized cells suggesting the inability of IL-4 to recapitulate the exact conditions in cancer tumour microenvironment in vivo.

Our HCK-specific gene signatures in TAM will allow us in the future to assess the extent of HCK signalling in human tumours and to better identify patients most likely to benefit from anti-HCK therapies.

1. Bhattacharjee, A., Pal, S., Feldman, G. M., & Cathcart, M. K. (2011). Hck is a key regulator of gene expression in alternatively activated human monocytes. Journal of Biological Chemistry, 286(42), 36709–36723. https://doi.org/10.1074/jbc.M111.291492
2. Pathria, P., Louis, T. L., & Varner, J. A. (2019). Targeting Tumor-Associated Macrophages in Cancer. In Trends in Immunology (Vol. 40, Issue 4, pp. 310–327). Elsevier Ltd. https://doi.org/10.1016/j.it.2019.02.003
3. Poh, A. R., Love, C. G., Chisanga, D., Steer, J. H., Baloyan, D., Chopin, M., Nutt, S., Rautela, J., Huntington, N. D., Etemadi, N., O'brien, M., O'keefe, R., Ellies, L. G., Macri, C., Mintern, J. D., Whitehead, L., Gangadhara, G., Boon, L., Chand, A. L., … Ernst, M. (2022). Therapeutic inhibition of the SRC-kinase HCK facilitates T cell tumor infiltration and improves response to immunotherapy. In Sci. Adv (Vol. 8). https://www.science.org
4. Poh, A. R., Love, C. G., Masson, F., Preaudet, A., Tsui, C., Whitehead, L., Monard, S., Khakham, Y., Burstroem, L., Lessene, G., Sieber, O., Lowell, C., Putoczki, T. L., O'Donoghue, R. J. J., & Ernst, M. (2017). Inhibition of Hematopoietic Cell Kinase Activity Suppresses Myeloid Cell-Mediated Colon Cancer Progression. Cancer Cell, 31(4), 563-575.e5. https://doi.org/10.1016/j.ccell.2017.03.006
5. Poh, A. R., O'Brien, M., Chisanga, D., He, H., Baloyan, D., Traichel, J., Dijkstra, C., Chopin, M., Nutt, S., Whitehead, L., Boon, L., Parkin, A., Lowell, C., Pajic, M., Shi, W., Nikfarjam, M., & Ernst, M. (2022). Inhibition of HCK in myeloid cells restricts pancreatic tumor growth and metastasis. Cell Reports, 41(2). https://doi.org/10.1016/j.celrep.2022.111479

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Exploring soluble HLA peptidome for cancer biomarkers through immunopeptidomics.

Erwin Tanuwidjaya[1, 2, 3], Terry Lim[1, 2, 3], Joel R Steele[2], Ralf B Schittenhelm[2], Pouya Faridi[1, 2, 3]

1. Department of Medicine, Translational Antigen Discovery Lab, Clayton, Victoria, Australia

2. Biochemistry and Molecular Biology, Monash Proteomics & Metabolomics Platform, Clayton, Victoria, Australia

3. Hudson Institute of Medical Research, Clayton, Victoria, Australia

Introduction:

Using circulating molecular biomarkers to screen for cancer and other debilitating disorders in a high-throughput and low-cost fashion is becoming increasingly attractive in medicine. One major limitation of investigating protein biomarkers in plasma is that antigenic cancer protein are often invisible through routine plasma proteomics. In contrast, Human Leukocyte Antigen (HLA) presents peptides from the entire proteome on the cell surface. While peptide-HLA complexes are predominantly membrane-bound, a fraction of HLA molecules is released into body fluids which is referred to as soluble HLAs (sHLAs). Recent

developments in mass spectrometry (MS)-based proteomics have enabled the acquisition of ever smaller input amounts and therefore enabling valuable information to be extracted out of the peptidome of these circulating sHLA.

Methods:

Plasma from healthy donors were collected and subjected through various sample development methods, from the sample preparation side to the mass spectrometry acquisition methods, via incorporation of recent immunopeptidomics approaches. Once method optimisation is completed, the final method would then be applied to 92 plasma samples of rare cancer patients undergoing immune check point inhibitor therapy to investigate whether there are shared immunopeptidomic profiles among them that would predict patient response to the therapy. In this poster presentation I would only highlight the results from method optimisation.

Preliminary Data:

To ensure reproducibility, we sought to optimize a semi-automated sample preparation based on a modified SAPrIm method on these plasma samples which minimize manual handling time. Improvement in sample preparation and mass spectrometry workflow has allowed for deeper plasma immunopeptidomics coverage without sacrificing the purity of the immunopeptidome in plasma from healthy donor.

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mRNA vaccine against malaria tailored for liver-resident memory T cells

Lauren Holz[1], Mitch Ganley[2], Jordan Minnell[3], Sarah Draper[2], Yu Cheng Chua[1], Zhengyu Ge[1], Anton Cozijnsen[4], Stephanie Gras[5], Geoffrey McFadden[4], Gavin Painter[2], Ian Hermans[3], William Heath[1]

1. The Peter Doherty Institute, Melbourne, VIC, Australia

2. The Ferrier Research Institute, Wellington, New Zealand

3. Malaghan Institute of Medical Research, Wellington, New Zealand

4. School of BioSciences, University of Melbourne, Parkville, VIC, Australia

5. Viral and Structural Immunology Laboratory, La Trobe Institute For Molecular Science, La Trobe University, Bundoora, VIC, Australia

Malaria is caused by *Plasmodium* species transmitted by *Anopheles* mosquitoes. Following a mosquito bite, *Plasmodium* sporozoites migrate from skin to liver, where extensive replication occurs, emerging later as merozoites that can infect red blood cells and cause symptoms of disease. As liver tissue-resident memory T cells (Trm) have recently been shown to control liver-stage infections, we embarked on an mRNA-based vaccine strategy to induce liver Trm to prevent malaria. While a standard mRNA vaccine was unable to generate liver Trm, or protect against challenge with *Plasmodium berghei* sporozoites in mice, addition of an agonist that recruits T cell help from type I Natural Killer T cells under mRNA-vaccination conditions resulted in significant liver Trm generation and effective protection. Moreover, while prior exposure of mice to blood-stage infection impaired traditional vaccines based on attenuated sporozoites, mRNA vaccination was unaffected, underlining the potential for such a rational mRNA-based strategy in malaria-endemic regions.

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T-bet high expressing CD4 T cell progenitors seed TRM cells in the peripheral tissues

Amania A. Sheikh[1, 2], Tom Weber[1, 2], Hanna Abberger[1, 2], Carolina Alvarado[1, 2], Benjamin Broomfield[1, 2], Sara Tomei[1, 2], Shalin H. Naik[1, 2], Joanna R. Groom[1, 2]

1. Division of Immunology, Walter Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

2. Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia

CD4+ tissue resident memory T cells (TRM) are the most abundant T cells in the memory pool that are permanently retained in peripheral tissues and have the ability to mount direct and rapid immune response against re-invading pathogens. However, cellular pathways underlying the formation and maintenance of TRM cells are unclear and is of significant therapeutic interest. Using an in vivo cellular barcoding strategy, we investigated the relatedness of T cell pool at the peak of immune response with memory population including TRM cells upon resolution of infection. We show that the T cells including T helper 1 cells (TH1) and mixed memory TH1 expressing high levels of TH1-associated transcription factor T-bet seed TRM cells. We and others have previously demonstrated that T-bet is important for TH1 differentiation and survival in LCMV infection. Here, we show T-bet deficient LCMV-specific cells vs controls have impaired ability to form T cell memory including TRM cells in the periphery. Further validating our cellular barcoding investigation that T-bet high expressing T cells seed TRM cells including memory cells and T-bet is crucial for the maintenance of immune memory. A better understanding of the pathways that dictate TRM formation is crucial for the development of both improved vaccines against pathogens and new therapeutics for malignancies.

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Deciphering the role of IgM in blocking human malaria transmission

Jo-Anne Chan[1], Ashley Lisboa-Pinto[1], Shirley Lu[1], Alexander Harris[1], Matthew Dixon[2], Kazutoyo Miura[3], Adam Thomas[1], Damien Drew[1], Sandra Chishimba[1], Herbert Opi[1], Linda Reiling[1], Kiprotich Chelimo[4], Michael Theisen[5], Carole Long[3], Arlene Dent[6], James Kazura[6], James Beeson[1]

1. Burnet Institute, Melbourne, VIC, Australia
2. Walter & Eliza Hall Institute, Melbourne
3. National Institute of Allergy and Infectious Disease, National Institutes of Health, Rockville, USA
4. Maseno University, Kisumu, Kenya
5. Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark
6. Center for Global Health and Diseases, Case Western Reserve University, USA

Background: Although there has been major progress in reducing the global malaria burden, this decline has recently plateaued. There are still over 200 million cases annually, exacerbated by the impact of COVID-19. As such, the development of vaccines that specifically block the transmission of malaria is a primary goal of WHO and Gates Foundation. Transmission-blocking vaccines are designed to target an essential biological bottleneck in the malaria lifecycle by generating antibodies that block parasite transmission from humans to mosquitoes. When a mosquito feeds on an infected person, whole blood containing antibodies and transmission stage parasites is ingested. Antibodies of the right functional properties that specifically target gametocytes can inhibit parasite development within the mosquito and prevent their subsequent transmission to humans. However, major knowledge gaps in our understanding of how antibodies block transmission represents a critical roadblock to vaccine development. In our study we aim to determine the major targets and function of antibodies, with a key focus on the role of IgM.

Methods: Here, we assessed antibody responses in samples from malaria-exposed children and adults from Kenya. We measured serum antibody levels and functional immune mechanisms (such as complement fixation and activation) and examined their correlation with clinical data available from the study cohorts.

Results/Conclusions: We detected high levels of IgM in serum samples tested from both children and adults. Further we showed that IgM purified from immune sera was capable of fixing and activating human complement. Our findings directly support a key role for IgM in mediating functional immunity that block malaria transmission. Our findings have major implications to further understand how the acquired human immune response potentially interrupt the transmission of malaria and accelerate the development of transmission-blocking vaccines crucial for malaria elimination.

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Age-related differences in mRNA vaccine adjuvancy and immunogenicity

Shivali Savita Chinni[1], Patrick Leung[1], Jonathan L McQualter[1], Joanne E Davis[2, 3], Rachel M Koldej[2, 3], David S Ritchie[2, 3], Colin W Pouton[4], Harry Al-Wassiti[4], Kylie M Quinn[1, 5]

1. School of Health and Biomedical Sciences, Royal Melbourne Institute of Technology (RMIT) University, Bundoora, VIC, Australia
2. Australian Cancer Research Foundation (ACRF) Translational Laboratory, Royal Melbourne Hospital, Melbourne, VIC, Australia
3. Department of Medicine, University of Melbourne, Melbourne, VIC, Australia
4. Monash Institute of Pharmaceutical Sciences (MIPS), Parkville, VIC, Australia
5. Department of Biochemistry, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Older individuals are both at increased risk of disease with infections and less likely to mount robust adaptive immunity to vaccines. Given this increased vulnerability, tailoring new mRNA vaccines specifically for the older immune system would be a significant advance. To design age-optimised mRNA vaccines, we first need to understand how current formulations perform in an aged setting. We therefore aimed to characterise how an mRNA vaccine performs in an aged mouse model.

We first validated that age-related differences in mRNA vaccine-induced adaptive responses are also seen in a mouse model. We vaccinated young (<5 months old (mo)) and aged (19 mo) C57BL/6 mice with an mRNA vaccine encoding the SARS-CoV-2 Spike protein at day 0 and day 21. Serum was harvested throughout the timecourse to track B cell responses, with this analysis still underway. T cell responses were assessed at peak (day 28) and memory (day 90) timepoints, using intracellular cytokine staining and activation-induced marker assays. Aged mice exhibited significantly lower antigen-specific CD8 and CD4 T cell responses with regard to nearly all markers assessed (IFNγ, TNF and IL-2 production, CD69+CD25+ CD8 T cells and CD154+OX40+ CD4 T cells), consistent with differences seen in older humans. Of note, aged mice had markedly higher background with all activation-induced markers assessed, consistent with previous observations of T cell hyperactivation in advanced age.

We then defined age-related differences in adjuvancy mechanisms that could impair adaptive responses. To track vaccine uptake and antigen expression by DCs, we vaccinated young and aged mice with mRNA vaccines, using either a fluorescently labelled lipid nanoparticle (LNP) or mRNA encoding a fluorescent protein. Draining lymph nodes were harvested 16 hours later, and uptake or expression were assessed using magnetic enrichment of DCs followed by flow cytometry. Serum was also harvested to track changes in innate immune activation, with this analysis still underway. Vaccine uptake by young and aged DCs was comparable (83.4% *vs* 87.3%) but intact antigen was significantly higher in aged DCs (12.33% *vs* 18.3%) and DC numbers were reduced in aged mice both prior to and after vaccination by at least 10-fold.

Our data both validates that aged mice are a viable model for mRNA vaccination and highlights age-related changes in adjuvancy (antigen retention, deficit in DC number) that may contribute to poorer adaptive immune responses in aged individuals. Strategies that circumvent these age-related deficits could improve vaccine outcomes for older individuals.

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Identification of cancer pathways and biomarkers in mouse models of spontaneous chronic colitis: From inflammation to cancer

Ramya Ephraim[1], Sarah Fraser[1], Rhian Stavely[1], rajaraman Eri[2], Kulmira Nurgali[1], Vasso Apostolopoulos[1]

1. Institute for Health and Sport, Immunology and Translational Research Group, Melbourne, Vic, Australia

2. RMIT University, Bundoora, VIC, Australia

Chronic inflammation is a key driver of oncogenesis, and inflammatory bowel disease is strongly associated with the development of cancer. In this study, the Winnie mouse model of inflammatory bowel disease is used to show that the severity of inflammation leads to the expression of a wide range of cancer genes. This study provides important insights into the genetic basis for malignancy in inflammatory bowel disease, as well as identifying markers that could be used to screen for the development of cancer in patients. The presence of checkpoint markers in cancer cells aids in immune escape. The identification of checkpoint markers and early cancer markers is of utmost importance to gain clarity regarding the relationship between colitis and progressive inflammation leading to cancer. Herein, the gene expression levels of checkpoint makers, cancer-related pathways, and cancer genes in colon tissues of mouse models of chronic colitis (Winnie and Winnie-Prolapse mice) using next-generation sequencing are determined. Winnie mice are a result of a Muc2 missense mutation. The identification of such genes and their subsequent expression and role at the protein level would enable novel markers for the early diagnosis of cancer in IBD patients. The differentially expressed genes in the colonic transcriptome were analysed based on the Kyoto Encyclopedia of Genes and Genomes pathway. The expression of several oncogenes is associated with the severity of IBD, with Winnie-Prolapse mice expressing a large number of key genes associated with development of cancer. This research presents a number of new targets to evaluate for the development of biomarkers and therapeutics.

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Developing multi-antigen and multi-species mRNA vaccines against malaria

Adam Thomas[1, 2], Timothy Ho[1, 2], Lee Yeoh[1, 3], Colin Pouton[4], Stewart Fabb[4], Che Ling[4], James Beeson[1, 3]

1. Burnet Institute, Melbourne, VICTORIA, Australia

2. Monash University, Melbourne, Victoria, Australia

3. University of Melbourne, Melbourne, Victoria, Australia

4. Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia

Malaria is still a leading health problem with high numbers of clinical cases and deaths recorded annually, the majority of which are children aged under 5 years. There is an urgent need for effective vaccines to reduce the disease burden and morbidity and enable malaria elimination. Malaria is largely caused by two species, *Plasmodium falciparum* and *Plasmodium vivax*. In recent years, recombinant protein vaccine platforms in conjunction with multiple adjuvants have been extensively used in vaccine development for different parasite targets. This includes the RTS,S vaccine, which targets sporozoites that initiate liver infection. However, it has modest and short-lived efficacy requiring regular booster doses and only acts against *P. falciparum*. Targeting antigens of additional parasite stages in vaccines could achieve greater protection against clinical illness and mortality, and reduce transmission. It is likely that multiple antigens in single vaccine formulations will be needed to improve efficacy. Working towards this gaol, we are evaluating blood-stage Merozoite Surface Proteins (MSP) in the mRNA vaccine platform. This group of proteins have shown promise in early clinical trials and vaccines can induce antibody-dependant complement-mediated and cellular inhibition activity against the parasite. We designed MSP mRNA constructs and successfully expressed them in human HEK293 cells, following which we packaged the mRNA in Lipid Nanoparticles (LNPs) delivery vehicles for mice immunisation and vaccine efficacy studies. Our constructs have shown induction of IgG in mice, confirming the immunogenicity of the expressed antigen *in vivo*. Functional and inhibitory studies will be performed to confirm the potential protective activity and we will evaluate vaccines designs that achieve long-lasting immunity. The mRNA approach could surpass the current recombinant Virus-Like Particle vaccines and achieve multi-antigen multi-species vaccines to accelerate malaria elimination.

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A precision approach to treatment enhancement by adjuvant Smac-mimetics in oral squamous cell carcinoma (OSCC).

Elham Moslemi[1], Anu Sakthianandeswaren[1], Natasha Silke[1], Jun Ting Low[1], Michael McCullough[2], Oliver Sieber[1], John Silke[1], Antonio Celentano[2], Tami Yap[2], Lorraine O'Reilly[1]

1. WEHI, Parkville, VIC, Australia

2. Melbourne Dental School, Faculty of Medicine, Dentistry and Health Science, University of Melbourne, Melbourne, VIC, Australia

Recent genomic analyses have identified cell death signalling and NF-kB pathways as molecular drivers in oral squamous cell carcinoma (OSCC), a major sub-type of head and neck cancers. Multiple components of these pathways are amplified, such as Inhibitor of Apoptosis (IAP) proteins or mutate (CASP8), allowing cancer cells to evade apoptosis, and contribute to treatment resistance. Smac-mimetics are a new drug class and target the IAPs (e.g cIAP1/cIAP2) and have been shown to restore treatment sensitivity, possibly through the promotion of apoptosis but also work as an adjuvant to immunotherapy checkpoint inhibitors. Adjuvant Smac-mimetics therapy may enhance treatment response and open avenues to improve patient survival and quality of life by altering survival/death signalling and thus OSCC immunobiology. We therefore aimed to first establish models to test adjuvant Smac-mimetics response in 2-D cell models and 3D tumouroids.

Human OSCC cell lines with known CASP8 allele status were tested by proliferation assay comparing response to traditional chemotherapy, Smac-mimetics (birinapant and compound A) and in combination. Using the genome editing tool CRISPR/CAS9, CASP8 was removed for testing in a parallel manner. A 4-NQO oral carcinogenesis model was established in wild-type and Casp8 genetically diverse C57BL/6 mice. Fresh tumour tissue was harvested and matched to confirm OSCC histopathology, for the development of tumour organoid. Human OSCC cell lines with functional CASP8 were significantly more sensitive to Smac-mimetic therapies (birinapant and compound A) in combination with chemotherapy. A workflow for murine organoid harvesting, culture and expansion was tested and optimised. Both murine wild-type and modified Casp8 sourced organoids were successfully expanded.

We have also established methods to grow and establish OSCC tumour organiods from both wt and Casp8- modified 4-NQO treated mice. Human cell lines with modified CASP8 are anticipated to respond further to adjuvant Smac-mimetics. Step follow include mutation-associated response to high throughput screening methods using a low-viscosity matrix suspension method to test sensitivity to chemotherapy, Smac-mimeticsor irradiation in single, double and triple combinations.

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Characterisation of Novel Cytokine Interferon Epsilon in the Murine Peritoneal Cavity

Jasmine Chuah[1], Nicole Campbell[1], Paul Hertzog[1]

1. Hudson Institute of Medical Research, Oakleigh South, VIC, Australia

The novel type I interferon, interferon epsilon (IFNε) possesses a unique manner of spatiotemporal expression and regulation. Largely studied in the female reproductive tract (FRT), it has been shown to maintain homeostatic conditions and mediate protective immunity against common FRT pathogens. Many FRT pathologies including cancer, endometriosis, and infection can extend detrimentally into the peritoneal cavity, and are characterised by dysregulated immune responses. Therefore, it is hypothesised that IFNε may have protective immunoregulatory effects that influence the phenotype, activity and composition of peritoneal immune cells under both steady state and inflammatory conditions.

Previous research utilizing syngeneic mouse models of high grade serous ovarian cancer (HGSOC) has indicated that IFNε administration alleviates tumour burden and ascites development, accompanied by changes in the activity of peritoneal immune cell populations. Following these observations, the peritoneal immune cells present in both male and female wild-type (WT) and *Ifnε-/-* mice in steady-state conditions and during inflammation were investigated via immunophenotyping. The results indicated that in homeostatic states, endogenous IFNε maintains a basal immunity in the peritoneal cavity by regulating the peritoneal myeloid cells through their recruitment into the peritoneal cavity, differentiation and proliferation, as well as through the modulation of the activation states in peritoneal lymphoid compartment.

Intraperitoneal administration of exogenous IFNε as a pretreatment prior to the induction of peritonitis in WT mice showed that IFNε may act similarly to IFNβ in the peritoneal cavity during infection, potentially by regulating the migration and activation of the peritoneal immune cells in response to infection. Collectively, these data underline the role of IFNε in maintaining the basal immunity of the murine peritoneal cavity, which may prompt the peritoneal immune response during bacteria-induced inflammation. Thus, IFNε may have potential as a future immunotherapy for peritoneal pathologies that induce aberrant activity in the peritoneal immune cells, primarily peritonitis and HGSOC metastases.

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Epigenetic-targeted CRISPR screens identify PRMT1 as a suppressor of MHC-I and anti-tumour immunity

Tirta (Mario) Djajawi[1], Lizzy Pijpers[2], Ricky Johnstone[2], Conor Kearney[1]

1. Olivia Newton John Cancer Research Institute, Heidelberg, VIC, Australia

2. Department of Oncology, Sir Peter MacCallum, Parkville, Victoria, Australia

Cancer immunotherapies have demonstrated remarkable success; however, the majority of patients fail to respond or develop resistance, often through disruption of pathways that promote neo-antigen presentation on MHC I molecules. Here, we conducted a series of epigenetic gene-targeted CRISPR/Cas9 screens to identify epigenomic factors that limit CD8+ T cell cell-mediated anti-tumour immunity. We identified that the arginine methyltransferase PRMT1 suppressed interferon-gamma (IFNγ) induced MHC-I expression, thus dampening CD8+ T cell-mediated killing. Indeed, CRISPR/Cas9 induced knockout of *PRMT1* or pharmacological targeting of type I PRMT with the clinical inhibitor GSK3368715 enhanced IFNγ-induced MHC-I expression in human and mouse tumour cells through enhanced STAT1 expression and activation, while re-introduction of PRMT1 in PRMT1 deficient cells reversed this effect. Furthermore, TCGA analysis revealed that PRMT1 expression in human melanoma is inversely correlated with expression of HLA molecules, infiltration of CD8+ T cells and overall survival. Taken together, we identify PRMT1 as a negative regulator of anti-tumour immunity, unveiling clinical PRMT1 inhibitors as novel immunotherapeutic agents or as adjuncts to existing immunotherapies where sub-optimal MHC-I expression may reduce therapeutic efficacy.

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Expanding neutralising antibody breadth with a polyvalent SARS-CoV-2 mRNA vaccine expressing three linked-RBD domains from different variants

Yadana Zaw[1], Marvin Holz[1], Georgia Deliyannis[1], Chinn Yi Wong[1], Julie McAuley[1], Sarah Collins[1], Samantha Grimley[1], Leonard Lim[2], Colin Pouton[2], Damian Purcell[1]

1. Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC, Australia

2. Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, Victoria, Australia

The current SARS-CoV-2 vaccines have successfully prevented severe disease, however the immune-protection they induce has not provided sufficient durability or breadth to prevent transmission of highly diverged new variants. Consequently, booster-vaccine updates targeting new spike variants aim for longer-lasting immunity. Our study introduces an mRNA vaccine candidate, termed 3RBD, which focuses immunity on the receptor binding domain (RBD) of spike, the primary target of neutralising antibodies, by presenting a polyvalent antigen linking three RBDs via a flexible linker. This design allows each RBD segment to maintain its unique strain-specific antigenicity, fostering antibody maturation toward common epitopes presented across each segment of the 3RBD polyvalent antigen.

Our proof-of-concept 3RBD antigen, incorporating Beta, Delta, and BA.1 variant RBDs with a C-terminal transmembrane domain, was expressed using optimised *in vitro* transcribed mRNA and delivered via lipid nanoparticle (LNP) formulation. C7BL/6 mice were immunised intramuscularly with three doses of 1 µg, 3 µg, or 10 µg, and subsequently challenged with a mouse-adapted strain of SARS-CoV-2 not represented in the vaccine.

The optimal antibody response was obtained after three doses of 10 µg of the vaccine, as assessed by ELISA binding and neutralising antibody assays. Mice elicited broad polyspecific RBD-antibodies against each variant expressed in the 3RBD antigen, indicating that the linkers do not interfere with antibody binding to individual RBDs. Mouse sera equally reacted with divergent strains, including a mouse-infectious ancestral strain not present in the vaccine. Importantly, vaccinated mice were protected from infection upon challenge with the non-homologous mouse-infectious strain.

Our study highlights that an mRNA vaccine expressing a membrane-tethered 3RBD antigen can induce robust polyspecific antibody responses against multiple variants of SARS-CoV-2, including those absent in the vaccine. Thus, the 3RBD mRNA vaccine emerges as a promising candidate for broad-spectrum protection against evolving SARS-CoV-2 variants, warranting further clinical investigation.

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Targeting Clec9A on type-I conventional dendritic cells to induce broad and durable systemic and mucosal immune responses against sarbecoviruses.

Nicholas YZ Cheang[1, 2], Peck Szee Tan[3], Kiren Purushotorma[2, 4], Wee Chee Yap[5], Kirsteen McInnes Tullett[3], Aileen Ying-Yan Yeoh[5], Xinlei Qian[2, 4], Yee Joo Tan[1], Paul A Macary[2, 4], Chee Wah Tan[5], Mireille H Lahoud[3], Sylvie Alonso[1, 2]

1. Infectious Diseases Translational Research Programme, Department of Microbiology and Immunology, National University of Singapore, Singapore

2. Life Sciences Institute, Immunology Programme, National University of Singapore, Singapore

3. Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Australia

4. Department of Microbiology and Immunology, Immunology Translational Research Programme, National University of Singapore, Singapore

5. Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

COVID-19 mRNA vaccines face limitations in their immune response generated, which include waning immunity, poor induction of mucosal immunity, and limited breadth across the different sarbecovirus clades. We have been developing a dendritic cell (DC) targeting vaccine delivery platform that consists of an anti-Clec9A monoclonal antibody and the vaccine antigen candidate fused at the heavy chains. Here, we engineered two Clec9A-RBD constructs harboring SARS-CoV-2 or SARS-CoV-1 spike receptor binding domain (RBD), and we evaluated their immunogenicity when administered either systemically or mucosally to mRNA (Pfizer BioNTech)-vaccinated mice. Boosting with either Clec9A-RBD-CoV2 or Clec9A-RBD-CoV1 induced strong systemic neutralizing antibody responses against pre-Omicron and Omicron SARS-CoV-2, and clade 1b animal sarbecoviruses that were non-inferior to boosting with Pfizer BioNTech Omicron BA.4/5 bivalent mRNA vaccine. Furthermore, boosting with Clec9A-RBD-CoV1 induced cross-clade neutralizing antibodies against SARS-CoV-1 and clade 1a sarbecoviruses. Uniquely, mucosal delivery of the Clec9A-RBD constructs resulted in RBD-specific IgA and the abovementioned neutralizing antibody responses within lung tissues, which were undetectable in mRNA-boosted mice. While waning occurred following mRNA boosting, the systemic and mucosal antibody responses remained highly persistent in Clec9A-RBD-CoV2 boosted mice. On the contrary, the antibody responses induced upon Clec9A-RBD-CoV1 boosting waned significantly over time. These observations correlated well with the magnitude of T cell recall responses. To overcome the limited T cell activation observed with Clec9A-RBD-CoV1, both constructs were combined into a single bivalent formulation, which demonstrated potent, sustained, and cross-clade systemic and mucosal neutralizing antibody and T cell responses. Taken together, Clec9A-RBD immunization has the potential to trigger robust, broad, and sustained systemic and mucosal immune responses against sarbecoviruses through the targeting of cDC1 residing in systemic and mucosal tissues. This work supports Clec9A-RBD as a promising booster shot to enhance the breadth, durability, and mucosal immunity of COVID-19 mRNA vaccines.

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Micro-projection array patch delivery of live-attenuated Measles and Rubella vaccine in a Phase I Clinical Trial

Imogen Bermingham[1], Angus Forster[1]

1. Vaxxas Pty Ltd, Hamilton, QLD, Australia

Microarray patches (MAPs) offer the possibility of improved vaccine thermostability and dose-sparing potential as well as the potential to be safer, more acceptable, easier to use and more cost-effective for the administration of vaccines than injection by needle and syringe. Here, we report a Phase I trial using the Vaxxas high-density MAP (HD-MAP) to deliver a live-attenuated Measles (Edmonston-Zagreb) and Rubella (RA27/3) vaccine into the upper arm of healthy young adults (18 to 50). The primary objective was to evaluate the safety and tolerability of a single high- and low-dose of Measles and Rubella vaccine by the high-density microarray patch (HD-MAP), compared to an uncoated (placebo) HD-MAP, and subcutaneous (SC) administration of a standard adult human dose of MR-Vac (Serum Institute of India). Secondary objectives were to evaluate the immune response by assessing IgG and neutralisation antibody titres and to assess the HD-MAP skin penetration by scanning electron microscopy (SEM). Vaccination was well tolerated and no serious adverse advents, or severe adverse events related to study treatment were reported. Local reactions at the application site were observed, such as erythema. Immune response was evident in all non-placebo groups at Day 28 and 56 and comparable between HD-MAP and MR-Vac groups, with pre-vaccination titre predictive of the immune response. Live-attenuated MR vaccine on the HD-MAPs demonstrated excellent thermostability at 2-8 °C over 24 months, including controlled temperature excursions of 3 days at 40 °C at the start and end of shelf life. Overall, this trial demonstrated that delivery of live-attenuated MR by a HD-MAP is safe, tolerable and induces an immune response comparable to SC injection in healthy adults1.

1. Baker B, Bermingham IM, Leelasena I, Hickling J, Young PR, Muller DA, Forster AH. Safety, Tolerability, and Immunogenicity of Measles and Rubella Vaccine Delivered with a High-Density Microarray Patch: Results from a Randomized, Partially Double-Blinded, Placebo-Controlled Phase I Clinical Trial. Vaccines (Basel). 2023 Nov 17;11(11):1725. doi: 10.3390/vaccines11111725. PMID: 38006057; PMCID: PMC10675090.

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Identification of regulators of the immune microenvironment in non-small cell lung cancer

Marina Leiwe[2, 1], Claire Marceaux[2, 1], Daniel Batey[2], Velimir Gayevskiy[2], Tracy Leong[3], Marie-Liesse Asselin-Labat[2, 1]

1. Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia

2. Walter and Eliza Hall Institute of Medical Research, Parkville, VICTORIA, Australia

3. Olivia Newton John Cancer Research Institute and Austin Health, Heidelberg, Victoria

Although immunotherapy has shown major success in the clinic, most lung cancer patients fail to respond long-term to the current treatment options. Immune cell composition and spatial organisation of the tumour microenvironment (TME) determine patient response to immunotherapy. However, how cancer cells modulate cellular arrangements within the TME remains poorly understood. Here, we combine a novel *in vivo* spatial CRISPR screen with spatial omic technologies to identify genes regulating the TME in non-small cell lung cancer and decipher mechanisms of resistance to immunotherapy.

We curated a candidate gene library based on differentially expressed genes in two RNA-seq datasets of lung adenocarcinoma patient biopsies: 1) comparing tumours sensitive or resistant to anti-PD-L1 therapy and 2) comparing tumours with high or low immune infiltration. Correlating these two transcriptomic datasets identified genes associated with both, response to immune checkpoint blockade and immune infiltration, suggesting these gene products might be involved in immunotherapy resistance via modulation of the tumour immune microenvironment.

Using a combinatorial protein barcoding system, called Perturb-map, this project aims to functionally validate these potential TME regulators and evaluate their function in an *in vivo* pooled CRISPR screen. Perturb-map uses protein-barcoded sgRNA vectors that can be resolved by imaging, hence allowing the identification of extracellular gene functions. Contrary to conventional *in vivo* CRISPR screens that can only assess how depletion of individual target genes impact tumour cell fitness, the protein barcodes add spatial resolution and additionally enable the assessment of potential effects on the tumour architecture. Finally, combining Perturb-map with multiplex-ion beam imaging and spatial transcriptomics enables us to investigate how individual gene perturbations influence the TME and immune cell composition, providing detailed mechanistic insights into these important determinants of immunotherapy efficacy.

Defining the spatial relationships within the tumour microenvironment is necessary to fully understand cell-cell interactions and mechanisms controlling immune infiltration. Our findings may help identifying patients responsive to immunotherapy, enabling more targeted and personalised therapy. Further, regulators of the TME may provide potential targets for future cancer treatments. Genes identified in our study may suggest novel drug targets and improve patient outcome in combination with conventional immunotherapies.

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Vaccines in The New Era: What Have We Learnt in The Last 30 Years?

Vasso Apostolopoulos[1]

1. Institute for Health and Sport, Immunology and Translational Research Group, Melbourne, Vic, Australia

The basis of T cell stimulation is via the specific interaction of an immunogenic peptide in complex with MHC by a T cell receptor. Other co-stimulatory molecules such as CD80, CD86 on antigen presenting cells, are recognised by T cells via CD28 and CTLA-4 which results in T cell activation. In recent years the identification of checkpoint markers such as PD-L1, PDL2 on antigen presenting cells, epithelial cells etc and their interaction with PD-1 on activated T cells results in apoptosis of T cells and immune escape mechanisms, in the case of cancer. The role of checkpoint markers in a range of disorders including autoimmune disorders, inflammatory disorders and cancer are being studied with a plethora of information being published in the last 5 years. In addition, peptide alterations of T cell epitopes with 1-2 amino acid mutations can have drastic effects on the outcome of this recognition. Such peptides are termed, altered peptide ligands that can act as modulators of immune responses as they can down-regulate or upregulate responses. Over the last 30 years, there has also been an emphasis on carriers, adjuvants, and delivery systems to modulate immunity *in vitro*, *in vivo* in animal models of disease and in human clinical trials. With this information we have develop several immune modulators / therapeutics / vaccines for cancer, with phase I, II and pilot phase III human clinical trials; one having 25 years clinical follow-up; for MS currently in phase I human clinical trial; and several immune modulators for type-1 diabetes and methamphetamine (ice) drug addiction; all of which will be discussed in the presentation.

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Investigating role of non-VAR2CSA specific antibodies in protection from placental malaria

Yvonne Dube[1], Wina Hasang[1], Mwayiwawo Madanitsa[2, 3], Victor Mwapasa[4], Jonathan Kurtis[5], Morten Nielsen[6], Stephen Scally[7], Herbert Opi[8], Kamija Phiri[9], Feiko O ter Kuile[3], Elizabeth H. Aitken[10, 1], Stephen J. Rogerson[1, 11]

1. Department of Infectious Diseases, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

2. Department of Clinical Sciences, Academy of Medical Sciences, Malawi University of Science and Technology, Thyolo, Malawi

3. Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

4. Department of Epidemiology and Biostatistics, School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi

5. Department of Pathology and Laboratory Medicine, Brown University Medical School, Providence, RI, USA

6. Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

7. Walter and Eliza Hall Instituite of Medical Research, The University of Melbourne, Melbourne, Victoria, Australia

8. Burnet Institute, Melbourne, Victoria, Australia

9. Training and Research Unit of Excellence, Blantyre, Malawi

10. Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

11. Department of Medicine (RMH), The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

Placental malaria (PM) is caused by *Plasmodium falciparum*-infected erythrocytes (IEs) sequestration in placenta via chondroitin sulphate A [1] , and antibodies to VAR2CSA have been associated with protection from PM and adverse pregnancy outcomes [2,4]. There are no VAR2CSA specific vaccines available, whereas vaccines based on other parasite antigens have progressed to clinical trials [3,5]. Their role in protection against PM is unclear.

We investigated whether antibodies to antigens other than VAR2CSA contributed to protection from PM. We also evaluated opsonic phagocytosis of whole IEs and merozoites by THP-1 cells and neutrophils respectively. IgG and adhesion-blocking IgG to placental binding IE were also assessed.

Plasma collected mid-pregnancy from infected pregnant women from Malawi, who had (n=75) or did not have evidence of PM (n=88) at delivery, was used to measure antigen-specific IgG, IgG1-4, IgA1, IgA2, IgM and interactions with Fc features and C1q to thirteen *P. falciparum* recombinant antigens including 8 merozoite, 2 schizont stage; 2 pregnancy specific (VAR2CSA) and 1 sporozoite antigen. IgG binding to merozoites and to placental binding IE and phagocytosis of merozoites and placental binding IE by THP1 cells and neutrophils was also measured. Levels of adhesion-blocking IgG to placental binding IE were also measured. Welch's t test was performed, and a volcano plot generated to visualize distribution of differences in antibody features between the two groups. A correlation network was generated to visualize the data.

Twenty-seven antibody features were higher in women with PM ($P \leq 0.05$), with nineteen being antibody features to merozoites (IgG to MSP3, PfRh5; IgG1to AMA1, EBA175, MSP1-p19, MSP2, PfRh2a1; IgG3 to MSP3; FcγRIIA to AMA1; FcγRIIIA to AMA1; FcγRIIB to AMA1, MSP1-p19, MSP3, FcγRIIIB to AMA1, MSP3; IgA2 to AMA1, MSP1-p19; C1q to AMA1, PfRh2a1. Five antibody features were higher in pregnant women with no placental malaria ($P \leq 0.05$), including IgA1 antibodies to MSP2, MSP9, PfRh2a1, PfRh5 and complement binding antibodies to PfRh5, as were antibodies to VAR2CSA and adhesion-blocking antibodies on IEs. Phagocytosis of opsonised IEs by THP-1 cells or of merozoites by neutrophils did not significantly differ between the groups.

Little evidence regarding the role of antibodies towards non-VAR2CSA proteins in protection from PM was observed. The recognition and binding inhibition assays indicate that the antibodies to VAR2CSA in the no PM group are protective. Univariate analysis suggests that antibodies to non-VAR2CSA proteins may be markers of exposure to PM rather than markers of protection.

1. Fried, M. and Duffy, P.E., 1996. Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. Science, 272(5267), pp.1502-1504.

2. Lloyd, Y.M., Fang, R., Bobbili, N., Vanda, K., Ngati, E., Sanchez-Quintero, M.J., Salanti, A., Chen, J.J., Leke, R.G. and Taylor, D.W., 2018. Association of antibodies to VAR2CSA and merozoite antigens with pregnancy outcomes in women living in Yaoundé, Cameroon. Infection and immunity, 86(9), pp.e00166- 18
3. Minassian, A.M., Silk, S.E., Barrett, J.R., Nielsen, C.M., Miura, K., Diouf, A., Loos, C., Fallon, J.K., Michell, A.R., White, M.T. and Edwards, N.J., 2021. Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. Med, 2(6), pp.701-719.
4. Ndam, N.T., Denoeud-Ndam, L., Doritchamou, J., Viwami, F., Salanti, A., Nielsen, M.A., Fievet, N., Massougbodji, A., Luty, A.J. and Deloron, P., 2015. Protective antibodies against placental malaria and poor outcomes during pregnancy, Benin. Emerging infectious diseases, 21(5), p.813.
5. Stoute, J.A., Slaoui, M., Heppner, D.G., Momin, P., Kester, K.E., Desmons, P., Wellde, B.T., Garçon, N., Krzych, U., Marchand, M. and Ballou, W.R., 1997. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparum malaria. New England Journal of Medicine, 336(2), pp.86-91.

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Association of *Plasmodium falciparum* specific afucosylated IgG with placental malaria protection

HongHua Ding[1], Wina Hasang[1], Bruce Wines[2], Mwayiwawo Madanitsa[3, 4], Victor Mwapasa[5], Kamija Phiri[6], Feiko O ter Kuile[4], Elizabeth Aitken[1, 7], Stephen Rogerson[1, 8]

1. Department of Infectious Diseases, The University of Melbourne, The Peter Doherty Institue, Melbourne, VIC, Australia

2. Immune Therapies Group, Burnet Institute, Melbourne, VIC

3. Department of Clinical Sciences, Academy of Medical Sciences, Malawi University of Science and Technology, Thyolo, Malawi

4. Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

5. Department of Epidemiology and Biostatistics, School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi

6. Training and Research Unit of Excellence, Blantyre, Malawi

7. Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute, Melbourne, Australia

8. Department of Medicine (RMH), The University of Melbourne, Melbourne, Australia

Malaria is a life-threatening disease that causes over 600,000 deaths annually, with the most severe form caused by *Plasmodium falciparum*. In addition to neutralizing activities, naturally acquired immunoglobulin G (IgG) antibodies serve a critical role in the activation of immune-protective functions via the Fc-gamma receptors (FcγR). The recent focus on IgG fucosylation highlighted the heightened binding affinity of afucosylated IgG to FcγRIIIa compared to the normally fucosylated IgG, resulting in enhanced antibody dependent cellular cytotoxicity (ADCC). This is due to the absence of fucose on the highly conserved N-linked glycan located in the Fc domain of IgG. In this study, we utilized the Fucose-sensitive Enzyme-linked immunosorbent assay (ELISA) for Antigen-Specific IgG (FEASI), an immunoassay that is capable of quantifying Fc fucosylation of antigen-specific IgG antibodies. FEASI consists of two ELISA assays; the first is to measure the levels of antigen-specific IgG independent of fucosylation using total IgG, while the second gives FcγRIIIa specific binding readouts which is highly sensitive to IgG fucosylation. The output of both ELISAs is converted into a ratio that represents the level of afucosylated IgG in a given sample. Here we examined the plasma from N=163 *P. falciparum* infected pregnant Malawian women with or without evidence of placental malaria at delivery using FEASI. Our results showed significantly higher levels of antigen-specific afucosylated IgG in women without evidence of placental malaria at delivery ($p < 0.001$). This finding suggests that afucosylated IgG levels could be a marker for protection and further experiments will explore the association between the levels of afucosylated IgG with neutrophil phagocytosis and NK cell activation against malaria infected erythrocytes, and validate these observations in a further sample set. These results have important implications in the understanding of naturally acquired protection against malaria in pregnant women.

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Role of IgG antibodies in protection from placental malaria birth outcomes

AKACHUKWU ONWUKA[1], Elizabeth Aitken[2, 1], Wina Hasang[1], Mwayiwawo Madanitsa[3, 4], Victor Mwapasa[5], Kamija Phiri[6], Feiko O ter Kuile[4], Stephen Rogerson[1, 7]

1. Department of Infectious Diseases, The Peter Doherty Institute of Infection and Immunity , University of Melbourne, Melbourne, Victoria, Australia

2. Department of Microbiology and Immunology, UNIVERSITY OF MELBOURNE, melbourne, Victoria, Australia

3. Department of Clinical Sciences, Academy of Medical Sciences, Malawi University of Science and Technology,, Thyolo, Malawi

4. Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

5. Department of Epidemiology and Biostatistics, School of Global and Public Health, Kamuzu, Blantyre, Malawi

6. Training and Research Unit of Excellence, Blantyre, Malawi

7. Department of Medicine (RMH), The Peter Doherty Institute of Infection and Immunity, University of Melbourne, Melbourne, Melbourne, Victoria, Australia

Placental malaria (PM) is a public health issue linked to poor pregnancy outcomes. Antibodies against VAR2CSA, a variant surface protein found on infected erythrocytes, protect against *Plasmodium falciparum* infections in pregnant women. This study aims to associate VAR2CSA antibody levels with poor pregnancy outcomes: low birthweight (LBW), preterm delivery, small for gestational age (SGA), and maternal anaemia. Pregnant Malawian women (n=466) were recruited at mid-pregnancy (16-28

gestation weeks). The pregnant women were subdivided into women infected at enrolment and infected later in pregnancy. Total IgG levels to recombinant VAR2CSA (DBL1X-ID2a) domains were measured at enrolment and delivery via indirect ELISA. The IgG antibody levels were log-transformed, and the arbitrary results (AU) were represented as mean + SEM. There were higher log IgG antibody levels in multigravid women who were infected at enrolment (3.50 ± 0.10 AU) than primigravid women (3.38 ±0.10 AU). Higher antibody levels were observed at enrolment (3.45 ±1.09 AU) compared to delivery (2.89 ± 0.97 AU). However, log IgG antibody levels measured at enrolment were not associated with reduced LBW (aOR=1.16, 95%CI 0.81-1.67, *p*= 0.4), preterm delivery (aOR=1.24, 95%CI 0.81-1.88, *p*=0.32), SGA (aOR=0.96, 95%CI 0.74-1.25, *p*=0.75) and maternal anaemia (aOR=1.08, 95%CI 0.84-1.40, *p*=0.55). This shows no significant associations between the antibody levels at enrolment with protection from poor pregnancy outcomes. The antibody responses to VAR2CSA are likely markers of placental malaria rather than protection from the infection.

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Fc receptor binding of naturally acquired antibodies to placental binding *Plasmodium falciparum* infected erythrocytes

Elizabeth Aitken[1], Natasha Sharma[1], Wina Hasang[1], Kwok Zi Rou[1], P Mark Hogarth[2], Bruce Wines[2], Maria Ome-Kaius[3], Mwayiwawo Mandanitsa[4], Victor Mwapasa[5], Kamija Phiri[5], Ken Maleta[5], Feiko ter Kuile[6], Holger Unger[7], Stephen Rogerson[1]

1. Department of Infectious Diseases, Peter Doherty Institute, University of Melbourne, Melbourne, Victoria, Asutralia

2. Burnet Institute, Melbourne, Victoria, Australia

3. Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

4. College of Medicine University of Malwi, Malawi

5. Kamuzu University of Health Sciences, Malawi

6. Liverpool School of Tropical Medicine, Liverpool

7. Menzies School of Health Research, Darwin

Malaria causes significant child and maternal morbidity and mortality. The identification of antibody responses which protect against malaria can assist with vaccine and therapeutic monoclonal antibody design, and antibodies that facilitate uptake or destruction of infected erythrocytes (IEs) by leukocytes or complement-mediated lysis may play important roles. We developed novel assays to measure binding of leukocyte receptors FcγRI, FcγRIIA and FcγRIIIA on IEs after opsonization with plasma from pregnant Papua New Guinean (n=77) and Malawian women (n=40). FcγRI, FcγRIIA and FcγRIIIA binding antibodies to placental binding IEs were acquired in a gravidity-dependent manner (P≤0.063). FcγRI binding antibodies were associated with protection from placental malaria (P=0.02). FcγRI, IIA and IIIA binding antibody on the IE were weakly to moderately correlated with IgG levels to the IE, and only FcγRI binding antibody on the IE was correlated with FcγRI, IIA or IIIA binding antibodies on recombinant placental binding PfEMP1. Measuring functional antibodies to IEs provide valuable information on antibody functions and quality in malaria as clinically relevant correlates of protective immunity.

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Searching for broadly reactive monoclonal antibodies to placental malaria antigen, VAR2CSA

Vivin R Kokuhennadige[1], Christopher A Gonelli[2], Wina Hasang[3], Holger W Unger[4, 5, 6], Paula Tesine[7], Alice Mengi[7], Benishar Kombut[7], Amy W Chung[2], Adam K Wheatley[2], Elizabeth H Aitken[2, 3], Stephen J Rogerson[1, 3]

1. Department of Medicine (RMH), The Peter Doherty Institute of Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

2. Department of Microbiology and Immunology, The Peter Doherty Institute of Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

3. Department of Infectious Diseases, The Peter Doherty Institute of Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

4. Department of Obstetrics and Gynaecology, Royal Darwin Hospital, Darwin, Nothern Territory, Australia

5. Menzies School of Health Research, Charles Darwin University, Darwin, Nothern Territory, Australia

6. Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

7. Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

Pregnant mothers are susceptible to malaria during pregnancy despite pre-existing immunity. Malaria during pregnancy is responsible for nearly 200,000 infant and 10,000 maternal deaths every year. Placental malaria, a mechanism by which malaria in pregnancy causes adverse perinatal outcomes, is characterised by the sequestration of *Plasmodium falciparum*-infected erythrocytes in the placenta via chondroitin sulphate A (CSA) cellular receptor. A *P. falciparum* erythrocyte membrane protein 1 family member, VAR2CSA, is the primary antigen responsible for the sequestration of parasites via CSA. Antibodies to VAR2CSA are acquired over successive pregnancies and have been associated with protection against placental malaria and adverse pregnancy outcomes. Current placental malaria vaccines that target VAR2CSA, PRIMVAC and PAMVAC, can only generate neutralising antibodies to homologous parasite strains, rendering them ineffective against heterologous strains in the field. Recently, there has been growing interest in the use of monoclonal antibodies (mAbs) both as therapeutics and as tools for characterisation of antibody-antigen interactions. To date, no broadly reactive antibodies have been identified against VAR2CSA that can neutralise parasite adherence and induce parasite clearance via phagocytosis by innate immune cells.

In this study, we hypothesized that monoclonal antibodies from multigravida women are cross-reactive across multiple *P. falciparum* strains and functional in preventing CSA adhesion of infected erythrocytes and inducing antibody-mediated phagocytosis by innate immune cells. So far, 10 multigravida women with high Ab titers for full-length VAR2CSA have been

identified in malaria-endemic Madang, Papua New Guinea. VAR2CSA-specific memory B cells were isolated, and variable regions of the corresponding antibodies were used for generating IgG monoclonal antibodies. Developed monoclonals will be characterised for reactivity and functionality across 7 parasite strains expressing heterologous forms of VAR2CSA.

Assays exploring monoclonal recognition of native VAR2CSA from heterologous strains and opsonic phagocytosis by THP-1 cell line and isolated neutrophils will be measured using flow cytometry. Furthermore, antibody-mediated inhibition of opsonised infected erythrocyte binding to CSA will be measured via binding inhibition assay.

Identification of broadly reactive monoclonal functional antibodies with the ability to induce parasite clearance via opsonic phagocytosis of innate immune cells and neutralise parasite sequestration by inhibiting CSA adhesion could inform future vaccine design and therapeutics against placental malaria.

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Recombinant Listeria Monocytogenes Vaccine Anti-tumor Response is Independent of the Host Inflammatory Cell Death Machinery

Abolaji S Olagunju[1, 2], Andrew V Sardinha[1], Aamir Rana[3], Gustavo P Amarante-Mendes[1, 2]

1. Institute of Biomedical Sciences, Sao Paulo, CIDADE UNVERSITARIA, Brazil

2. Instituto de Investigação em Imunologia, Instituto Nacional de Ciência e Tecnologia (INCT), São Paulo, SP, Brazil

3. Department of Immuno-Oncology, Beckman Research Institute, City of Hope, California, USA

Recombinant *Listeria monocytogenes* (LM) emerges as a promising candidate for cancer vaccination, owing to its unique ability to replicate within the cytoplasm of host cells and its inherent accessibility to genetic manipulation, facilitating the expression of desired proteins. Vaccination strategies employing live LM have demonstrated significant efficacy in eliciting robust CD8 T cell responses and fostering substantial immune priming. Leveraging LM's capacity to provoke potent and targeted immune reactions against cancer, alongside the activation of *in vivo* antigen-specific CD8+ T cells, holds considerable promise. Notably, LM has been observed to activate inflammatory and immunogenic forms of cell death, including necroptosis and pyroptosis, thereby modulating immunological responses in a favorable manner. Importantly, the impact of host deficiencies in inflammatory cell death pathways on the effectiveness of LM-mediated anti-tumor immune responses remains unclear. In this study, we hypothesized that deficiencies in regulatory or effector molecules associated with pyroptosis or necroptosis would hinder the host's response to LM and consequently attenuate the stimulation of CD8+ T cell-mediated immunity. To test this hypothesis, we vaccinated intravenously wild-type (WT) C57Bl/6 mice alongside those lacking caspase-1/11, GSDMD, RIPK3, or MLKL with recombinant *L. monocytogenes* carrying the ovalbumin gene (LM.OVA). Subsequently, we conducted an *in vivo* cytotoxicity assay to assess the efficacy of OVA-specific CD8+ T lymphocytes in eliminating target cells. Additionally, we monitored the *in vivo* growth of B16 and B16.OVA melanoma cell lines in both control and vaccinated mice. Our findings indicate that although Caspase-1/11 and GSDMD participate at the control of LM-OVA infection, the deficiency of each of these molecules as well as RIPK3 or MLKL did not impair the antigen-specific anti-tumor response elicited by LM-OVA.

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Next generation proteomic profiling of a pan-cancer cohort for the development of screening tools for cancer

Renu Balyan[1], Marijana Rucevic[2], María Bueno Álvez[3], Ryan Lamers[2], Ola Caster[2], Hilda Andersson[2], Fredrik Edfors[3], Linn Fagerberg[2], Mathias Uhlen[3]

1. Olink Proteomics, Singapore

2. Olink Proteomics, Uppsala, Sweden

3. Science for Life Laboratory, Department of Protein Science, KTH Royal Institute of Technology, Stockholm, Sweden

A comprehensive characterization of blood proteome profiles in cancer patients could provide a better understanding of disease biology, enabling earlier diagnosis, risk stratification and better monitoring of the different cancer subtypes.

Here, we describe the use of next generation protein profiling to explore the proteome signature in blood across patients representing 12 major cancer types. Plasma profiles of 1463 proteins from more than 1400 cancer patients of the Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN) biobank was measured at the time of diagnosis and before treatment. Using machine learning methods, the differentially expressed proteins identified were used to derive models to discriminate among different cancer types. A panel of 83 proteins was found to identify the correct cancer types with AUCs ranging between 0.93 and 1. Preliminary analysis indicated that the protein panel was able to discriminate all cancers from healthy controls and showed promising performance in both staging some of the cancer types, and in detecting very early-stage cancer. The data from this study was made available via the Disease Blood Atlas, an open-access resource.

The results were used as a foundation to establish the Olink Insight platform, an open-access digital data resource to accelerate adoption of proteomics in the research community. In Olink Insight, we are creating a collection of proteomic profiles for important diseases, beginning with cancer. Olink Insight and the Human Disease Blood Atlas represent a significant step towards uncovering human disease proteome and will be a valuable resource for researchers in many areas of medicine and biology.

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Engineering of an intrinsically stable SARS CoV-2 soluble spike trimer.

Christine Langer[1], Irene Boo[1], Tasnim Zakir[1], Heidi E Drummer[1, 2, 3], Andy Poumbourios[1, 2]

1. Burnet Institute, Melbourne, VIC, Australia

2. Department of Microbiology, Monash University, Clayton, Vic, Australia

3. Department of Microbiology at The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Vic, Australia

SARS CoV-2 vaccines, which deliver full-length S either as mRNA or as recombinant protein have saved over 20 million lives since their roll out. However, the periodic emergence of global variants has necessitated the updating of vaccines to maintain effectiveness against the new viral strains. The S protein-based vaccine that is currently licensed for human use is produced by a complex process that reconstitutes S in an adjuvanted nanoparticle. The advent of a simple method for producing stable soluble S trimers that can be stored and distributed without the need of an ultracold chain would fill a significant gap in the current arsenal of COVID-19 vaccines. The aim of this study was to produce an intrinsically stable soluble S trimer with improved antigenic characteristics. This was achieved by first modifying the stem/HR2 region of a soluble, trimeric prefusion-stabilized omicron BA.4 glycoprotein containing the core-filling mutations, A1016V/A1020I, to increase the yield of trimer. The thermostability of the stem-modified glycoprotein was then increased by the introduction of artificial intermolecular disulfide bonds between subdomain 1 and heptad repeat region 1 of the spike. Neutralizing antibody epitopes involving the receptor-binding motif (RBM) were occluded to varying extents by the engineered disulfides, whereas conserved NAb epitopes located on the flanks of the RBD remained exposed, and a conserved neutralization epitope within the stem was stabilized by the mutations. Similar results were obtained with the omicron BA.2.86 spike. The intrinsically stable soluble trimeric S glycoproteins represent novel vaccine candidates in which variable epitopes overlapping the RBM can be occluded to potentially redirect antibody responses to more conserved regions of S.

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Single-cell sequencing combined with artificial intelligence assists in COVID-19 vaccine antigen design and preclinical efficacy evaluation

Zhongyi Zhu[1], Lei Zhang[1], Shuangbao Li[1], Yang Gao[1], Yuwei Wang[1], Zhonglin Chen[1], Siyu Wu[1], Yonghui Zhang[1], Mengyuan Zhang[1], Zhihao Xie[1], Weijun Chen[1], Fuxing Zeng[1], Jinmin Ma[1]

1. BGI Huo-Yan Engineering Technology, BGI-Shenzhen, Shenzhen, China

Variability in antibody responses among individuals following vaccination is a universal phenomenon. So a well designed vaccine whose antigen should elicit a strong protective immune response in variety of individuals. Single-cell transcriptomics offers a potential avenue to understand the underlying mechanisms of these variations and improve our ability to evaluate and predict vaccine effectiveness that facilitates vaccine antigen design. Artificial intelligence tools make antigenic protein structure design more efficient and convenient than ever before. Based on the individual diversity of immune response characteristics revealed by single-cell sequencing results, we designed several antigenic variants of SARS-CoV-2, and ultimately verified that several mutant designs were able to efficiently provoke highly effective neutralizing antibodies to more than 11 Omicron variants, includes mutants that appear after the antigen has been designed. What's more, the antigens we designed stimulated strong humoral and cellular immunity, with a neutralizing antibody titers of >1*10 4 at 28 days and a *Th1*-biased cellular immune response in BALB/C mice. And the result also indicates that the S–6P-GSAS variant elicits superior immunogenicity at lower doses compared to the S–2P variant.

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Contribution of long non-coding RNAs to the Paediatric Diffuse Midline Glioma immunopeptidome

Tima Shamekhi[1, 2], Terry Lim[3, 4], Dongbin Jin[2], Liesl Bramberger[1, 2], Grace Huang[1, 2], Nivedhetha Selvakumar[1, 2], Claire Sun[2], Paul Daniel[2], Erwin Tanuwidjaya[1, 2], Gabriel Goncalves[4], Riccardo Dolcetti[5], Ron Firestein[2], Pouya Faridi[1, 3, 2]

1. School of Clinical Sciences, Department of Medicine, Monash University, Clayton, Victoria, Australia

2. Centre for Cancer Research , Hudson Institute of Medical Research, Clayton, Victoria, Australia

3. Monash Proteomics and Metabolomics Facility, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

4. Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

5. Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Paediatric diffuse midline glioma (pDMG) represents the most aggressive brain tumour in children, with a 9-month survival rate and no successful treatment. Peptides derived from cancer-specific antigens and presented by HLA molecules are ideal targets for precision cancer immunotherapy. Recent studies have highlighted the contribution of antigens originating from long non-coding RNA (lncRNA)s, which play pivotal roles in tumour immune recognition. In this study, we present a comprehensive immunopeptidomics analysis to identify peptide antigens derived from lncRNAs in a cohort of DMG cell lines.

Using transcriptomics data from 52 different DMG cell lines, we constructed an in-house database consisting of over 38,000 RNAs that are specifically overexpressed in pDMG compared to healthy tissues. We then studied the immunopeptidome of 20 DMG cell lines. In brief, 10 million cells from each cell line were subjected to immunopeptidomics using a modified SAPrlm protocol, followed by mapping against both the human proteome and our in-house pDMG-specific lncRNA database. Data were analysed by PEAKS 11, and the FDR was calculated at 1%. In total, we identified 162 peptides across all the cells presented by 14 types of HLA. Less than 1% of the identified peptides in each cell line are derived from lncRNA. We validated the authenticity of 39 lncRNA pHLA by synthetic peptides.

pDMG is a low-mutation burden tumour; therefore, there is a need to identify cancer antigens beyond classical somatic mutation-derived neoantigens. Our findings underscore the considerable contribution of lncRNA-derived tumor antigens in pDMG's immunopeptidome, presenting a promising avenue for pDMG immunotherapy.

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Anti SARS COV 2 IgG levels a year after vaccination in healthcare workers at Prof Dr R D Kandou Hospital Manado

Geri Waldi Setiawan[1], Eko Edi Surachmanto[2], Juwita Soekarno[2], Eulis Alwi Datau[2], Agung Nugroho[3]

1. Department of Internal Medicine, Faculty of Medicine University of Sam Ratulangi Manado, Manado, North Sulawesi, Indonesia

2. Division of Allergy Immunology, Department of Internal Medicine, Faculty of Medicine, Sam Ratulangi University/Prof. Dr. R. D. Kandou Hospital Manado, Manado, North Sulawesi, Indonesia

3. Division of Tropical Medicine and Infectious Disease, Department of Internal Medicine Faculty of Medicine, Sam Ratulangi University/Prof. Dr. R. D. Kandou Hospital, Manado, North Sulawesi, Indonesia

Objective: COVID-19 vaccination has emerged as the most effective method to mitigate the likelihood of severe symptoms. It is crucial to evaluate individuals after vaccination in order to establish the duration of the protection period and determine the optimal timing for the booster dose. This study compares the levels of anti-SARS-CoV-2 IgG antibodies one year after receiving the fourth dosage of BNT162b2 and mRNA-1273 vaccines.

Methods Cross-sectional observational study on healthcare workers at Prof. Dr. R.D. Kandou Hospital Manado was carried on December 2023. All healthcare workers took the first and second dose of Coronavac vaccine, third dose of mRNA-1273 vaccine, and fourth dose of the BNT162b2 or mRNA-1273 vaccine in 2022, these were included in the study. Participants with history of flu like syndrome and COVID-19 infection in the last 6 months were excluded from the study. The anti-SARS-CoV-2 IgG level was determined one year after vaccination by using chemilumiscent microparticle immunoassay (CMIA). The data were not normally distributed so the Mann-Whitney U test was used. The p-value of ≤0.05 was considered significant.

Results: A total of 40 subjects were included in the study, most were males (67,5%) with an average age of 30,52 years old. It consists of 20 subjects in the BNT162b2 group and 20 subjects in the mRNA-1273 group. The mean level of anti-SARS-CoV-2 IgG was higher in female group (10287.03 ± 6247.78) compared to male group (8157.77 ± 7363.54) but the difference was no statistically significant (p=0.252). The mean level of anti-SARS-CoV-2 IgG was higher in BNT162b2 group (9424.38 ± 8029.25 AU/mL) compared to mRNA-1273 group (8275.19 ± 5981.76 AU/mL), but the difference was not statistically significant (p=0.779). The mean level of anti-SARS-CoV-2 IgG was higher in age group of <30 years (9525.54 ± 9060.15 AU/mL) compared to age group of ≥30 years (8485.91 ± 5800.02 AU/mL), but the difference was no statistically significant (p=0.922).

Conclusions: The anti-SARS-CoV-2 IgG levels were higher in healthcare workers who took the fourth BNT162b2 vaccination, female group, and who are at the age below 30 years old.

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In-depth characterisation of the tumour immune microenvironment in paediatric solid cancers – PFA ependymoma and osteosarcoma

Charlotte Dr Chen[1]

1. Peter MacCallum Cancer Centre, Melbourne, VICTORIA, Australia

The tumour immune microenvironment (TME) provides substantial information on the tumour progression, metastasis which gives insights on the resistance mechanisms to current available immunotherapies [1-4]. At present, paediatric solid tumours pose significant challenges due to the limited understanding of their TME, and thus, unfavourable treatment outcomes with immunotherapies like immune checkpoint blockade (ICB) [5-9]. To advance the development of immunotherapies for paediatric solid tumours, our current studies specifically investigate the TME of two prevalent and challenging cases: posterior fossa A ependymoma (PFA-EPN) and osteosarcoma (OS) with lung metastasis, both associated with dismal survival.

We are conducting retrospective cohort studies comprising 30 patient samples for each disease. In the OS study, we hypothesize the existence of OS-specific immunosuppressive factors in the TME, contributing to lung metastasis. Our goal is to investigate the TME dynamic between the primary tumour and its lung metastasis. In the PFA-EPN study, we hypothesize distinct TME characteristics contributing to the poorest prognosis within paediatric ependymoma. Our aim is to uncover these features. In both studies, we seek to identify potential treatment resistance mechanisms to ICB by exploring on the TME.

The samples for both studies are sourced from various biobanks within the ANZCHOG, comprising formalin fixed paraffin embedded (FFPE) samples, fresh frozen (FF) samples, and paired frozen PBMCs. Initial profiling involved transcriptomic and genomic analyses of immune subsets and tumour cells. To enhance our insights, we will conduct spatial proteomic and single-cell level transcriptomic analyses on FFPE tissue sections. Integrating bulk transcriptomic data with spatial analysis will provide a deeper understanding of the interplay between tumour and immune/stromal cells within the TME of individual tumours.

Our current data on PFA-EPN suggested the tumour has "cold" TME with little T cell infiltration, substantial PD-L1+ immunosuppressive myeloid subsets. There is no significant difference in TME profiles between primary and the recurrent tumour.

The next step is to conduct single nuclear RNAseq and spatial neighbourhood analysis. We aim to find potential treatment resistance mechanisms through deep interpretation of the interplay between distinct tumour molecular subtypes and the neighbourhood immune subsets. For the OS study, in lung metastasis samples, transcriptomic profiling showed the co-existence of activations on both anti-tumour immunity and myeloid cells mediated immunosuppression. The Spatial analysis of the involved immune subsets and WES analysis are ongoing, which will provide more detailed insights on the mechanisms of metastasis into the lung, and its contribution to treatment resistance.

1. 1. Binnewies, M. et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 24, 541–550 (2018).
2. 2. Fu, T. et al. Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response. J Hematol Oncol 14, 98 (2021).
3. 3. Xu, L. et al. Reshaping the systemic tumor immune environment (STIE) and tumor immune microenvironment (TIME) to enhance immunotherapy efficacy in solid tumors. J Hematol Oncol 15, 87 (2022).
4. 4. Terry, R. L. et al. Immune profiling of pediatric solid tumors. J Clin Invest 130, 3391–3402 (2020).
5. 5. Merchant, M. S. et al. Phase I Clinical Trial of Ipilimumab in Pediatric Patients with Advanced Solid Tumors. Clin. Cancer Res. 22, 1364–1370 (2016).
6. 6. Davis, K. L. et al. A Phase I/II Trial of Nivolumab plus Ipilimumab in Children and Young Adults with Relapsed/Refractory Solid Tumors: A Children's Oncology Group Study ADVL1412. Clin. Cancer Res. 28, 5088–5097 (2022).
7. 7. Tilburg, C. M. van et al. Phase I/II intra-patient dose escalation study of vorinostat in children with relapsed solid tumor, lymphoma, or leukemia. Clin. Epigenetics 11, 188 (2019).
8. 8. Geoerger, B. et al. Pembrolizumab in paediatric patients with advanced melanoma or a PD-L1-positive, advanced, relapsed, or refractory solid tumour or lymphoma (KEYNOTE-051): interim analysis of an open-label, single-arm, phase 1–2 trial. Lancet Oncol. 21, 121–133 (2020).
9. 9. Meftahpour, V., Aghebati-Maleki, A., Fotouhi, A., Safarzadeh, E. & Aghebati-Maleki, L. Prognostic significance and therapeutic potentials of immune checkpoints in osteosarcoma. EXCLI J. 21, 250–268 (2022).

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Differential responses of mouse and human dendritic cells to clinical inhibitors used to treat melanoma

Zoe Magill[1], Ee Shan Pang[2], Jamie Gearing[3], Angus Shoppee[1], Nazneen Jahan[1], Mariam Bafit[1], Peck Tan[1], Jung Hee Kang[1], Mark Shackleton[4], Miles Andrews[4], Meredith O'Keeffe[1]

1. Biomedicine Discovery Institute, Monash University , Clayton, VIC , Australia

2. CSL, Parkville, VIC, Australia

3. Hudson Institute of Medical Research, Clayton, VIC, Australia

4. Central Clinical School, Monash University, Melbourne, VIC, Australia

Mutations in the mitogen-activated protein kinase-extracellular signal-regulated kinase (MAPK-ERK) signalling pathway are common in melanoma. The BRAF serine/threonine kinase is a key regulator of this pathway. Approximately 60% of melanoma patients have a gain-of-function $BRAF^{V600E}$ mutation, leading to uncontrolled proliferation. BRAF inhibitors have shown clinical benefit, however, emerging evidence suggests that they diminish responses to subsequent immunotherapy, indicating unknown effects on the immune system Given the relatively high incidence of resistance and treatment-related toxicities, it is important to better understand the mechanisms through which BRAF inhibitors work and how they affect immune cells.

Dendritic cells (DC) are professional antigen presenting cells integral to strong anti-tumour T cell responses. However, the effects of BRAF inhibitors on DC function are not understood. Our work utilises *in vitro* models and *ex vivo* murine and human primary DCs to explore the functional effects of BRAF inhibitors on DC function, including surface activation marker expression, cytokine production and T cell stimulation. Our preliminary data show that BRAF inhibitors can enhance *ex vivo* DC activation in mouse models. In stark contrast, *ex vivo* treatment of human DCs with BRAF inhibitors impedes DC activation by inhibiting proinflammatory cytokine production and downregulating surface activation markers. These data suggest that BRAF inhibitors hinder human DC activation, which may impair subsequent anti-tumour T cell responses.

Importantly, this work demonstrates the divergent responses of mouse and human primary DCs to BRAF inhibition. This highlights a need to elucidate the mechanisms of immune effects, as this may underlie variability between preclinical and clinical data on BRAF inhibitors. Additionally, this may enhance our understanding of the interactions between BRAF inhibitors and immunotherapy.

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Exploiting the Clec9A targeting vaccine platform to develop a safe and effective DENV vaccine

Geraldine Nadya Putri[3, 1, 2], Yee Hwa Wong[4], Julien Lescar[4], Mireille Lahoud[5], Sylvie Alonso[3, 1, 2]

1. Department of Microbiology & Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

2. Immunology programme, Life Sciences Institute, National University of Singapore, Singapore

3. Infectious Diseases Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

4. Institute of Structural Biology, Nanyang Technological University, Singapore

5. Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia

With approximately 3.8 billion people at risk of infection in tropical and sub-tropical regions, dengue ranks among the top ten threats worldwide. It is caused by dengue virus, a flavivirus with four distinct serotypes. Dengue places a large economic burden on endemic countries and has the potential for severe disease manifestation in the form of Dengue Haemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). With no approved antivirals to treat infected patients, vaccines have been recognised to be the foundation to reduce DENV burden. However, dengue vaccine development has proven to be a challenge as an imbalanced immune response towards one serotype over the other could lead to antibody dependent enhancement (ADE) and eventual severe dengue. There are two live-attenuated dengue vaccines (Dengvaxia, Sanofi and QDENGA, Takeda Pharmaceuticals) that have been approved for human use, but only in specific age groups and have varied efficacies against the four DENV serotypes.

DENV envelope domain III (EDIII) has been a leading subunit dengue vaccine candidate, shown to induce strongly neutralising and predominantly serotype-specific antibodies, thereby minimising the risk of ADE. However, EDIII weak immunogenicity has represented a major bottleneck in the development of EDIII-based vaccines.

We have explored a dendritic cell-targeting vaccine approach to deliver EDIII to the cDC1 dendritic cell subset. cDC1s are highly efficient in processing antigens to present them on both MHC I and MHC II, thereby inducing potent and sustained cellular and humoral immune responses. The vaccine construct consists of a rat anti-mouse Clec9A antibody fused with EDIII at the C-terminus of each heavy chain. Clec9A is a C-type lectin receptor that is specifically expressed on cDC1 . We show that a homologous prime-boost immunization regimen induced sustained anti-EDIII IgG titres and neutralising antibody titres up to 9 months post-boost. Clec9A-EDIII immunisation also generated EDIII-specific spleen T_{fh} cell response and poly-functional $CD4^+$ T cells secreting IFN-γ, IL-2, and TNF-α. These promising results hence support that the Clec9A targeting approach may overcome the weak immunogenicity of EDIII vaccine antigen and advance its clinical development. .

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Febrile temperatures differentially modulate the binding of antibodies to Staphylococcal Enterotoxin B (SEB) and to Cytotoxic T-lymphocyte associated protein 4 (CTLA-4)

Razvan C Stan[1], Puneet K Singh[1]

1. Chonnam National University, Gwangju, JEOLLANAM, South Korea

Background

The role of febrile temperatures on the formation of antibody: antigen complexes has not been a topic of research. While most infections can induce systemic fever in humans, most cancers are afebrile. However, inflamed tissues, as present in the tumor regions, are warmer by 2-3℃ than the average core body temperature of 37℃.

Objectives

To determine the effect of physiologic and febrile temperatures (38℃ - 41℃) on the binding affinity of two monoclonal antibodies (6d3 and 14g8) against SEB from the high-fever causing *S. aureus*, and of ipilimumab against its target CTLA-4.

Methods

In vitro calorimetry experiments in conjunction with molecular dynamics simulations were used to assess the binding affinity of antibodies against their targets at pertinent temperatures.

Results

Moderate fever (38℃ - 39℃) has a net positive role on the antibody binding affinity against SEB, while hyperpyrexia (≥40℃) inactivates these antibodies. In contrast, the activity of ipilimumab is adversely affected by moderate fever temperatures, down to an order of magnitude at 39℃.

Conclusion

Small temperature changes, as encountered during fever or local inflammation, may influence the binding of antibodies against their targets, and may lead to the judicious use of fever-range temperatures in a hospital setting, depending on the clinical condition.

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Analysis of T-cell responses to the epitopes derived from SARS-CoV-2 nucleocapsid protein presented by HLA Class II alleles in Indonesian population

Marsia Gustiananda[1], Caroline Nadia Bahana[1], Ellen Budiono[1], Fanie Tansil[1], Michael Jonathan[1], Jyothsna Girish[1], Vivi Julietta[1], Sita Andarini[2], Yacine Maringer[5, 3, 4], Annika Nelde[5, 3, 6], Jaturong Sewatanon[7], Juliane Walz[5, 3, 4]

1. Department of Biomedicine, School of Life Sciences, Indonesia International Institute for Life Sciences, Jakarta, Indonesia

2. Department of Pulmonology and Respiratory Medicine, Faculty of Medicine University of Indonesia -Persahabatan Hospital, Jakarta, DKI Jakarta, Indonesia

3. Department of Peptide-based Immunotherapy, University and University Hospital Tübingen, Tübingen, Germany

4. Cluster of Excellence iFIT (EXC2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, Tübingen, Germany

5. Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), Department of Internal Medicine, University Hospital Tübingen, Tuebingen , Germany

6. Institute for Cell Biology, Department of Immunology, University of Tübingen, Tübingen, Germany

7. Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok, Thailand

Cell-mediated immunity provided by T-cells is very important to combat SARS-CoV-2 infection. CD8+ T-cells or cytotoxic T-cells (CTL) kill the virus-infected cell thereby removing the virus reservoir and CD4+ T-cells or helper T-cells (HTL) orchestrating the appropriate immune responses and help B-cells to produce antibody. T-cells recognize SARS-CoV-2 peptide presented by human leukocyte antigen (HLA) on the surface of the infected cells. Knowledge about what viral peptides seen by T-cells is very important for vaccine development, immunotherapy and diagnostic. However, as sought in the Immune Epitope Database (IEDB) there remains limited research on the peptide presented by the HLA alleles of the Indonesian population. We conducted immunoinformatics analysis to predict peptides from the SARS-CoV-2 nucleocapsid protein (NP) that are presented by HLA Class II alleles of Indonesian population such as HLA-DRB1*12:02 (allele frequency 36.8%), HLA-DRB1*15:02 (24.1%), and HLA-DRB1*07:01 (13.7%). The immunogenicity of six nucleocapsid protein peptides, predicted to be presented by HLA class II, was validated through interferon gamma enzyme-linked immunospot (IFN-γ ELISpot) assay. One peptide (NP_263-280) has been reported in IEDB, while the remaining five (NP_104-122, NP_125-144, NP_166-183, NP_352-371, and NP_387-406) are new. The IFN-γ ELISpot assay on 23 peripheral blood mononuclear cells (PBMC) samples revealed positive T-cell responses for NP_104-122, NP_125-144, NP_166-183, NP_263-280, and NP_352-371 peptides. Kinetic analysis of T-cell responses toward each epitope shown negative correlation between the number of spots and time since a COVID-19 positive test, suggesting a gradual decline. Enzyme-linked immunosorbent assay (ELISA) was performed on the same samples as ELISpot to measure anti-SARS-CoV-2 NP IgG antibody levels. ELISA on 24 serum samples exhibited significant anti-SARS-CoV-2 NP IgG differences between donors with a history of COVID-19 infection (47.17±22.54 µg/ml) and those without (26.76±12.91 µg/ml) (p=0.036). Kinetic analysis on the antibody level was conducted and compared to the T-cell responses. It was revealed that antibody responses declined more than T-cell responses, emphasizing the durability of T-cells and the importance of understanding long-term T-cell responses.

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CCR5-Mediated Monocyte Interaction and IL-2-Signaling Orchestrate T helper 1 cell differentiation in vivo.

Takahiro Asatsuma[1, 2], Marcela L. Moreia[1, 2], Cameron G. Williams[1, 2], Hyun Jae Lee [1, 2], Oliver P. Skinner [1, 2], Saba Asad [1, 2], Shihan Li[1, 2], Thomas Burn[1, 2], Lynette Beattie[1, 2], Ashraful Haque[1, 2]

1. Microbiology and Immunology, University of Melbourne, located at the Peter Doherty Institute for Infection and Immunity, Melbourne

2. Doherty Institute, Carlton, VICTORIA, Australia

CD4⁺ T helper cells can protect against many infectious agents, including malaria parasites. The T-helper-1 (Th1) subset, for example, can protect by boosting the antimicrobial activity of phagocytes via IFNγ. Discovery of mechanisms controlling Th1 differentiation may offer opportunities for improving immunity to many pathogens. Previously, we observed early co-expression of chemokine receptors CXCR3 and CXCR5 by *Plasmodium*-specific TCR transgenic PbTII cells in *P.chabaudi*-infected mice, prior to their bifurcation towards either Th1 or T follicular helper (Tfh) fate [1]. We hypothesized here that early competition between CXCR3 and CXCR5 influenced Th1/Tfh fate. To test this, genes encoding CXCR3, CXCR5, or CXCR6 were disrupted in naïve PbTIIs via CRISPR/Cas9 and examined for effects on differentiation *in vivo.* None of these chemokine receptors, either alone or in combination, substantially influenced either PbTII expansion or Th1-differentiation, while interactions via LFA-1, or IL-2-signalling via CD25 were required for optimal clonal expansion and Th1 differentiation. In addition, consistent with our spatial transcriptomic analysis, which suggested a role for monocyte-Th1 interactions via CCR5, CRISPR/Cas9-mediated disruption *of Ccr5* reduced clonal expansion and Th1-differentiation in our model. Hence, we propose that CCR5-dependent monocytic-interactions of IL-2-primed CD4⁺ T cells promotes Th1 immunity in malaria. These data provide mechanistic insight into how Th1-reponses are optimally generated *in vivo*.

1. 1. Lönnberg, T., et al., Single-cell RNA-seq and computational analysis using temporal mixture modeling resolves TH1/TFHfate bifurcation in malaria. Science Immunology, 2017. 2(9): p. eaal2192.

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Targeting of Regulatory T cells: A therapeutic paradigm for preventing Cutaneous Squamous Cell Carcinoma

Shoaib Anwaar[1], Jazmina-Libertad Gonzalez-Cruz[1], James Wells[1]

1. Frazer Institute, Faculty of Medicine, The University of Queensland, Woolloongabba, QLD 4102, .

Cutaneous squamous cell carcinoma (cSCC) is one of the most prevalent cancers in Caucasian populations, and its aggressive form poses a high risk of metastasis resulting in increased rates of mortality and morbidity. Recurrent and chronic exposure to ultraviolet (UV) radiation from the sun plays a crucial role in the initiation, development, and perpetuation of cSCC. Accumulating evidence suggests that regulatory T (Treg) cells are associated with UV-induced immunosuppression, however, their direct contribution to the establishment of cSCC remains elusive. When mice were exposed to 5 consecutive days of UVB (dose rate 150mJ/cm^2), they showed reduced ear swelling in response to ovalbumin challenge in a contact hypersensitivity assay, suggesting that functional antigen specific Tregs were induced. However, ear swelling responses did not show signs of suppression when mice were treated with anti-CTLA-4 and anti-TIGIT antibodies following the cessation of UV treatment. Phenotypically, the expression of Foxp3, FR4, CTLA-4 and TIGIT on Tregs in the inguinal lymph nodes and spleen of UV-exposed mice did not differ from those in non-UV-exposed mice. Following the treatment of mice with UVB 5 days per week for different time periods (2w, 4w, 6w, 8w), it was determined that only UVB treatment for eight weeks consistently allowed the establishment and growth of tumours following the adoptive transfer of cSCC tumour fragments from donor mice. We aim to target Tregs in these tumour models in our future studies to determine whether Treg depletion or manipulation will reverse the capacity of UV to

enable cSCC tumour establishment. Overall, this study will examine the plausibility of Treg manipulation as a preventative strategy to prevent UV-induced cSCC tumour establishment.

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Humoral and cellular responses to SARS-CoV-2 vaccination in Australian adults with cancer.

Amy Body[1, 2], Elizabeth Ahern[1], Luxi Lal[1], Stephen Opat[2, 3], Michael F Leahy[4], Noemi Fuentes-Bolanos[5], Bhavna Padhye[6], Nada Hamad[7], Corey Smith[8], Stuart Turville[9], William Rawlinson[10, 11], Raina MacIntyre[9], Sriganesh Srihari[8], Eva Segelov[12]

1. Medical Oncology, Monash Health, Clayton, VIC, Australia

2. Monash University, Clayton, VIC, Australia

3. Haematology, Monash Health, Clayton, VIC, Australia

4. Haematology, Royal Perth Hospital, Perth, WA, Australia

5. Oncology, Sydney Children's Hospital, Sydney, NSW, Australia

6. Paediatric Oncology, Westmead Hospital, Sydney, NSW, Australia

7. Haematology, St Vincent's Hospital, Sydney, NSW, Australia

8. QIMR Berghofer, Brisbane, QLD, Australia

9. The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

10. NSW Health Pathology, Sydney, NSW, Australia

11. University of New South Wales, Sydney, Australia

12. Faculty of Medicine University of Bern and Department of Radiation Oncology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

Background

Patients with cancer have increased risk of severe outcomes from SARS-CoV-2 infection. Vaccination incompletely reduces risk; vaccinated cancer patients have a higher chance of hospitalisation and death due to COVID-19 than healthy controls. Neutralising antibodies are a correlate of protection. SerOzNET is a prospective study seeking to elucidate nuanced detail about immune response to vaccination in Australians with cancer.

Methods

Participants with cancer were enrolled prior to first SARS-CoV-2 vaccination. Comprehensive information regarding diagnosis and treatment was collected. Blood was sampled at baseline and serial timepoints until 3 months after 5th vaccine dose, and analysed for neutralising antibody response, quantitative IgG response (Abbott), and T-cell cytokine production (IFN-γ).

Results

There were 395 patients enrolled, mean age 57 years (range 20-85), 60% were female. Cancer diagnoses were solid organ cancers (SOC) in 257/395 patients (65%), and haematological cancers (HC) in 138/395 (35%). Cytotoxic chemotherapy was received by 49% of SOC and 62% of HC patients. Neutralising antibody was detectable in SOC and HC respectively in 84% and 43% after 2 doses, 96% and 65% after 3 doses, 98% and 69% after 4 doses and 100% and 90% after 5 doses. T cell IFNγ response was detected in SOC and HC respectively in 72% and 49% after 2 doses, 75% and 90% after 3 doses and 82% and 87% after 4 doses. On multivariate analysis, antibody response rate was higher in SOC (vs HC, OR 1.38, 1.06-1.8, p=0.017), and lower in patients on anti-B cell therapies (OR 0.19, 0.13-0.27, p<0.001). T cell response was predicted by SOC (vs HC, OR 1.76, 1.27-1.43, p=0.001). There was a non-significant trend toward negative effect of chemotherapy on antibody (OR 0.81, 0.64-1.03) and T cell response (OR 0.8, 0.6-1.06). Median absolute IgG titre was lower at serial timepoints in patients with HC, or on steroids, and higher with hybrid immunity post infection. B cell count correlated with IgG titre and was lower in patients on anti-B-cell therapies.

Conclusion

Most patients with cancer develop protective antibodies after SARS-CoV-2 vaccination, proportion of responders increases after each dose. After 3 doses, almost all patients with SOC respond however a third of HC patients do not. T cell response may provide an additional means of protection for patients with suboptimal antibody response; however, HC patients have reduced T cell and antibody response, placing them at higher risk. Patients with HC may require additional boosters and precautions against SARS-CoV-2 infection.

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Is BTN3A1 a regulator of αβ T cell responses?

Kezia Christilla Singgih[1], Rachel Deanne Pascoe[1, 2], Caroline Soliman[1], Shihan Li[1], Zheng Ruan[1], Dale Godfrey[1], Celine Gubser[3, 4], Sharon Lewin[1, 3, 5, 2], Adam Uldrich[1], Tom Fulford[1]

1. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute of Infection and Immunity, Melbourne, VIC, Australia

2. Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

3. Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute of Infection and Immunity, Melbourne, VIC, Australia

4. Service des Maladies Infectieuses, CHUV, Lausanne, Switzerland

5. Victorian Infectious Diseases Service, Royal Melbourne Hospital at the Peter Doherty Institute of Infection and Immunity, Melbourne, VIC, Australia

The role of butyrophilins (BTNs) as mediators of human γδ T cell activation has been well-characterised. However, a recent study reported that the overexpressed BTN member 3A1 (BTN3A1) on ovarian cancer tumours may suppress human $CD4^+$ and $CD8^+$ αβ T cell responses through engagement with CD45RO. BTN3A1 has also been shown to be elevated in chronic HIV infection with an overexpression of BTN3A1 on $CD4^+$ T cells enriched with total HIV DNA, and when coupled with persisting impaired HIV-specific $CD8^+$ T cell responses, may suggest a role for BTN3A1 in facilitating impaired HIV-specific responses. We, therefore, aimed to elucidate the potential immunosuppressive role of BTN3A1 towards $CD4^+$ and $CD8^+$ αβ T cells, and investigate the therapeutic benefits of disrupting BTN3A1 interactions with BTN3A-directed neutralising monoclonal antibodies (mAbs) to reinvigorate HIV-specific $CD8^+$ T cell immunity *in vitro*. Using a redirected presentation assay, we were not able to demonstrate BTN3A1-mediated suppression of $CD4^+$ and $CD8^+$ αβ T cell activation, proliferation, cytokine secretion and cytotoxicity. In line with these findings, treatment of peripheral blood mononuclear cells from people living with HIV on suppressive antiretroviral therapy with anti-BTN3A blocking mAbs failed to reinvigorate HIV-specific $CD8^+$ T cell cytotoxicity and proliferative capacity. Furthermore, we failed to observe an interaction between BTN3A1 and CD45RO ectodomains using tetramers. Collectively, our findings are inconsistent with the concept that BTN3A1 can suppress $CD4^+$ and $CD8^+$ αβT cells *in vitro* or in HIV infection, and moreover, we were unable to detect an interaction between BTN3A1 and CD45RO in contrast to a previous report. The reasons for the discrepancy in results are currently unclear and further studies are required to establish whether targeting BTNs with agonist or antagonist mAbs confer any therapeutic benefit for αβ T cell responses in acute or chronic viral infections.

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Age-related dynamics and spectral characteristics of the TCRβ repertoire in healthy children: implications for immune aging

Mingyan Fang[1], Stephanie Sun[2]

1. BGI Research, BGI Shenzhen, Shenzhen, Guangdong, China

2. BGI Australia, Brisbane, QLD, Australia

Background: The T cell receptor (TCR) is crucial for adaptive immunity, providing a diverse repertoire for antigen response. However, despite its clinical importance, baseline characterization of the TCR in the pediatric population remains limited. Methods: We conducted TCRβ sequencing on 325 healthy Chinese children aged 0-18 years, categorized into six age groups, to understand dynamic changes and establish a reference database of TCRβ rearrangements for this demographic. Furthermore, we used cell sorting on 81 samples with flow cytometry to analyze cellular composition and associations, focusing specifically on age-related variations in the TCRβ repertoire. Results: Our study revealed an age-related decline in TCRβ repertoire diversity, marked by increased high-frequency and in-frame clonotypes. This shift in diversity is likely due to age-related variations in CDR3 length and V(D)J gene usage. The analysis of dynamic clonal changes in TCRβ, especially in public clones, suggests a link to perinatal influences and the immune system's evolving personalization. Early-life vaccinations and antigen exposures play a pivotal role in shaping this immune evolution. Notably, a significant association was found between the decrease of CD4+ T naive cells and the diminishing TCRβ repertoire diversity along with age. Furthermore, our predictive models highlight specific TCRβ features as potential biomarkers for biological age, supported by their significant correlation. Conclusion: This study provides essential insights into age-related variations in the TCRβ repertoire among children, enriching our understanding of pediatric immune system evolution and informing potential disease prevention strategies.

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Single-cell sequencing combined with artificial intelligence assists in COVID-19 vaccine antigen design and preclinical efficacy evaluation

Jinmin Ma[1], Stephanie Sun[2], Mingyan Fang[1]

1. BGI Research, BGI Shenzhen, Shenzhen, Guangdong, China

2. BGI Australia, Brisbane, QLD, Australia

Variability in antibody responses among individuals following vaccination is a universal phenomenon. So a well designed vaccine whose antigen should elicit a strong protective immune response in variety of individuals. Single-cell transcriptomics offers a potential avenue to understand the underlying mechanisms of these variations and improve our ability to evaluate and predict vaccine effectiveness that facilitates vaccine antigen design. Artificial intelligence tools make antigenic protein structure design more efficient and convenient than ever before. Based on the individual diversity of immune response characteristics revealed by single-cell sequencing results, we designed several antigenic variants of SARS-CoV-2, and ultimately verified that several mutant designs were able to efficiently provoke highly effective neutralizing antibodies to more than 11 Omicron variants, includes mutants that appear after the antigen has been designed. What's more, the antigens we designed stimulated strong humoral and cellular immunity, with a neutralizing antibody titers of >1*10 4 at 28 days and a Th1-biased cellular immune response in BALB/C mice. And the result also indicates that the S–6P-GSAS variant elicits superior immunogenicity at lower doses compared to the S–2P variant.

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Interferon regulatory factors 1 and 2 regulate expression of programmed cell death-ligand 1 in dendritic cells

Ashley M Firth[1], Christophe Macri[1], Annabelle Blum[1], Cassandra Ouslinis[1], Nishma Gupta[2, 3], Justine D Mintern[1]

1. Bio21 Institute of Molecular Science and Biotechnology, Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville, Victoria, Australia

2. Inflammation Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

3. ACRF Chemical Biology Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Background

Programmed cell death-ligand 1 (PD-L1) is a regulatory molecule which is overexpressed in many cancers and plays a major role in suppressing the immune system. As well as tumour cells, PD-L1 is expressed by all haematopoietic cells. In particular, the upregulation of PD-L1 on dendritic cells (DCs), one of the primary cells which presents antigen to T cells, can lead to T cell inhibition through binding to programmed cell death-1 (PD-1). Many immunotherapies have traditionally focussed on the T cell response to cancer, including blocking PD-1 expression by T cells. However, the importance of DCs in the immunological response to cancer is now better appreciated, and targeting of immunotherapies to DCs is an emerging area in cancer research. Still, the regulation of PD-L1 and other checkpoint inhibitor molecules expressed in DCs remains poorly understood.

Methods and results

A genome-wide CRISPR/Cas9 screen searching for regulators of PD-L1 in DCs identified the transcription factor, interferon regulatory factor 2 (IRF2), as a promotor of PD-L1 cell surface expression. Production of a single-gene knockout of IRF2 in the MuTu DC line (*Irf2*$^{-/-}$) allowed confirmation that *Irf2*$^{-/-}$ DCs have lower cell surface expression of PD-L1 when compared to wild type, without affecting the DC's ability to stimulate adaptive immunity. Reduced PD-L1 expression was also observed on *Irf2*$^{-/-}$ DCs following stimulation with a toll-like receptor agonist, CpG, which is a potent inducer of PD-L1 upregulation. In contrast, when *Irf2*$^{-/-}$DCs were activated with interferon-γ, a major driver of PD-L1 expression in the tumour microenvironment, there was no discernible difference in PD-L1 expression relative to wild type cells. Instead, the upregulation of another interferon regulatory factor, IRF1, is observed. Production of a knockout of IRF1 in MuTu DCs confirmed that it is the main transcriptional driver of PD-L1 expression in interferon-γ-activated DCs. This mirrors the role of IRF1 as a known promoter of PD-L1 in tumour cells.

Conclusion

This study supports that in DCs, PD-L1 expression is regulated by the transcription factors IRF1 and IRF2, which play interchangeable roles dependent on the stimuli encountered in the microenvironment. Ongoing research aims to confirm the roles of IRF1 and IRF2 as PD-L1 regulators in primary DC cultures and mouse models. Further expansion on the roles of IRF1 and IRF2 will advance our understanding of the DC response to tumours and could lead to the generation of more effective immunotherapies.

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In vivo Programming of Immune Cells Using mRNA-LNP Chimeric Antigen Receptors

Jian Ding[1], Shannon Argueta[1], Franciele K Melber[1], Michael Gorgievski[1], Hongyun Zhao[1], Neha Divangi[1], Edward Cochran[1], Thach Chu[1], Thomas Prod'homme[1], Yuxiao Wang[1], Robert Hofmeister[1], Daniel Getts[1]

1. Myeloid Therapeutics, Cambirdge, MASSACHUSETTE, United States

Adoptive cell therapies have provided significant clinical benefits to cancer patients. However, *ex vivo* cell therapies are costly and associated with long vein-to-vein times. To overcome these limitations, we deliver cancer-targeting chimeric antigen receptor (CAR) mRNA via lipid nanoparticles (LNPs) to reprogram immune cells directly *in vivo*. To avoid expression of the CAR in other than myeloid cells when adminsterd systemically, we created a new FcaR I (CD89) based CAR construct whose expression and function depends on the co-expression the FcR γ-chain, a signal adaptor protein that is primarily restricted to myeloid cells. Using the same design principle, we also generated CARs that are only operative in NK and T cells, respectively.

Upon uptake of TROP2 CAR mRNA/LNPs *in vitro*, TROP2 CARs primarily expressed on mouse and human myeloid cells triggered CAR-dependent cytokine release and tumor cell lysis. Systemic mRNA/LNP delivery of the CAR showed robust anti-tumor efficacy against multiple tumor antigens in xenograft mouse models of human breast cancer, hepatocyte carcinoma, and ovarian cancer. Furthermore, using gp75-targeted CD89-based CAR/LNPs in the B16/F10 immunocompetent melanoma mouse tumor model, we demonstrated robust anti-tumor immune responses and profound changes in the tumor microenvironment characterized by tumoral infiltration of activated CD8+ T cells, diminished tumor-associated Tregs, systemic enhancement of dendritic cell activation and anti-tumor IgG production.

Encouraged by our preclinical data, we advanced the TROP2-targeting CAR to a phase I clinical trial to assess the safety and efficacy in epithelial malignancies.

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Trends in viral replication and lung pathogenesis of influenza A(H1N1)pdm09 viruses from 2009 to 2022 in the ferret model.

Harry Stannard[1], Paulina Koszalka[1], Smitha Georgy[2], Nikita Deshpande[1], Saira Hussain[1], Kanta Subbarao[3, 1], Patrick Reading[3, 1], Ian Barr[1]

1. WHO Collaborating Centre for Reference and Research on Influenza, at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

2. Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Melbourne, VIC, Australia

3. Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC, Australia

Background:

Ferrets are widely considered to be the gold-standard small animal model to study the pathogenesis of influenza viruses, as well as the impact of vaccines and antiviral drugs on influenza infections. This study aims to explore trends in viral replication and lung pathogenesis of A(H1N1)pdm09 viruses from their emergence in 2009 to 2022 using the ferret model. In addition, we aimed to identity contemporary influenza virus strains that demonstrate robust influenza disease, and lower respiratory tract involvement in the ferret, updating the current ferret model for A(H1N1)pdm09 infection.

Methods:

Five cell passaged, plaque-purified human influenza A(H1N1)pdm09 viruses selected from 2009 to 2022 vaccine recommendations (A/California/07/2009, A/Michigan/45/2015, A/Victoria/2570/2019, A/Sydney/5/2021, and A/Victoria/4897/2022) and a A(H3N2) virus, A/Darwin/6/2021 for comparison, were inoculated intranasally into ferrets. At 3- or 5-days post-infection, nasal wash and respiratory tissue (including individual lung lobes) samples were collected to determine viral titres and/or histopathological analysis.

Results:

Our results revealed consistent viral tires in the nasal wash, nasal turbinate, and soft palate tissues of ferrets in all viruses tested. Area under the curve analysis indicated A/Sydney/5/2021 had higher total viral shedding over five days than A/California/07/2009 infected animals. Viral replication in the lung lobes differed between the five strains. A/Sydney/5/2021 also demonstrated the greatest lung tropism at day 5 post infection, with a 1.2, 2.1 and 2.1 $\log_{10}TCID_{50}$ higher mean lung viral titre than the three older strains, although only 0.6 $\log_{10}TCID_{50}$ higher than the most recent A/Victoria/4897/2022 virus. Lung histopathology showed that A/Sydney/5/2021 had significantly higher histopathology scores than earlier strains, with a prominent feature of bronchiolitis and peribronchiolar lymphocyte cuffing. A recent human A(H3N2) virus (A/Darwin/6/2021) in contrast, was not detected in ferret lungs and displayed no histological changes in their lungs.

Conclusions:

While upper respiratory tract viral replication remained similar across the five A(H1N1)pdm09 viruses isolated from 2009 to 2022, the two most recent viruses tested (2021 and 2022) had higher viral titres in the lungs of ferrets and more severe lung pathology compared to the other earlier viruses. This was not observed with a recent A(H3N2) virus (A/Darwin/6/2021), which like other previous A(H3N2) viruses did not replicate in the lungs of ferrets. The A/Sydney/5/2021 ferret infections displayed robust viral loads and histopathology, making it an ideal contemporary virus for exploring the protective efficacy of current and future anti-influenza treatments or vaccines in the ferret model.

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Cancer Neutrophil Encyclopedia: A Deep Dive into Antigen-Presenting Warriors

Yingcheng Wu[1], Jiaqiang Ma[1], Qiang Gao[1]

1. Fudan University, Xuhui, SHANGHAI, China

Introduction

Neutrophils, the most efficient defenders against pathogens, are essential for tumor microenvironment balance and homeostasis. However, given their plasticity and short half-life which made them too fragile to be profiled, it poses complex challenges regarding how neutrophils are imprinted and adapt specific fates across cancers.

Material and Methods

Here we designed a one-two-punch sorting strategy, generated the neutrophil atlas from 225 samples of 144 patients from 17 cancer types, and further developed a computational pipeline to recover both shared and specific transcriptional programs.

Results and Discussions

Unexpectedly, neutrophils harbored extraordinary complexity composed of 10 cell states and showed sharp tissue or phenotypic specialty. We observed and verified that cancer neutrophils are dramatically arranged along tumor-specific terminal differentiation paths such as inflammation, angiogenesis and antigen-presenting. In particular, the antigen-presenting program was associated with better patient outcomes in the majority of cancers. Such a program can be evoked by leucine metabolism and is dependent on mitochondrial remodeling, acetyl-CoA generation, and preferable epigenetic histone H3K27ac modification. Functionally, antigen-presenting neutrophils invoked expanded T cell response and neoantigen-specific reactiveness. We finally designed the antigen-presenting neutrophil immunotherapy (adoptive transferring and leucine diet) which fine-tunes the microenvironment balance and fuels anti-PD-1 immunotherapy.

Conclusion

In summary, these data not only lay the groundwork for future neutrophil research, and open the black box of neutrophil state divergence across cancers, but also unravel minimally invasive therapeutic opportunities including adoptive transferring antigen-presenting neutrophils.

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PVM infection after bone marrow transplantation leads to increased mortality which is associated with impaired viral antigen-specific T cell responses and IL-6-mediated pathogenic Th17 differentiation in the lung

Sophie C Hamann[1], Alika D Collinge[1], Rachel D Kuns[1], Stuart D Olver[1], Albert C Yeh[2], Andrew D Clouston[3], Kirsten M Spann[4], Geoffrey R Hill[2, 5], Mariapia Degli-Esposti[6], Simon Phipps[7, 8], Seweryn Bialasiewicz[7, 9], Antiopi Varelias[1, 4, 7, 10]

1. Transplantation Immunology, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

2. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

3. Envoi Specialist Pathologists, Brisbane, QLD, Australia

4. School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia

5. Division of Medical Oncology, University of Washington, Seattle, WA, USA

6. Infection and Immunity Program and Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

7. Australian Infectious Diseases Research Centre, University of Queensland, St Lucia, QLD, Australia

8. Respiratory Immunology, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

9. Australian Centre for Ecogenomics, School of Chemistry and Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia

10. Faculty of Medicine, University of Queensland, St Lucia, QLD, Australia

Respiratory viral infections are a major global public health problem. RSV-induced bronchiolitis and pneumonia are the leading cause of hospitalization in infants and young children worldwide, while in adult allogeneic hematopoietic stem cell transplant (HSCT) recipients, the incidence of progression from upper to lower respiratory tract infection is 40-60%, with mortality rates as high as 80%. The effectiveness of antivirals or immunoglobulins to curb RSV infection in HSCT recipients remains controversial and represents an unmet clinical need. Given the paucity of mechanistic data to guide clinical studies or define the basis of disease, we established a unique murine model of RSV infection after bone marrow transplantation (BMT) using pneumonia virus of mice (PVM), the murine homologue of human RSV, to address this. B6.WT grafts were transplanted into lethally irradiated B6D2F1 (alloBMT) or B6.WT (synBMT) mice and infected with PVM on day 21 post-BMT. In contrast to syngeneic BMT, alloBMT recipients displayed a high incidence of mortality after PVM infection with fulminant lung pathology, recapitulating the outcome seen in patients. Notably, IL-6 levels in lung tissue were significantly elevated in PVM infected alloBMT recipients compared to uninfected alloBMT and synBMT recipients at day 12 post-infection and correlated with lung pathology. We hypothesized the elevated IL-6 levels would trigger activation of the Th17/Tc17 differentiation program. We performed transplants using grafts comprised of T cells from B6.IL-17eYFP fate-map reporter mice with PVM infection at day 21 post-BMT. This revealed an increase in the frequency and number of Th17 cells, but not Tc17 cells, in lung tissue of PVM-infected alloBMT recipients compared to uninfected alloBMT and synBMT recipients, suggesting that blockade of the IL-6/Th17 axis may serve as a logical therapeutic pathway. Furthermore, to identify PVM-specific T cell responses, we designed MHC-I and MHC-II tetramers directed to epitopes of PVM matrix (M_{37-47}) and RNA polymerase ($L_{1052-1060}$) proteins. A reduction in the frequency and number of PVM-specific $CD4^+$ and $CD8^+$ T cells was observed in lung and lymphoid tissues of PVM-infected alloBMT recipients versus synBMT recipients at days 9-12 post-infection. Taken together, these data suggest that in the presence of GVHD impaired antiviral T cell responses may lead to a lack of viral control and thereby exacerbate disease. Ongoing mechanistic experiments are focussed on interrogating the relationship between elevated IL-6 levels, defective antiviral T cell responses and viral control in PVM-infected alloBMT recipients.

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Inhibition of nuclear ACE2 translocation guards against SARS-CoV-2 replication and lung injury via epigenetic imprinting

Taniya Ahuja[1]

1. QIMR Berghofer, Brisbane, QLD, Australia

Publish consent withheld

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Characterisation of viral interactions between Influenza, SARS-CoV-2 and other circulating respiratory viruses

Nikita Deshpande[1], Rubaiyea Farrukee[2], Paul Whitney[1], Daniel Steinfort[3], Sarah Londrigan[2], Mariana Baz[1]

1. WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, VIC, Australia

2. Department of Microbiology and Immunology, University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

3. Department of Respiratory and Sleep Medicine, Royal Melbourne Hospital, Parkville, VIC, Australia

Introduction: Respiratory viruses (RVs) represent a significant global health threat. The prevalence of RVs has surged due to the relaxation of public health measures and reduction in non-pharmaceutical interventions in communal settings. RVs have the capacity to induce concurrent or sequential infections. This study aims to explore potential viral interactions and assess the

efficacy of a broadly active antiviral drug against prevalent RVs, including Influenza (IAV), SARS-CoV-2, Respiratory Syncytial Virus (RSV), rhinovirus, human metapneumovirus (HMPV), and parainfluenza virus (PIV).

Methods: A biobank of primary bronchial epithelial cells (pBECs) obtained via bronchoscopy was established, and Air-liquid interface (ALI) cultures were subsequently developed. Single virus infections were performed using strains of SARS-CoV-2 (including ancestral, delta, omicron BA.1, and BA.5 variants), Influenza A virus (pdmH1N1 and H3N2 subtypes), clinical isolates of Respiratory Syncytial Virus (RSV) types A and B, as well as laboratory reference strains of rhinovirus, human metapneumovirus (HMPV), and parainfluenza virus (PIV) in the ALI cultures. Transepithelial electrical resistance (TEER), serving as a proxy for tissue integrity, and cellular damage, indicated by lactate dehydrogenase (LDH) levels, were monitored daily. Additionally, cytokine production and induction of interferon-stimulated genes (ISGs) were assessed at 24 hours and 48 hours post-infection.

Results: TEER measurements for all viruses reached their peak at 24 hours post-infection, likely attributed to increased mucus production. The growth kinetics of all viruses indicated peak titres ranging from 10^1.7 to 10^7.95 $TCID_{50}$/mL. Notably, despite the decrease in TEER and increased virus growth, the ALI monolayer remained relatively intact up to ten days post-infection. Molnupiravir, an antiviral drug effective against SARS-CoV-2, IAV, and RSV, demonstrated dose-dependent inhibition of SARS-CoV-2 in a proof-of-concept experiment using Calu-3 ALI cells. Quantification of interferon responses (Type I and Type II) and levels of ISG induction following viral infections is underway through optimization of qPCR protocols. Current investigations are focused on characterizing sequential respiratory virus infections.

Conclusion: This study aims to offer valuable insights into viral interactions among epidemiologically relevant respiratory viruses (RVs) and the effects of molnupiravir on both single RV infections and sequential infections.

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Immunity to zoonotic influenza viruses in individuals vaccinated with seasonal influenza vaccines

Malet Aban[1], Heidi Peck[1], Monica Bobbitt[1], Ian Barr[1]

1. WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, VICTORIA, Australia

Introduction: There is an ongoing threat of an influenza pandemic emerging from zoonotic infections. As part of ongoing risk assessments for susceptibility to potential pandemic viruses it is useful to examine population immunity both before and after seasonal influenza vaccinations to see what the level of immunity is present and if it is boosted by vaccination with the annual influenza vaccine. There is little data on human antibody levels of Australians against local animal influenza viruses that are circulating.

Methods: Human serum panels (n=192) taken from paediatric, adults and elderly subjects prior to and following vaccination with the 2023 Southern Hemisphere influenza vaccine were tested by haemagglutination inhibition assay (HI) against recent animal and zoonotic influenza viruses isolated in Australia. Influenza viruses tested were A/Emu/Vic/23-02903/2023 (A(H10N7)) and two swine-origin viruses isolated from humans, A/South Australia/85/2018 and A/South Australia/1/2021 A(H3N2v).

Results: Increased geometric mean titres against recent circulating seasonal human influenza viruses were observed in the cohort post vaccination as expected. Responses to the animal influenza virus A/Emu/Vic/23-02903/2023 (A(H10N7)) showed no detectable antibody levels in any of the human serum panels. Serum antibody levels with a HI titre of ≥40 against A/South Australia/85/2018 A(H3N2v) were detected in 43% of pre-vaccination and 51% of post-vaccination sera with similar results seen against A/South Australia/1/2021 A(H3N2v) 34% (pre-vaccination) and 41% (post-vaccination).

Conclusion: Low seroprevalence is a risk factor for the human population against potential zoonotic influenza viruses. Vaccinating with seasonal influenza vaccine will in general not boost antibody levels against many of these viruses, especially those of avian origin. No cross-reactive antibodies were detected against the A(H10N7) virus, however good levels of cross-reacting antibody were seen against the swine A(H3N2v) viruses, and these were boosted following seasonal influenza vaccination and should offer good levels of protection against infection.

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Bhlhe40 Deficiency in CD8⁺ T Cells Attenuates Alloreactive Immunopathology and Modulates Cytokine Responses in Graft-versus-Host Disease

Amir Shamshirian[1, 2], Karshing Chang[1], Graham Magor[1], Kate Gartlan[1, 2]

1. QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

2. School of Medicine, University of Queensland, Herston, Brisbane, QLD, Australia

Stem cell transplantation (SCT) is a key therapeutic approach to treat those who suffer from blood cancers. SCT aims to re-establish the patient's defective immune system with donor-derived hematopoietic stem cells and provide the graft-versus-leukaemia effect (GVL), whereby donor immune cells target and eliminate malignant cells within the host due to donor/host genetic disparity. However, a major challenge associated with SCT is the onset of Graft-versus-Host-Disease (GVHD), a severe complication characterised by donor alloreactive immunopathology in healthy recipient tissues. This adverse event is primarily attributed to the same genetic disparities that facilitate the GVL effect, highlighting a significant difficulty in the successful application of SCT.

Bhlhe40 is a basic helix-loop-helix (bHLH) transcription factor involved in a range of biological processes, including circadian rhythm regulation, cell differentiation, and immune responses. In the immune system, Bhlhe40 has been shown to influence the function and differentiation of immune cells, including T cells, which play a significant role in the pathology of GVHD. Recent studies have identified Bhlhe40 as an important transcriptional regulator that regulates the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) in CD4⁺ T cells, playing a key role in mediating pathological damage within the gastrointestinal (GI) tract during GVHD. However, the role of this transcription factor in CD8⁺ T cells remains unclear and is currently undefined in allotransplantation.

In this study, we identified that the absence of Bhlhe40 in donor $CD8^+$ T cells within the allogeneic SCT setting substantially mitigates the severity of GVHD, characterised by significantly prolonged survival rates and reduced clinical manifestations. This improved clinical outcome was associated with a series of significant immunological changes, including lower expression of pathogenic cytokines linked with T cell activation and cytotoxicity, revealing a reduced cytotoxic and inflammatory response. Also, we observed significant increase in naive T-cells, and lower frequencies of short-lived-effector cells (SLECs) suggesting a tempered immune response that potentially moderates the severity of GVHD. Hence, our findings suggest that Bhlhe40 plays an important role in regulating the pathogenic potential of $CD8^+$ T cells in GVHD, through its influence on T cell differentiation and cytokine production. Thus, this study provides novel insights into the immunoregulatory functions of Bhlhe40 in $CD8^+$ T cells and presents a potential therapeutic target for GVHD treatment.

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Glycoengineering of Antigens for Focusing The Immune Response

Sacha Zinn[1, 2], Jake Y Henry[1, 2], Febrina Sandra[3], Pall Thordarson[3], Daniel Christ[1, 2]

1. Garvan Institute of Medical Research, Sydney, NSW, Australia

2. Faculty of Medicine, St Vincent's Clinical School, UNSW, Sydney, NSW, Australia

3. RNA Institute, UNSW, Sydney, NSW, Australia

Engineering antigens to direct immune responses is a promising methodology that has the potential to address shortcomings in vaccinology. Cellular and humoral immune responses tend to bias towards specific antigens and particular epitopes, in a phenomenon known as immunodominance. During the SARS-CoV-2 pandemic, the immunodominance of key viral antigens (such as the spike protein) were shown to play a role in the emergence of highly virulent variants that drove significant increases in morbidity and mortality.

The receptor binding domain (RBD), is an immunodominant region within the spike protein that mediates contact with hACE2 receptors, thus making it vulnerable to antibody neutralisation. The epitopes of the RBD are organised into classes, ranging from I – V. Class I & II epitopes are designated contact points for ACE2 and are mutational hotspots. Emergent strains of SARS-CoV-2 have demonstrated significant mutability within these regions, helping to facilitate escape from prior established immunity. Therefore, directing immune responses away from highly mutable epitopes towards more conserved regions of the spike and RBD, was a promising avenue to developing more variant-resistant vaccines.

Skewing of immune responses was achieved by masking immunodominant regions (Class I/II epitopes) through glycan obstruction. Glycans elicit immunosuppressive effects on regions of protein they are conjugated to and cover, a strategy utilised by several pathogenic viruses such as SARS-CoV-2 & HIV.

Membrane-bound RBD constructs were profiled for N-linked glycosylation motifs and a selection of single and double glycan motif knock-ins were designed (n=15). Designed RBD constructs were transfected into HEK-293 cells *in vitro* and then analysed through flow cytometry against a panel of class I, III and V binding antibodies. RBD constructs that demonstrated significant knockdowns in class I binding, but no effect on class III or V binding were determined to have effective glycan shielding of class I/II sites and viable protein expression.

Successful glycan RBD variants (n=2) were then transcribed into mRNA for encapsulation in Lipid Nanoparticles (LNP) formulation for *in vivo* immunisation. BALB/c mice (n=5) were immunised with 4ug of either WT-RBD or Glyc-RBD mRNA-LNP formulations via IM injections on days 0 and 21, with serum responses measured at day 35.

Immunised mice were sacrificed and spleens were harvested for lymphocyte immunophenotyping and antigen-positive B-cell quantification via flow cytometry. Antigen-positive lymphocytes will be stained with labelled ACE2-Fc to ascertain the level of class I skewing achieved by glycan blockade.

1. Wellington D, Yin Z, Kessler BM, Dong T. Immunodominance complexity: lessons yet to be learned from dominant T cell responses to SARS-COV-2. Curr Opin Virol. 2021 Oct;50:183-191. doi: 10.1016/j.coviro.2021.08.009. Epub 2021 Sep 8. PMID: 34534732; PMCID: PMC8424056.
2. Buettner, M.J., Shah, S.R., Saeui, C.T., Ariss, R. & Yarema, K.J. Improving Immunotherapy Through Glycodesign. Frontiers in Immunology 9(2018).
3. Bajic, G., et al. Influenza Antigen Engineering Focuses Immune Responses to a Subdominant but Broadly Protective Viral Epitope. Cell Host & Microbe 25, 827-835.e826 (2019).
4. Shinnakasu, R., et al. Glycan engineering of the SARS-CoV-2 receptor-binding domain elicits cross-neutralizing antibodies for SARS-related viruses. J Exp Med 218(2021).
5. Hauser, B.M., et al. Rationally designed immunogens enable immune focusing following SARS-CoV-2 spike imprinting. Cell Reports 38, 110561 (2022).

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Enhancing thermal stability of live virus vaccine using metal organic frameworks

Aimee Talbot[1, 2], Daniel Layton[1], Kerri Bruce[1], Ruhani Singh[3], Cara Doherty[3], Tania de Koning-Ward[2]

1. CSIRO, Geelong, VIC, Australia

2. Deakin University , Waurn Ponds , VIC

3. CSIRO Manufacturing , Clayton south, VIC

Vaccines have proven to be one of the most effective strategies in controlling infectious diseases and are a key component towards the protection of our health and economy. However, the success of vaccination programs often face considerable challenges in resource-limited regions, primarily due to the necessity of maintaining cold chain throughout transportation and

storage. The demand for temperature-stable vaccines has spurred research into innovative strategies for preserving vaccine efficacy under harsh environmental conditions for equitable distribution without the dependence of 'cold chain' logistics. Here, we report biomimetic-mineralization of a live viral vaccine using metal organic frameworks (MOFs) to enhance their storage stability. Using Newcastle disease virus vaccine NDV V4 as our candidate, we investigate the impact of different molar concentrations of MOF composites on encapsulation, reporting that an increase in molar concentrations influences recovery of virus. We describe the impacts of lyophilisation on MOF structural integrity, viral titre, and report on alternative techniques such as air drying or column drying, evaluating these strategies with a storage experiment at ambient temperatures. Additionally, we describe using a haemagglutination assay as a quick and easy method to measure MOF encapsulation of NDV, as well as report on a new cell line used in a tissue culture infectious dose assay (TCID50) that can accurately displays cytopathic effect of NDV without immunofluorescent staining.

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Circulating CXCR3+ CCR4+ and regulatory high-risk HLA class II-restricted islet-specific T cell phenotypes predict poor clinical outcome in children with T1D.

Gayathri M[2, 1], Hanno Nel[2, 1], Emma Hamilton-Williams[2, 1], Hugh Reid[3], Mai Thao T Tran[3], Jamie Rossjohn[4], Kerry Buchanan[5], Ranjeny Thomas[2, 1]

1. Frazer Institute, The University of Queensland, Brisbane

2. Translational Research Institute, South Brisbane, QUEENSLAND, Australia

3. Biochemistry and Molecular Biology, Monash University, Melbourne

4. Biochemistry & Molecular Biology, Monash Biomedicine Discovery Institute, Melbourne

5. Children's Health Queensland Clinical Unit, The University of Queensland, Brisbane

There is a growing need to identify biomarkers of Type 1 diabetes (T1D) progression to determine the efficacy and durability of therapies in clinical trials. Studies have found that a stronger proinflammatory immune landscape predicted faster beta cell decline during partial remission in subjects with T1D. Due to the well-documented, disease-causing role of islet-specific T cells, we aimed to characterise these cells in relation to clinical outcome in subjects with T1D. Using multiplexed HLADR4/DQ8-restricted proinsulin (PI) and PI- hybrid insulin peptide (HIP) tetramers in a comprehensive T cell phenotyping spectral flow cytometry panel, we stained frozen PBMC samples from patients, *ex vivo*. We found a disease-specific enrichment of CXCR3+ CCR4+ and exhausted regulatory T cell (Treg) phenotypes within islet-specific T cells. Interestingly, these phenotypes which were significantly correlated with shorter partial remission duration in children with T1D. This suggests that the upregulation of specific chemokine receptors on islet-specific T cells may induce their migration to inflamed islets to exacerbate chronic inflammation. This association of CXCR3+ CXCR4+ islet-specific T cells with decreased length of partial remission was also reflected in the global CD4+ and CD8+ T cell compartments, highlighting the potential of these circulating double-positive T cells as robust biomarkers of disease progression and response to therapy in clinical trials.

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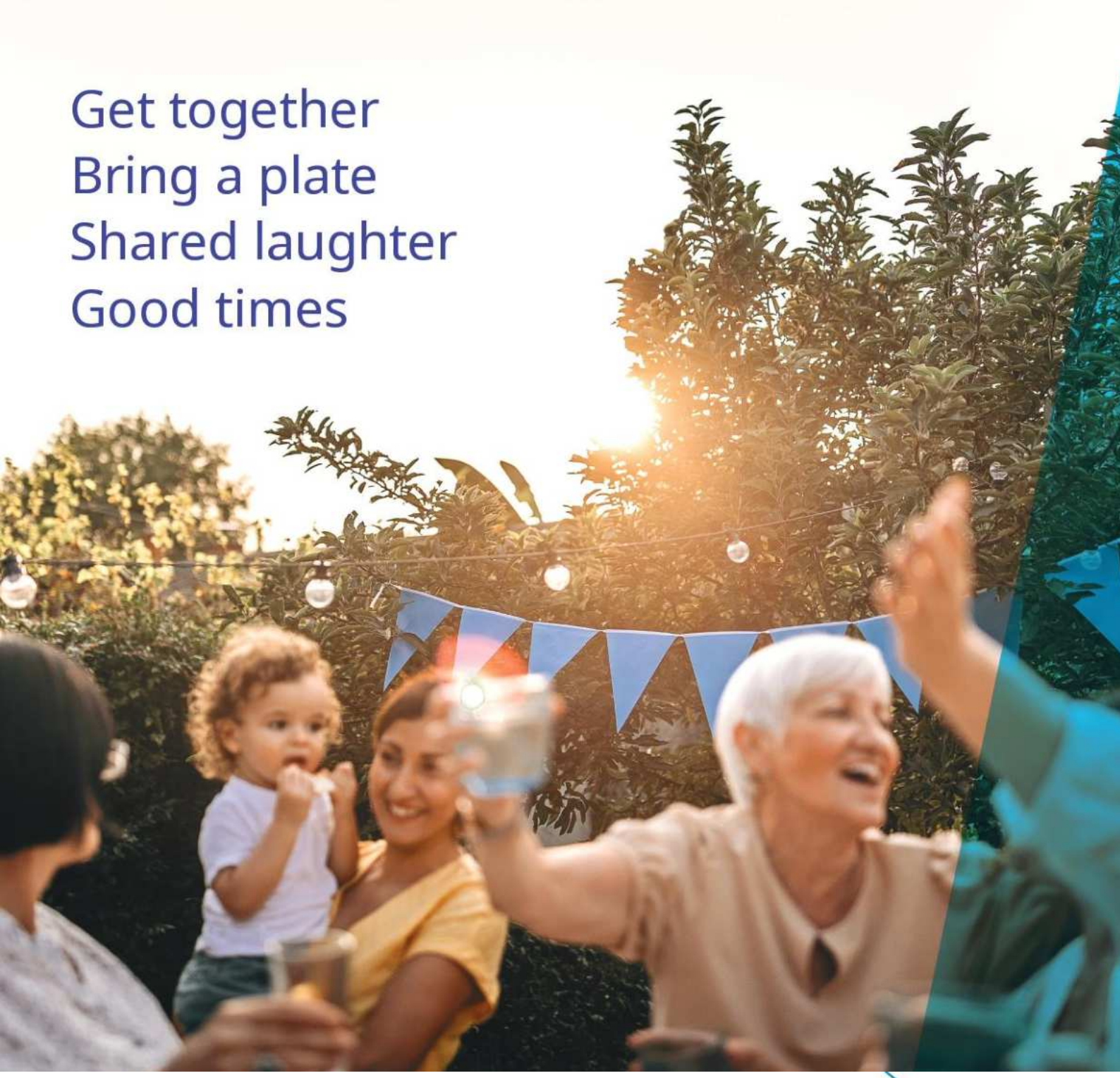

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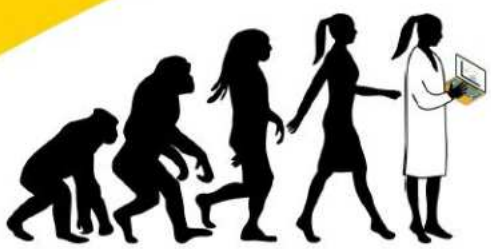

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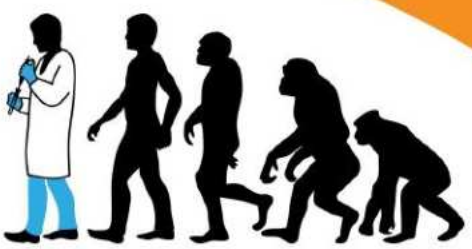

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