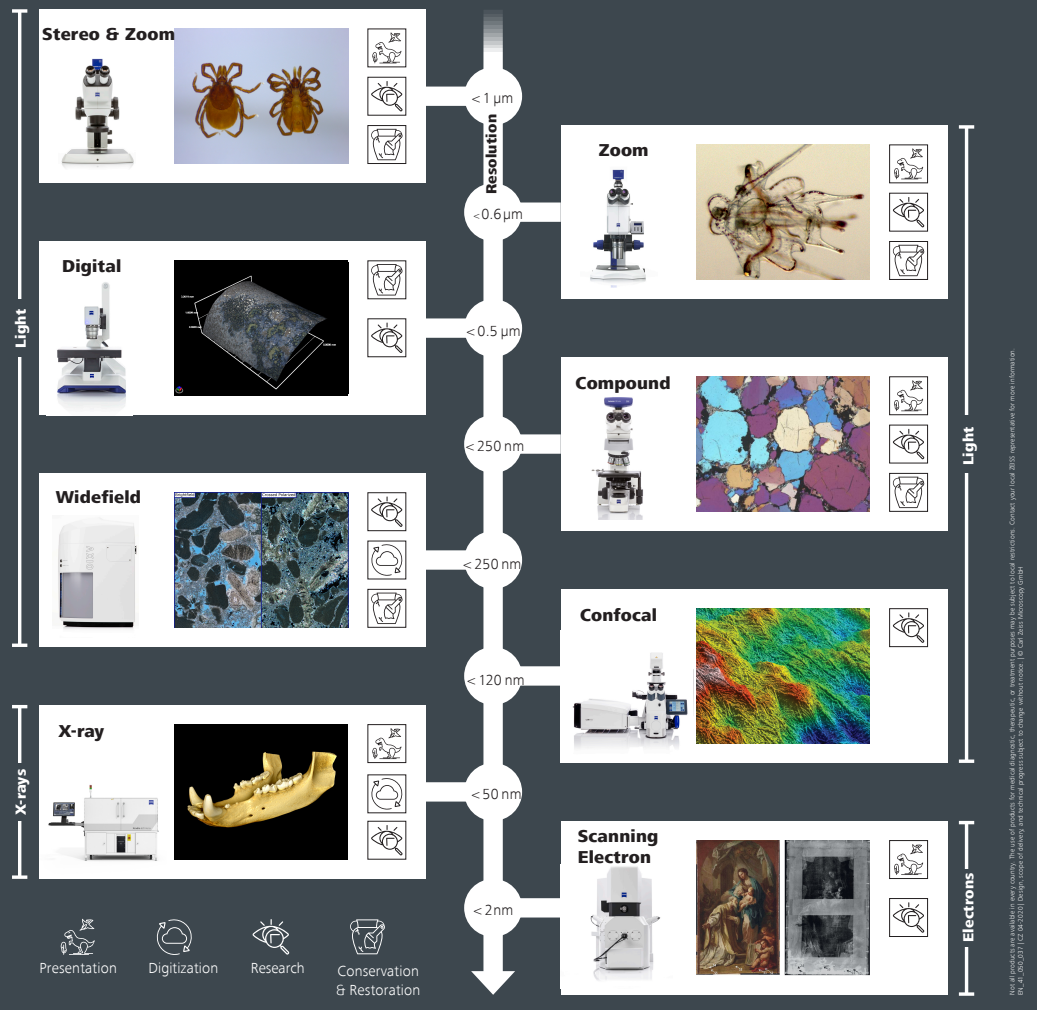
Cell Signalling
to Cancer Medicine

10th Barossa Meeting

ZEISS Microscopy Technologies for Your Museum



Carl Zeiss Microscopy GmbH
microinfo.au@zeiss.com
zeiss.com/microscopy

ZEISS

Seeing beyond

Contents

Meeting Secretariat

Lara Malcolm
The Meeting People Pty Ltd
PO Box 764, Mitcham SA 5062

T: 08 8177 2215
E: lara@themeetingpeople.com.au
W: www.themeetingpeople.com.au

Audio Visual + Website

Frank Stromski
https://www.barossameeting.org/

Welcome

Welcome to the 10th Barossa Meeting: *Cell Signalling* to Cancer Medicine

The biennial Barossa Meetings aim to bring together some of the world's best biomedical scientists across different disciplines in a convivial atmosphere conducive to open and energetic discussions. This year's theme of *Cell Signalling* to Cancer Medicine aims to continue this tradition and bring into focus the major new paradigms arising from fundamental discoveries and their impact on new therapeutic strategies in different malignancies. In addition to the numerous international delegates, the conference will be attended by many of the key Australian researchers in cell signalling and cancer medicine.

As with other meetings in this series, the 10th Barossa Meeting will also be the occasion to award the Clifford Prize for Cancer Research, recognising major contributions to the global fight against cancer.

We welcome you to what promises to be an exciting and enjoyable meeting.

Stuart Pitson
Co-Convenor, on behalf of the Organising and Program Committees

Organising Committee

Stuart Pitson (Co-Convenor)

Jose Polo (Co-Convenor)

Michael Brown

Lisa Butler

Briony Forbes

Yeesim Khew-Goodall

Angel Lopez

Michael Samuel

Program Committee

Claudine Bonder

Cameron Bracken

Michael Brown

Loretta Dorstyn

Briony Forbes

Luke Isbel

Yeesim Khew-Goodall

Luciano Marletto

Angel Lopez

Stuart Pitson

Jose Polo

Michael Samuel

Quenten Schwarz

Luke Selth

Sponsors

The 10th Barossa Meeting gratefully acknowledges the support of the following companies and organisations:

Bronze Sponsor

CSL

Sponsors

The Hospital Research Foundation Group

Leica MICROSYSTEMS

Lanyard Sponsor

MERCK

Exhibitors

NEW ENGLAND BioLabs

Cell Signaling TECHNOLOGY

TransnetYX Serving research. Saving time.

AUSTRALIAN biosearch

revvity

Integrated Sciences 40 YEARS

TECAN

nanoString

STORZ KARL STORZ—ENDOSKOPE

CYTEK TRANSCEND THE CONVENTIONAL

BioTools Innovative Tools for Life Sciences

Lastek Photonics Technology Solutions

Nikon

SAGC SOUTH AUSTRALIAN GENOMICS CENTRE

decode science enabling discovery

Hosted by

Centre for Cancer Biology

SAiGENCI SOUTH AUSTRALIAN IMMUNOGENOMICS CANCER INSTITUTE

Flinders University

General Information

Venue
Novotel Barossa Valley
42 Pioneer Avenue, Rowland Flat SA 5352
T: +61(08) 8524 0000
E: h3026-re1@accor.com

The plenary sessions will be held in the Shiraz Rooms. The exhibition, catering and posters will be located in the foyer and the Cabernet Room.

Registration Desk
The registration desk will be open at the following times:

Tuesday 14th November 11:00 - 17:00
Wednesday 15th November 08:00 - 17:00
Thursday 16th November 08:00 - 17:00
Friday 17th November 08:00 - 12:00

Name Badges
Each conference delegate will receive a name badge on registration. The badge will be your official pass and must be worn to gain entry to all sessions, lunch and refreshment breaks.

Speaker Preparation
All speakers must report to Frank Stromski, our Audio Visual Technician located in the Shiraz Rooms. Please load your talk with Frank during the breaks prior to your session. It is preferable to load at least two sessions prior to your session.

Poster Presenters
All Posters are up for the duration of the meeting. Posters should be portrait and no more than 1 metre wide x 1.2 metres long. Posters can go up from Tuesday afternoon from 12 pm and should be removed by the end of morning tea at 11 am on Friday. Poster authors should stand by their posters during from 5:00 pm – 7:00 pm on Thursday afternoon to answer queries in relation to your research. Velcro will be provided to affix your poster to the boards.

Abstract Book
All abstracts are available online for downloading prior to the start of the Meeting. Please refer to the link Meeting Handbook to obtain a copy to save to your device. No printed abstracts will be provided during the meeting.

WIFI
WIFI will be available at the Novotel Barossa Valley. A code will be given to you at the time of the Meeting.

Catering Breaks and Special Diets
All catering breaks will be located in the foyer with the exhibitions. We are very grateful for the support of our sponsors and encourage you to take the time to visit them during the breaks. The waiting staff have been advised of any special diets to date. Please see the staff at the Registration Desk or the wait staff to locate your requirements.

Mobile Phones
Please ensure that all mobile phones are switched to silent mode during scientific sessions.

Social Events

Welcome BBQ Mixer
Date: Tuesday 14 November 2023
Time: 7:00 pm
Venue: Poolside, Novotel Barossa Valley
Cost: Inclusive for full and student registrations.
Extra Tickets: $75 per person

Gala Dinner
Date: Wednesday 15 November 2023
Time: 7:00 pm (coaches depart Novotel Barossa Valley and return between 10:30-11:00 pm)
Dinner: 7:30-11:00 pm
Venue: Lambert Estate, 55 Long Gully Road, Angaston
Cost: Inclusive for full and student registrations.
Extra Tickets: $190 per person (includes coach transfers to dinner)

Final Dinner
Date: Thursday 16 November 2023
Time: 7:00 pm
Venue: Shiraz Rooms, Novotel Barossa Valley
Cost: Inclusive for full and student registrations.
Extra Tickets: $130 per person

Invited Speakers

Maté Biro
University of New South Wales

Kristin Brown
Peter MacCallum Cancer Centre

Andrew Cox
Peter MacCallum Cancer Centre

David Croucher
Garvan Institute

Raelene Endersby
Telethon Kids Institute

Matthias Ernst
Olivia Newton-John Cancer Research Institute

Alistair Forrest
Harry Perkins Institute

Laura Mackay
Peter Doherty Institute

Ravindra Majeti
Stanford University

James Murphy
Garvan Institute

Heidi Neubauer
University of Veterinary Medicine Vienna

Antonella Papa
Monash University

Enrico Petretto
Duke-National University of Singapore

Jasmine Plummer
St Jude's Children's Research Hospital

John Scott
University of Washington

Alex Swarbrick
Garvan Institute

Valerie Weaver
University of California San Francisco

Program

Tuesday 14th November 2023

13:00–14:30	*Registration*	Foyer
14:30–14:40	***Welcome** – Stuart Pitson*	Shiraz Rooms
14:40–16:20	**Session 1: Cell Reprogramming in Cancer** Chairs: Richard D'Andrea and Tom Gonda	
14:40-15:10	**Ravindra Majeti** (Stanford University, USA) "Stem cells and reprogramming in human acute leukemia"	
15:10-15:30	**Luke Isbel** (SAiGENCI, Adelaide) "Specificity in genome regulation through transcription factor sensitivity to chromatin"	
15:30-15:45	**Winnie Kan** (Centre for Cancer Biology, Adelaide) "Transcriptional regulation of stemness programs in acute myeloid leukaemia by IL-3 receptors with different stoichiometries"	
15:45-16:05	**Andrew Cox** (Peter MacCallum Cancer Institute, Melbourne) "The Keap1-Nrf2 pathway regulates lysosomal biogenesis and cell fate determination"	
16:05-16:20	**Saumya Samaraweera** (Centre for Cancer Biology, Adelaide) "Hypermethylation of the GADD45A promoter identifies cooperating mechanisms of altered RNA biology in IDH1 and 2-mutant AML"	
16:20–16:50	*Coffee break and exhibition*	Foyer
16:50–18:35	**Session 2: Cell Signalling Networks** Chairs: Stuart Pitson and Philip Gregory	Shiraz Rooms
16:50-17:20	**John Scott** (University of Washington, USA) "Recruitment of BAG2 to DNAJ-PKAc scaffolds promotes cell survival and resistance to drug-induced apoptosis in fibrolamellar carcinoma"	
17:20-17:40	**David Croucher** (Garvan Institute, Sydney) "Memory of stochastic single-cell apoptotic signalling promotes chemoresistance in neuroblastoma"	
17:40-18:00	**Theresa Hickey** (SAiGENCI, Adelaide) "Androgen receptor signalling in breast cancer and normal breast tissues"	
18:00-18:20	**Antonella Papa** (Monash University, Melbourne) "Decoding roles of the PTEN-P13K axis in breast cancer progression and therapeutic response"	
18:20-18:35	**Amelia Parker (**Garvan Institute, Sydney) "Prognostic extracellular matrix profiles are associated with pro-tumourigenic signalling in the squamous cell carcinoma subtype of non-small cell lung carcinoma"	
19:00	***Dinner – Pool-side BBQ at Novotel***	

Program

Wednesday 15th November 2023

09:00–10:10 **Session 3: Cell Fate in Development and Cancer** — Shiraz Rooms
Chairs: Pascal Duijf and Luke Selth

09:00-09:20 **Natasha Harvey** (Centre for Cancer Biology, Adelaide)
"TBA"

09:20-09:35 **Natalie Lister** (Monash University, Melbourne)
"A reprogramming strategy to restore normal gene networks in aggressive prostate cancer"

09:35-09:50 **Teresa Sadras** (Peter MacCallum Cancer Institute, Melbourne)
"Subconal mutations alter core signalling nodes and drug responses in acute lymphoblastic leukaemia"

09:50-10:10 **Quenten Schwarz** (Centre for Cancer Biology, Adelaide)
"Ubiquitination regulates key intercellular signalling pathways driving cardiac development"

10:10–10:40 *Coffee break and exhibition* — Foyer

10:40–12:35 **Session 4: Cancer Immunology and Signalling** — Shiraz Rooms
Chairs: Marcel Nold and Michael Brown

10:40-11:00 **Laura Mackay** (Doherty Institute, Melbourne)
"Deconvoluting tissue-resident memory T cells in tumours"

11:20-11:40 **Matthias Ernst** (Olivia Newton-John Cancer Research Institute, Melbourne)
"Inhibition of myeloid cell-specific HCK kinase activity confers immune check point blockade activity in pancreatic and ovarian cancer"

11:40-12:00 **Briony Forbes** (Flinders University, Adelaide)
"TBA"

12:00-12:15 **Sean Porazinski** (Garvan Institute, Sydney)
"Anti-fungal modulation of the immune-suppressive tumour microenvironment overcomes immunotherapy resistance in pancreatic ductal adenocarcinoma"

12:15-12:35 **Michael Brown** (Centre for Cancer Biology, Adelaide)
"GD2-CAR-T cell therapy for aggressive primary brain tumours"

12:35–13:35 *Lunch and exhibition* — Foyer

13:35–15:00 **Session 5: Spatial Profiling of Cancer** — Shiraz Rooms
Chairs: Luciano Martelotto and Greg Goodall

13:35-14:05 **Jasmine Plummer** (St Jude's Children's Research Hospital, USA)
"How will single cell and spatial analysis help with cancer medicine? A case study of triple-negative breast cancer"

14:05-14:25 **Alex Swarbrick** (Garvan Institute, Sydney)
"Systems immunology of breast cancer"

14:25-14:45 **Kristen Feher** (SAiGENCI, Adelaide)
"Decomposing gene expression using Gene Over-Representation Projection Pursuit"

14:45-15:00 **Brooke Pereira** (Garvan Institute, Sydney)
"Temporal mass spectrometry proteomics and advanced microscopy to resolve matrisomal drivers of pancreatic cancer"

Program

Wednesday 15th November 2023 continued…

15:00–15:30	*Coffee break and exhibition*	Foyer
15:30–17:00	**Session 6: Novel Therapeutics and Therapy Resistance** Chairs: Briony Forbes and Wendy Ingman	Shiraz Rooms
15:30-15:50	**Raelene Endersby** (Telethon Kids Institute, Perth) "Considering the Tumour Immune MicroEnvironment of children's brain cancer: Is it TIME for better models?"	
15:50-16:10	**Luke Selth** (Flinders University, Adelaide) "CDK9 inhibition disables multiple oncogenic transcriptional and epigenetic pathways in prostate cancer"	
16:10-16:30	**Nirmal Robinson** (Centre for Cancer Biology, Adelaide) "Unravelling the role of "Don't eat me signal" CD47 in regulating cellular and metabolic plasticity"	
16:30-16:45	**Mark Bunting** (SAiGENCI, Adelaide) "Advancing treatment options for triple negative breast cancer through NF-kappa B inhibition"	
16:45-17:00	**Ana Lonic** (Centre for Cancer Biology, Adelaide) "Targeting phosphorylation of specific proteins - A novel approach to overcome chemoresistance in TNBC"	
	Break	
18:00–19:00	**Clifford Prize Presentation and Lecture**	Shiraz Rooms
19:00-23:00	***Dinner – off-site at Lambert Estate*** *Coaches depart the Novotel Barossa Valley at 19:00. Coaches will return to the hotel and depart Lambert Estate between 22:30-23:00.*	

Program

Thursday 16th November 2023

09:00–10:45	**Session 7: Systems Biology and Cancer** Chairs: Chris Sweeney and Lan Nguyen	Shiraz Rooms
09:00-09:30	**Enrico Petretto** (Duke-National University of Singapore, Singapore) "WWP2, an unexpected therapeutic target for multiorgan fibrosis"	
09:30-09:45	**Katherine Pillman** (Centre for Cancer Biology, Adelaide) "Understanding the neuroblastoma transcriptome: identifying molecular drivers for better tumour stratification"	
09:45-10:05	**Alistair Forrest** (Harry Perkins Institute, Perth) "Studying ovarian cancer subclones and their microenvironments using spatial transcriptomics"	
10:05-10:25	**Fuyi Li** (SAiGENCI, Adelaide) "ProsperousPlus: a one-stop and comprehensive platform for accurate protease-specific substrate cleavage prediction and machine-learning model construction"	
10:25:10:45	**Chris Hahn** (Centre for Cancer Biology, Adelaide) "Genetic conundrums and complexities in familial haematological malignancies"	
10:45–11:15	*Coffee break and exhibition*	Foyer
11:15–12:25	**Session 8: Cancer Metabolism** Chairs: Lisa Butler and Joanna Woodcock	Shiraz Rooms
11:15-11:35	**Kristen Brown** (Peter MacCallum Cancer Institute, Melbourne) "Metabolic regulation of tumour cell MHC-I antigen presentation"	
11:35-11:55	**Julie-Ann Hulin** (Flinders University, Adelaide) "Inducing synthetic lethality in prostate and breast cancer via androgens and lipids"	
11:55-12:10	**Joshua Hodgson** (SAiGENCI, Adelaide) "Acquired resistance to CDK4/6 inhibition leads to metabolic re-writing and protection against ferroptosis in prostate cancer"	
12:10-12:25	**Alana White** (Flinders University, Adelaide) "Chronic lymphocytic leukaemia cells have a unique lipid profile and survive in vitro when cocultured with adipocytes"	
12:25–13:25	*Lunch* (includes presentation by Leica Microsystems)	
13:25–17:00	Free Discussion	
17:00-19:00	**Poster Session**	Cabernet Rooms
19:00	***Conference Dinner***	Shiraz Rooms

Program

Friday 17th November 2023

09:00–10:30	**Session 9: Cancer Biology to Therapy** Chairs: Yeesim Khew-Goodall and Teresa Sadras	Shiraz Rooms
09:00-09:20	**Heidi Neubauer** (University of Veterinary Medicine, Vienna) "Uncovering disease mechanisms and targeted therapies in hepatosplenic T cell lymphoma"	
09:20-09:40	**James Murphy** (WEHI, Melbourne) "Tales From the Crypt: how RIPK3 kinase unleashes the zombie protein, MLKL, to kill cells by necroptosis"	
09:40-09:55	**Jason Powell** (Centre for Cancer Biology, Adelaide) "Overcoming Bcl-2 inhibitor resistance in acute myeloid leukemia"	
09:55-10:10	**Kimberly Clark** (SAiGENCI, Adelaide) "Preventing resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer"	
10:10-10:30	**Claudine Bonder** (Centre for Cancer Biology, Adelaide) "A novel role for intercellular adhesion molecule (ICAM)-1 on breast cancer cells"	
10:30–11:00	*Coffee break and exhibition*	Foyer
11:00–12:25	**Session 10: Tumour Microenvironment** Chairs: Michael Samuel and Paul Timpson	Shiraz Rooms
11:00-11:30	**Valerie Weaver** (University of California San Francisco, USA) "Interplay between Inflammation, Anti-tumor Immunity and Tissue Tension"	
11:30-11:50	**Maté Biro** (University of New South Wales, Sydney) "Mechanobiology of cytotoxic lymphocyte-mediated solid tumour rejection"	
11:50-12:05	**Sarah Boyle** (Centre for Cancer Biology, Adelaide) "Compressive stress promotes mammary tumour progression via Piezo1-CaMKII-RhoA-ROCK mechanotransduction signalling"	
12:05-12:25	**Thomas Cox** (Garvan Institute, Sydney) "A novel first-in-class anti-fibrotic blunts tumour desmoplasia, rewires stromal signalling and augments gemcitabine response and survival in pancreatic cancer"	
12:25	***Closing Remarks – Jose Polo***	
12:30	***Conference End***	

Hosted by

Centre for Cancer Biology

SAiGENCI
SOUTH AUSTRALIAN IMMUNOGENOMICS CANCER INSTITUTE

Flinders University

Posters

P1 Targeting subcellular JNK activity in pancreatic cancer
Antonia Cadell, *Brooke Pereira, Marina Pajic, Paul Timpson, David Croucher*

P2 Rationalising the inclusion of HDAC inhibitors with standard-of-care chemotherapy for high-risk neuroblastoma
Monica Phimmachanh, *Jeremy Han, King Ho Leong, Sharissa Latham, David Croucher*

P3 CDK9 inhibition as a therapeutic strategy in advanced prostate cancer
Razia Rahman, *Muhammed Rahaman, Adrienne R. Hanson, Jianling Xie, Scott L. Townley, Nicholas Choo, Kaylene J. Simpson, Susanne Ramm, Ganessan Kichenadasse, Simon J. Conn, Gail P. Risbridger, Renea A. Taylor, Mitchell G. Lawrence, Wayne Tilley, Margaret M. Centenera, Lisa M. Butler, Shudong Wang, Luke A. Selth*

P4 Desmoglein-2 expression by multiple myeloma is an independent predictor of poor prognosis that can be rapidly identified by flow cytometry
Barbara McClure, *Charlotte Downes, Lisa Ebert, Kate Vandyke, Zahied Johan, Lih Tan, Giles Best, Kay K Myo Min, Andrew Zannettino, Stuart Pitson, Craig Wallington-Gates, Claudine Bonder*

P5 Monitoring AKT activity and targeting in live tissue and disease contexts revealed by the novel Akt-FRET biosensor mouse
James R.W. Conway, Sean C. Warren, Young-Kyung Lee, Andrew T. McCulloch, Astrid Magenau, Victoria Lee, Xanthe L. Metcalf, Janett Stoehr, Katharina Haigh, Lea Abdulkhalek, Cristian S. Guaman, Daniel A. Reed, Kendelle J. Murphy, Brooke A. Pereira, Pauline Melenec, Cecilia R. Chambers, r Sharissa L. Latham, Helen Lenthall, Elissa K. Deenick, Yuanqing Ma, Tri Phan, Elgene Lim, Anthony M. Joshua, Stacey Walters, Shane T. Grey, Yan-Chuan Shi, Lei Zhang, Herbert Herzog, David Croucher, Andy Philp, David Herrmann, Owen J. Sansom, Jennifer P. Morton, Antonella Papa, Jody J. Haigh, ***Max Nobis***, *Paul Timpson*

P6 Inter-patient heterogeneity across patient-derived glioblastoma explant organoids
Kaitlin Scheer, *Erica Yeo, Chloe Shard, Helen Palethorpe, Conor Ryan, Sakthi Lenin, Melinda Tea, Santosh Poonnoose, Minh-Son To, Rebecca Ormsby, Stuart Pitson, Lisa Ebert, Guillermo Gomez*

P7 Investigating a novel matrisomal target in pancreatic cancer to reduce fibrosis and improve standard-of-care chemotherapy.
Jessie Zhu, *Cecilia Chambers, Shona Ritchie, Morghan Lucas, Daniel Reed, Alice Tran, Kendelle Murphy, Diego Chacon-Fajardo, Benjamin Parker, Marina Pajic, Jennifer Morton, Thomas Cox, Brooke Pereira, David Herrmann, Paul Timpson*

P8 Advanced 3D cell culture platform for recapitulating intestine tissues
Chia-Chi Chien, *Chia-Lin Chien, Theodora Almond, Ilka Priebe, Chun-Hsien Chen, Rajvinder Singh, Kim Fung*

P9 Understanding the Dynamics of MEKK1 auto-regulation within Cellular Signalling Responses
Alex Bohles, *Boaz Ng, David Croucher, Peter Mace*

Posters

P10 Dual epithelial and stromal targeting in triple negative breast cancer using ROCK2 inhibition
***Daniel Reed**, Kendelle Murphy, Man Nobis, Brooke Pereira, Astrid Magenau, Cecilia Chambers, Anna Howell, Sunny Wu, Julia Chen, Kate Harvey, Denise Attwater, David Gallego-Ortega, Alex Swarbrick, Thomas Cox, Anthony Gill, Sandra O'Toole, Liz Caldon, Elgene Lim, Paul Timpson, David Herrmann*

P11 Lighting the fire: Turning up the heat in the prostate cancer immune microenvironment
***Sam Rollin**, Scott Townley, Adrienne Hanson, Michael Michael, Luke Selth*

P12 Generation and validation of anti-linker monoclonal antibodies for the surface detection of scFv-based CARs
Puiyi Tiffany Pang

P13 Single Cell Scoring of Molecular Phenotypes
***Malvika Kharbanda**, Dharmesh Bhuva, Melissa Davis*

P14 An orthotopic syngeneic mouse model of bortezomib-resistant multiple myeloma
Manjun Li

P15 Mouse models of venetoclax-resistant acute myeloid leukemia for pre-clinical evaluation of new therapeutic approaches
***Gus Nwosu**, Victoria Pope, Paul Moretti, John Toubia, Alexander Lewis, Melinda Tea, Richard D'Andrea, Stuart Pitson, Jason Powell*

P16 ROCK activation promotes tumour progression in the intestine
***Zahied Johan**, Natasha Pyne, Sarah Boyle, Michael Samuel*

P17 Understanding the role of ROCK signalling pathway in modulating the functional characteristics of the tumour extra-cellular matrix
***Chun-Hsien Chen**, Edward Jack Buckley, Claudine Bonder, Michael S. Samuel*

P18 Investigating the Role of Rho-ROCK Signalling in Breast Cancer Metastasis
***Moganalaxmi Reckdharajkumar**, Sarah T. Boyle, Gregory J. Goodall, Michael S. Samuel*

P19 Antibody Drug Conjugates and Pyroptosis: Using Fire to Fuel Anti-Tumour Immunity
***Nicole Wittwer**, Alexander H Staudacher, Vasilios Liapis, Michael P Brown*

P20 Understanding beta common cytokine pleiotropy through mimetic ligands
***Tim Hercus**, Winnie Kan, Karen Cheung Tung Shing, Tracy Nero, Ta-Yi Yu, Marc Exposit, David Baker, Michael Parker, Angel Lopez*

P21 Understanding the role of the Rho-ROCK pathway in modulating mammary tumour immunity
Edward J. Buckley, Natasha Kolesnikoff, M. Zahied Johan, Sarah T. Boyle and Michael S. Samuel

P22 A new understanding of the role of 14-3-3 proteins in lung cancer
Jo Woodcock, Rhys Hamon, Carl Coolen, Xin Jiang, Clifford Young, Angel Lopez and Stuart Pitson

P23 Examining the efficacy of targeting mutant TET2 in AML
Leeann Desouza, Keith Lau, Victoria Pope, Daniel Thomas, Jason Powell, Stuart Pitson

The Clifford Prize

The Clifford Prize recognizes international excellence in Cancer Research.

The Prize represents an appreciation by Australian Scientists of the outstanding scientific discoveries that have laid the foundation of new and significant cancer therapies. The Prize consists of a Perpetual Trophy and a glass sculpture (both manufactured by Nick Mount). The awardee also receives a magnum of Clarendon Hills 'Astralis'.

The presentation of the Prize will always be linked to the biennial Barossa signalling meetings. This conjunction recognizes the importance of high-powered small meetings as one of the major drivers of new ideas, gives the possibility for more junior scientists to interact closely with the recipients of the Prize, and emphasizes Australia, and in particular South Australia, as a leader in scientific as well as gastronomic and epicurean innovation.

The Prize was initiated by the organizers and their home Division of Human Immunology, in the Hanson Centre for Cancer Research, now a major component of the Centre for Cancer Biology, and is named after Bob Clifford, a previous Chairman of the Council of the Institute of Medical and Veterinary Science.

Previous recipients of the Clifford Prize for Cancer Research include:

2005: Axel Ullrich of the Max Planck Institute of Biochemistry, Germany, for his discovery of the HER2/neu oncogene, the basis of the therapeutic Herceptin for the treatment of breast cancer.

2007: Tony Hunter of the Salk Institute, La Jolla, USA, for his discoveries that proteins can undergo phosphorylation on tyrosine, and of tyrosine kinases.

2009: John Dick of University of Toronto, Ontario, Canada, for his work on cancer stem cells, their biology and their potential as therapeutic targets.

2011: Vishva Dixit of Genentech, South San Francisco, USA, for his work on the cell death pathway and pro-inflammatory signalling in cancer.

2013: Arul Chinnaiyan of University of Michigan Medical School, Ann Arbor, USA, for his work on the pathogenesis of breast and prostate cancer, and cancer bioinformatics.

2015: Joint winners Inder Verma of the Salk Institute for Biological Studies, La Jolla, USA and **Jane Visvader** of the Walter and Eliza Hall Institute, Melbourne, for their work in targeted cell therapies for cancer.

2017: **Joseph Schlessinger** of Yale University, New Haven, USA, for his work on the receptor tyrosine kinase (RTK).

2019: **Karen Vousden** of the Francis Crick Institute, London, UK, for her work on the the tumour suppressor gene, p53, that is disrupted in most human cancers.

This year's Prize was awarded by a committee comprised of Professors Mathew Vadas, Jenny Stow, and Angel Lopez, and Professors Tony Hunter, John Dick, Vishva Dixit, Arul Chinnaiyan, Inder Verma, Jane Visvader, and Karen Vousden as the previous winners.

The Clifford Prize

‘Discovery leaning on Learning’

The main part of the sculpture represents a seed pod and figuratively refers to the germination of ideas that underlie great discovery. Within the seed pod are white lines representing DNA – the main culprit of cancer, but also a source of continual regeneration.

The glass seed pod was made by traditional Italian Zanfirrico techniques and the ballottini technique was used to generate the helices. The outside of the seed pod was treated by the battuto method to gain its feeling of cellularity.

The main glass sculpture rests on three books - representing learning, two made of hard steel and one of hand-made paper.

A jarring wrought iron spike unites the two parts of the sculpture, representing the wounding of the natural harmonies by disease.

Designer Nick Mount is one of Australia’s preeminent glass artists. His career, spanning three decades, combines virtuoso technique with a keen instinct for design, freely adapting traditional Venetian decorative styles to his own distinctive sculptural approach. Recognised for his commissions, teaching, and exhibitions in Australia, Europe, South America, the United States and Japan, his work is held in many major public and private collections.

Awardee 2023

Professor Ravindra Majeti

The Clifford Prize for Cancer Research 2023 Recipient

Acute myeloid leukemia (AML) is the most common form of aggressive leukemia in adults and has had poor survival rates, especially for older patients. Dr. Majeti and his team are developing new treatments for AML based on their investigations of the identity and functions of leukemia stem cells (LSCs).

Dr. Majeti's team has enhanced the understanding of the role of CD47 as a dominant macrophage immune checkpoint and "do not eat me" signal on leukemic cells and their interactions with the innate immune system. This has led to the development of a first-in-class anti-CD47 antibody, known as magrolimab, that is being tested in clinical trials for AML patients. CD47 is a dominant macrophage immune checkpoint and "do not eat me" signal on AML and LSCs. AML cells upregulate CD47 to evade macrophage phagocytosis and elimination. Therapeutic blockade of the CD47 pathway with the anti-CD47 antibody leads to macrophage phagocytosis of AML cells and in preclinical models leads to disease clearance in vivo, including elimination of LSCs. Dr. Majeti's team is also unravelling the roles of mutations in AML, which develops from the sequential acquisition of multiple mutations in a single lineage of cells.

Dr. Majeti's team has found these mutations initially occur in pre-leukemic stem cells, and are enriched in genes that regulate the epigenome. They have modeled this pre-leukemic state by engineering these mutations into normal human hematopoietic stem and progenitor cells and observed that similar to humans, in some cases these engineered cells would spontaneously progress to myeloid disease in vivo. Their efforts to elucidate potential therapeutic vulnerabilities of the pre-leukemic HSCs raise the possibility of targeting these cells prior to progression. They are continuing to investigate the properties of AML stem cell subclones and have identified mutation-specific vulnerabilities, revealing multiple targets that respond to genetic and/or pharmacologic modulation in primary AML cells and xenograft models.

Oral Abstracts

Session 1: Cell Reprogramming in Cancer

Stem Cells and Reprogramming in Human Acute Leukemia

Ravindra Majeti
Department of Medicine, Division of Hematology, Cancer Institute, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, USA

AML is an aggressive blood malignancy driven by leukemic stem cells (LSCs) that must be eradicated for disease cure. Genomic studies have demonstrated that AML develops from the sequential acquisition of multiple mutations that initially occur in pre-leukemic HSCs. We have focused on understanding the biology of LSCs and pHSCs as keys to developing novel therapeutic strategies.

Separately, we have developed methods for the lineage reprogramming of B-ALL cells into APCs that can stimulate leukemia-eradicating T cell immunity. Therapeutic cancer vaccination seeks to elicit activation of tumor-reactive T cells capable of recognizing tumor-associated antigens (TAA) and eradicating malignant cells. Here, we present a cancer vaccination approach utilizing myeloid-lineage reprogramming to directly convert cancer cells into tumor-reprogrammed antigen-presenting cells (TR-APC). Using syngeneic murine leukemia models, we demonstrate that TR-APCs acquire both myeloid phenotype and function, process and present endogenous TAAs, and potently stimulate TAA-specific CD4+ and CD8+ T cells. *In vivo* TR-APC induction elicits clonal expansion of cancer-specific T cells, establishes cancer-specific immune memory, and ultimately promotes leukemia eradication. We further show that both hematologic cancers and solid tumors, including sarcomas and carcinomas, are amenable to myeloid lineage reprogramming into TR-APCs. Finally, we demonstrate the clinical applicability of this approach by generating TR-APCs from primary clinical specimens and stimulating autologous patient-derived T cells. Thus, TR-APCs represent a cancer vaccination therapeutic strategy with broad implications for clinical immuno-oncology.

Oral Abstracts

Session 1: Cell Reprogramming in Cancer

Specificity in genome regulation through transcription factor sensitivity to chromatin

Dr Luke Isbel[1]
[1]*SAiGENCI – South Australian immunoGENomics Cancer Institute. ACE – Adelaide Centre for Epigenetics, Adelaide, Australia*

There is a remarkable degree of specificity in the establishment and maintenance of cellular identity, given that our cells have the same DNA but vastly different gene expression profiles. The mechanistic basis of this is a complex network of gene activation and repression events. Profiling at the cellular and genomic level reveals extensive correlations and there is little doubt that 'epigenetic' forces contribute to normal and disease states. However, critical insights into such forces are necessary to move beyond correlation and establish causation.

Cancer represents a dramatic shift in cellular identity, in large part due to the deregulation of proteins called transcription factors (TFs), which read-out DNA sequence to activate genes. For instance, key TFs like p53 enable stress response and are frequently suppressed in cancer. At the same time, TFs navigate a chromatinized genome and it is generally assumed that chromatin structure works 'hand-in-hand' with TFs by tuning their potential to activate genes. Understanding the molecular basis of this process is critical.

Recent developments with *in vivo* and *in vitro* approaches now enable the dissection of TF binding and activity determinants at an unprecedented molecular level. We have utilized reductionist stem cell models to reveal that chromatin states act as barriers to TF function, including at the level of nucleosomes and histone modifications. Importantly, these insights suggest that TFs vary in their ability to navigate chromatin states in a manner that is highly protein dependent. Our findings provide a future framework to understand why some TFs but not others can drive cellular identity, so-called 'pioneering activity'. Additionally, they suggest how targeting TF activity in cancer may be achieved by focusing on chromatin binding cofactor proteins.

Oral Abstracts

Session 1: Cell Reprogramming in Cancer

Transcriptional regulation of stemness programs in acute myeloid leukaemia by IL-3 receptors with different stoichiometries

Dr Winnie Kan[1], Dr Kerstin Kaufmann[2], Dr Timothy Hercus[1], A/Prof Daniel Thomas[3], A/Prof Luciano Martelotto[4], Dr Adrienne Sullivan[4], Prof Jose Polo[4], Dr Saumya Samaraweera[5], Dr Denis Tvorogov[1], Prof Richard D'Andrea[5], Prof John Dick[2], Prof Michael Parker[6], Prof Angel Lopez[1]
[1]Cytokine Receptor Laboratory, Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, Australia, [2]Princess Margaret Cancer Centre, University Health Network, Toronto, Canada, [3]Department of Medicine, University of Adelaide, Adelaide, Australia, [4]Adelaide Centre of Epigenetics and South Australian Immunogenomics Cancer Institute, The University of Adelaide, Adelaide, Australia, [5]Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, Australia, [6]Department of Biochemistry and Pharmacology and the ACRF Facility for Innovative Cancer Drug Discovery, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Australia

Acute myeloid leukemia (AML) is a devastating disease with dismal prognosis. Leukemia stem cells (LSC) possess self-renewal and arrested differentiation properties that are responsible for disease emergence, therapy failure and recurrence in AML, however, the transcriptional programs that become unregulated in these cells are not understood.

We have recently showed that the pleiotropic cytokine interleukin-3 (IL-3) controls the balance between stemness and differentiation in LSCs. Using crystallographic, super-resolution FLIM-FRET, functional and transcriptomic approaches, we showed that LSC fate is controlled through the assembly of the IL-3 receptor (IL-3R) into distinct hexamer and dodecamer configurations due to different ratios of IL-3Rα vs IL-3Rβc expression. The hexamer activates distinct signalling and transcriptional programs to induce stemness whereas the dodecamer mediates differentiation. Importantly, the IL3Rα to βc ratio varies across cell types in the AML hierarchy, with the highest ratios in LSCs directly driving hexamer assembly and biasing activation of stemness programs and function(1). Our discovery raises tantalizing questions regarding the transcriptional and epigenetic regulation of stemness programs mediated by differential IL-3Rα/βc ratios and resulting alternative hexamer vs dodecamer assemblies.

Using our IL-3R hexamer vs dodecamer gene signatures, our current studies are using single cell RNA-seq in high vs low IL3Rα/βc ratio patient samples to dissect IL-3-induced hexamer and biased STAT signalling in distinct stem and progenitor cell subsets for stemness and cell state plasticity in the AML hierarchy. This will be integrated with ATAC-seq studies in our unique stem and progenitor cell models to identify the critical transcription factor regulatory networks underlying IL-3-driven cell fate decisions. Together with our work to define the transcriptional and epigenetic regulation of the IL3Rα/βc ratio, we aim to reveal how pathogenic genetic mutations work in synergy with hexameric signalling to drive leukaemogenesis.

References

1 Kan, W. L. et al. Cancer Discovery 13, 1922-1947, (2023).

Oral Abstracts

Session 1: Cell Reprogramming in Cancer

The Keap1-Nrf2 pathway regulates lysosomal biogenesis and cell fate determination

Andrew Cox
Peter MacCallum Cancer Institute, Melbourne

The maintenance of redox homeostasis is essential for cell survival and function. A central regulator of redox homeostasis is the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1). Recent studies have found that activating mutations in Nrf2 and Keap1 are frequent in patients with liver cancer. Our research aims to explore the cellular and molecular consequences of Nrf2 activation in the context of development and liver cancer. To interrogate this, we established a hepatocyte-specific inducible Nrf2 over-expression model in zebrafish (TO-Nrf2^{T80K}). Overexpression of the oncogenic mutant form of Nrf2 in adult zebrafish caused reversible transdifferentiation of hepatocytes to cholangiocytes. Mechanistically, we find that Nrf2 activation stimulates aberrant activation of the Tfeb/Tfe3 transcriptional program of lysosomal biogenesis. Using a complementary approach, we find that lysosomal biogenesis is also regulated by Keap1. Importantly, we find that the Nrf2-driven activation of Tfeb/Tfe3-dependent lysosomal biogenesis is evolutionarily conserved among vertebrates. Our studies identify a previously undescribed feature of Nrf2 involving activation of Tfeb/Tfe3-driven lysosomal biogenesis. More broadly, we hypothesize that lysosomal biogenesis and alteration of cell fate play a central role in Nrf2-driven cancers.

Oral Abstracts

Session 1: Cell Reprogramming in Cancer

Hypermethylation of the GADD45A Promoter Identifies Cooperating Mechanisms of Altered RNA Biology in IDH1 and 2-Mutant AML

Dr Saumya Samaraweera[1], Yaseswini Neelamraju[2], John Toubia[1,3], Duan Hassane[4], David Ross[1,5,6], Andrew Wei[7], Martin Carroll[8], Michael Becker[9], Ari Melnick[4], Francine Garrett-Backelman[2], Richard D'Andrea[1]

[1]Centre For Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia, [2]Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, USA, [3]Australian Cancer Research Foundation Cancer Genomics Facility, The Centre for Cancer Biology, SA Pathology, Adelaide, Australia, [4]Division of Hematology and Medical Oncology, Department of Medicine, Weill Cornell Medicine, New York, USA, [5]Department of Haematology and Bone Marrow Transplantation, Royal Adelaide Hospital, Adelaide, Australia, [6]Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, Australia, [7]Department of Haematology, Peter MacCallum Cancer Centre, Royal Melbourne Hospital, Walter and Eliza Hall Institute of Medical Research and University of Melbourne, Melbourne, Australia, [8]Division of Hematology and Oncology, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA, [9]Department of Medicine, University of Rochester, Rochester, USA

Hot-spot mutations in the Krebs cycle enzymes IDH1 and IDH2 occur recurrently in AML, result in production of the oncometabolite 2-hydroxyglutarate, and associate with a characteristic, profound global DNA hypermethylation due to reduced TET enzyme activity. The contribution of epigenetic and metabolic effects of the IDH1/2 mutations in tumorigenesis is not fully defined. We have previously reported the strong association of IDH1/2 mutations in AML with a highly specific DNA methylation mark in the GADD45A promoter. In independent AML cohorts this methylation mark, GADD45AmeHI, is detected in most IDH-mutant AMLs at diagnosis while also occurring at significant frequency in IDH wild type (WT) AML, consistent with hyper-methylation of this region in other cancers lacking IDH mutations. As hypermethylation at this site cannot be solely attributed to IDH1/2 mutations, and does not correlate with GADD45A mRNA expression, we postulated that the enrichment of this marker in IDH1/2 AML reflects its association with an aberrant leukaemia biology that cooperates with IDH1/2 mutations. We have performed global DNA methylation profiling and RNA-sequencing for an AML cohort relative to healthy controls and used gene set enrichment analyses to identify altered pathways associated with GADD45AmeHI AML. We show a distinct GADD45AmeHI DNA hyper-methylation phenotype in the samples lacking the IDH1/2 mutations. We observed negative enrichment of multiple RNA processing and splicing gene-sets with the gene expression for GADD45AmeHI AMLs, also detectable for IDH1/2-mutant AML, and we identified specific transcripts showing differential splicing in GADD45AmeHI AML. We identified an ATF4 intron retention event associated with GADD45AmeHI- and IDH-mutant AML, which suggests a role for mis-splicing of ATF4 in AML as a cooperating event with the IDH1/2-mutant phenotype. The implications for progression and treatment IDH1/2-mutant AML will be discussed.

Oral Abstracts

Session 2: Cell Signalling Networks

Recruitment of BAG2 to DNAJ-PKAc scaffolds promotes cell survival and resistance to drug-induced apoptosis in fibrolamellar carcinoma

John D Scott
Department of Pharmacology, University of Washington Medical Center, Seattle, WA 98195, United States

The DNAJ-PKAc fusion kinase is a defining feature of the adolescent liver cancer fibrolamellar carcinoma (FLC). A single lesion on chromosome 19 generates this mutant kinase by creating a fused gene encoding the chaperonin binding domain of Hsp40 (DNAJ) in frame with the catalytic core of protein kinase A (PKAc). FLC tumors are notoriously resistant to standard chemotherapies, with aberrant kinase activity assumed to be a contributing factor. Yet recruitment of binding partners, such as the chaperone Hsp70, implies that the scaffolding function of DNAJ-PKAc may also underlie pathogenesis. By combining proximity proteomics with biochemical analyses and photoactivation live-cell imaging we demonstrate that DNAJ-PKAc is not constrained by A-kinase anchoring proteins. The fusion kinase phosphorylates a unique array of substrates. One validated DNAJ-PKAc target is the Bcl-2 associated athanogene 2 (BAG2), a co-chaperone recruited to the fusion kinase through association with Hsp70. Immunoblot and immunohistochemical analyses of FLC patient samples demonstrate increased levels of BAG2 in advanced disease and metastatic recurrence. BAG2 is linked to the anti-apoptotic factor Bcl-2. Pharmacological approaches using the DNA damaging agent etoposide and the Bcl-2 inhibitor navitoclax show that the DNAJ-PKAc/Hsp70/BAG2 axis contributes to chemotherapeutic resistance in a cellular model of FLC. Wildtype AML12 cells were susceptible to each drug alone and in combination. In contrast, $AML12^{DNAJ\text{-}PKAc}$ cells were moderately affected by etoposide, resistant to navitoclax, but markedly susceptible to the drug combination. We implicate BAG2 as a marker for advanced FLC and a chemotherapeutic resistance factor in DNAJ-PKAc signaling scaffolds.

Oral Abstracts

Session 2: Cell Signalling Networks

Memory of stochastic single-cell apoptotic signalling promotes chemoresistance in neuroblastoma

David Croucher
Garvan Institute, Sydney

Many theories have been proposed describing the single-cell dynamics of chemotherapy response and expansion of resistant clones. These usually invoke the presence of low frequency somatic mutations or the *de novo* acquisition of new mutations. In contrast to these predominantly genetic mechanisms, we have now utilised mathematical modelling and longitudinal single-cell imaging to demonstrate that a chemoresistant population of cancer cells can emerge solely through the inherently noisy process of gene expression, which is amplified by the non-linear behaviour of apoptotic signalling pathways.

High-risk neuroblastoma is an aggressive, highly chemoresistant childhood tumour. We previously demonstrated that *in silico*, patient-specific modelling of apoptotic signalling could stratify neuroblastoma patient cohorts and provide robust biomarkers of patient survival. We now show that application of this patient-level model to single-cell populations also predicts the presence of this innately chemoresistant cell population, which cannot activate sufficient drug-induced signalling to reach an in-built apoptotic threshold.

Using JNK activity biosensors with longitudinal high-content and intravital imaging, we have now confirmed that this stochastic population of chemoresistant cells exist prior to treatment and are selected for during chemotherapy treatment. Furthermore, using matched PDX models established at diagnosis and relapse from the same patients, we demonstrate that a memory of this resistant state is maintained through epigenetic remodelling. Consequently, we show that priming neuroblastomas with an HDAC inhibitor cannot erase the memory of this resistant state within relapsed neuroblastomas, but improves response in the first-line setting by restoring drug-induced JNK activity within the chemoresistant population of treatment naïve tumours.

Oral Abstracts

Session 2: Cell Signalling Networks

Androgen Receptor Signalling in Breast Cancer and Normal Breast Tissues

A/Prof Theresa Hickey
Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide, Adelaide, Australia

The androgen receptor (AR) is expressed in all molecular sub-types of breast cancer and is of major interest as a therapeutic target, especially since AR antagonists and agonists are clinically used to treat other medical conditions. We have recently shown that AR plays a tumour suppressor role in estrogen receptor positive (ER+) breast cancer and that use of selective AR modulators (SARMs) that activate AR activity in breast tissues is the best therapeutic strategy. The advantage of non-steroidal SARMs over steroidal androgenic drugs that were historically used to treat breast cancer is their lack of virilizing capacity and their ability to promote bone and muscle health. In part, AR signalling trans-represses ER signalling to inhibit growth of ER+ breast tumours. This action in ER+ breast tumours likely reflects the role of AR signalling in normal breast tissues. We have shown that female breast tissues exposed to high dose testosterone for gender-affirming female-to-male transition undergoes dramatic changes that induce breast involution via direct actions in many different types of cells in the microenvironment including epithelial cells, fibroblasts, T-cells and adipocytes. AR is also expressed in different sub-types of breast cancer that lack ER, but its role in these contexts is unresolved and the best means to therapeutically target AR in those contexts remains to be determined.

Oral Abstracts

Session 2: Cell Signalling Networks

Decoding roles of the PTEN-PI3K axis in breast cancer progression and therapeutic response

Dr Antonella Papa
Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia.

Constitutive activation of the PI3K-AKT-mTOR signalling cascade is frequently observed in cancer where it promotes anabolic growth and cell survival. Inactivation of the tumour suppressor phosphatase PTEN and acquisition of oncogenic mutations in the kinase PI3K have been identified as the most common genetic events leading to PI3K pathway activation and tumour initiation. In particular, the co-occurrence of PTEN and PI3K mutations is associated with disease progression and poor response to systemic and targeted therapies across many cancer types, including breast cancer.
This presentation will provide an overview of the model systems and experimental approaches we use to study mechanisms promoting breast cancer malignancy caused by cancer-associated and germline mutations in PTEN and PI3K. It will also highlight ongoing work characterising new treatment modalities for resistance disease.

Oral Abstracts

Session 2: Cell Signalling Networks

Prognostic Extracellular Matrix Profiles are Associated with Pro-Tumourigenic Signalling in the Squamous Cell Carcinoma Subtype of Non-Small Cell Lung Carcinoma

Amelia Parker[1,2], Elise Bowman[3], Adriana Zingone[3], Brid Ryan[3], Wendy Cooper[4,5,6], Maija Kohonen-Corish[7], Curtis Harris[3], Thomas Cox[1,2]
[1]Matrix and Metastasis Laboratory, Cancer Ecosystems Program, Garvan Institute of Medical Research, Darlinghurst, NSW Australia, [2]School of Clinical Medicine, UNSW Sydney, NSW, Australia, [3]Center for Cancer Research, National Cancer Institute, Bethesda, USA, [4]Department of Tissue Pathology and Diagnostic Oncology, NSW Health Pathology, Royal Prince Alfred Hospital, Camperdown, NSW Australia, [5]Sydney Medical School, University of Sydney, NSW, Australia, [6]Discipline of Pathology, School of Medicine, Western Sydney University, NSW, Australia, [7]Woolcock Institute of Medical Research, Sydney, NSW Australia

Squamous cell carcinoma (SqCC) is a subtype of non-small cell lung cancer and patient prognosis remains poor. The extracellular matrix (ECM) is critical in regulating cell behaviour by activating outside-in signalling pathways that converge on oncogenic drivers. However, the importance of the ECM in promoting tumour aggressiveness remains to be fully characterized.

To comprehensively map the ECM features associated with initiation and recurrence, multi-omics data from SqCC human tumour specimens was combined and consensus clustering was applied to define prognostic matreotypes. These differentially prognostic matreotypes were independent of the mutational status of the tumours, with the worst prognosis conferred by a tumour microenvironment enriched in pro-fibrotic ECM, and with a diverse cellular ecosystem. In silico analysis indicates that matrix remodeling programs differentially activate intracellular signaling in tumor and stromal cells to both reinforce therapy resistance, and also amplify further ECM remodelling, promoting tumour progression. Overlap between poor prognosis SqCC matreotypes and ECM remodelling seen in the chronic lung disease idiopathic pulmonary fibrosis (IPF) also suggests that specific ECM remodelling programs may contribute to field cancerization, typically associated with elevated lung cancer risk. Furthermore, this suggests that a subset of SqCC patients with the worst prognosis may benefit from treatment with existing IPF drugs targeting these hyperactivated signalling pathways. By integrating spatial proteomics and transcriptomics analysis of human SqCC tumours, we are now revealing heterogeneous compartmentalization of these intercellular signalling nodes and their association with prognostic ECM remodelling programs.

Collectively, these analyses define matrix-driven signalling pathways that may be used to inform precision medicine treatment strategies towards improved patient outcome in SqCC.

Oral Abstracts

Session 3: Cell Fate in Development and Cancer

"TBA"

Natasha Harvey
Centre for Cancer Biology, Adelaide

Oral Abstracts

Session 3: Cell Fate in Development and Cancer

A reprogramming strategy to restore normal gene networks in aggressive prostate cancer

Dr Natalie Lister[1], Dr John Ouyang[2], Dr Bo Sun[1], Dr Sue Mei Lim[1], A/Prof David Pook[1], Professor Gail Risbridger[3], Professor Owen Rackham[4], Professor Renea Taylor[3], Professor Jose Polo[1,5]
[1]Monash University, Clayton, Australia, [2]Duke-National University of Singapore, Medical School, Singapore, [3]Cancer Program, Biomedicine Discovery Institute, Monash University, Clayton, Australia, [4]The Alan Turing Institute and University of South Hampton, United Kingdom, [5]The South Australian immunoGENomics Cancer Institute, Adelaide, Australia.

Background: Transcription factor-mediated reprogramming is the process of converting one cell-type into another by altering transcription factors (TF) that rewire gene networks and promote a cell-specific phenotype and function. Whilst there are many reports of successful reprogramming between normal cell-types, the ability to reprogram cancer cells back towards a 'normal' cellular state using TFs remains less defined. Here we demonstrate proof-of-concept that an aggressive patient-derived prostate cancer can undergo transcriptional reprogramming towards normal prostate lineage.

Methods: Human prostate tissues were obtained from consenting patients undergoing radical prostatectomy. Non-malignant regions were identified by a trained pathologist and 'normal' prostate luminal epithelial lineage was isolated by flow cytometry. Human prostate cancer cells were obtained from the Melbourne Urological Research Alliance (MURAL) cohort of patient-derived xenografts (PDXs). The Mogrify algorithm was applied to gene expression data to predict master transcription factors in normal versus malignant cells. Functional validation of transcription factors was assessed using lentiviral knockdown/overexpression vectors in patient-derived organoid models.

Results: Distinct sets of transcription factors were predicted to regulate normal versus malignant prostate cells. Knockdown of key TFs driving cancer-associated transcriptional networks, notably MYBL2, significantly reduced the growth and survival of cancer organoids *in vitro*, including adenocarcinoma and neuroendocrine lineage. In addition, we re-expressed combinations of TFs, predicted to be able to induce a "normal" prostate transcriptional network, in prostate cancer cells derived from a man with highly aggressive and therapy-resistant disease. After 7 days in culture, reprogrammed cancer cells upregulated differentiation markers found on normal prostate lineage and significantly reduced their proliferation and growth *in vitro*.

Conclusions: We observe that prostate cancer cells can undergo TF-mediated reprogramming towards normal programs of growth and differentiation. Future work will focus on understanding the mechanisms underlying transcriptional reprogramming, with the potential to reduce tumour growth and improve therapeutic sensitivity.

Oral Abstracts

Session 3: Cell Fate in Development and Cancer

Subconal mutations alter core signalling nodes and drug responses in acute lymphoblastic leukaemia

Dr Teresa Sadras[1]
[1]*The Peter Maccallum Cancer Centre, Melbourne, VIC* [2]*The Children's Cancer Institute, Melbourne, VIC*

B-cell acute lymphoblastic leukaemia (B-ALL) is a heterogeneous disease characterized by recurrent genomic aberrations that activate kinase signalling, disrupt tumour suppressors, and block B-cell differentiation. Alterations that drive deregulated expression of cytokine receptor-like factor 2 (CRLF2) are present in 5–15% of B-ALLs and represent a group of patients with poor prognosis and high-rates of relapse. These patients respond poorly to standard chemotherapy, and currently lack effective targeted therapies.

Approximately 50% of CRLF2+ B-ALLs harbor activating mutations in JAK2, most commonly JAK2-R683S/G. Co-expression of CRLF2 and mutant JAK2 results in constitutive STAT5 activation, and factor-independent transformation of B-cell progenitors. The current consensus is that activated JAK/STAT activation is the hallmark of CRLF2 B-ALL, however JAK2 inhibitors such as Ruxolitinib have shown very limited efficacy in this leukemia. Our work and that from others, shows that some CRLF2+ B-ALLs lacking JAK2 mutations instead harbor activating mutations in the RAS-ERK pathway (e.g. KRAS-G12D). Using single-cell approaches, here we show that in rare cases of patients with both STAT and ERK activating lesions, these mutations are present in competing subclones. This highlights that therapeutic approaches for CRLF2+ B-ALL are inherently more complex. It remains unknown how subclonal mutations alter the signalling properties and drug responses of CRLF2+ leukemias. To test this, we have established murine models expressing the human CRLF2 receptor complex and common JAK2 and RAS pathway mutations. We show for the first time that the combination of CRLF2 with RAS mutations behaves distinctly to the combination of CRLF2 and JAK2 (or to cells transformed with either mutation alone), and drives unique drug dependencies which can be therapeutically leveraged. While targeted therapy approach pipelines are generally built around the primary genetic lesion, our work demonstrates how the accumulation of competing subclonal mutations can provide signalling plasticity and likely contributes to relapsed disease.

Oral Abstracts

Session 3: Cell Fate in Development and Cancer

Ubiquitination regulates key intercellular signalling pathways driving cardiac development

Sophie Wiszniak, Iman Lohraseb, Peter McCarthy, Quenten Schwarz
Centre for Cancer Biology, University of South Australia and SA Pathology

Much of the complexity of heart development is underpinned by the interaction of multiple different cell types (second heart field, neural crest and endothelial cells) to orchestrate formation of the fully functional heart. Removal of the ubiquitin ligase Nedd4 specifically in neural crest cells (*Wnt1-Cre; Nedd4*$^{fl/fl}$ embryos) results in outflow tract defects reminiscent of those observed clinically, including double outlet right ventricle (DORV) and transposition of the great arteries (TGA). We have global proteomics analyses to uncover novel Nedd4 targets in neural crest cells that control heart development. Our work shows that cardiac precursors of the second heart field which sit adjacent to the migrating cardiac neural crest cells, exhibit premature cardiomyocyte differentiation leading to a reduced progenitor pool to contribute to correct lengthening of the outflow tract. Laser capture microdissection of the second heart field region, followed by mRNA sequencing (LMD mRNA-seq) revealed potential modulation of the Wnt signalling pathway in *Wnt1-Cre; Nedd4*$^{fl/fl}$ embryos, as well as an upregulation of the secreted Wnt signalling inhibitor Dkk1. Spatial expression analysis has revealed increased Dkk1 expression is localised to the neural crest cells. We further identify Dkk1 as a novel Nedd4 target and identify new loss of function Nedd4 genetic variants in patients with DORV. We propose that Nedd4 controls levels Dkk1 protein in neural crest cells, which modulates Wnt signalling in the second heart field to influence the balance of cardiomyocyte differentiation.

Oral Abstracts

Session 4: Cancer Immunology and Signalling

Deconvoluting tissue-resident memory T cells in tumours

Laura Mackay[1]
[1]*Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity at the University of Melbourne, Melbourne, VIC 3000, Australia*

Tissue-resident memory T (TRM) cells provide rapid and superior control of localised infections and are essential for tumour immunosurveillance. TRM cells are transcriptionally distinct from their circulating counterparts, and the TRM cell transcriptional signature derived from this distinction has been associated with positive prognoses in numerous tumour settings. However, the high antigen load and chronic inflammatory tumour microenvironment is conducive to driving dysfunctional exhausted T (TEX) cells. The transcriptional signatures of TRM and TEX cells are highly correlated, and as a result it is unclear whether these two cell types have been conflated in the tumour context, or whether they are truly distinct populations. Here we show that while TEX and TRM cells are transcriptionally and phenotypically similar, they are indeed distinct T cell lineages with divergent developmental trajectories, driven by disparate micro-anatomical location and antigen specificities. We therefore provide a conceptual framework from which to interrogate tumour-associated TRM cells and assess their therapeutic capacity.

Oral Abstracts

Session 4: Cancer Immunology and Signalling

Inhibition of myeloid cell-specific HCK kinase activity confers immune check point blockade activity in pancreatic and ovarian cancer

Professor Matthias Ernst[1,2], Ashleigh R Poh[1,2], Elizabeth Christie[3], Marina Pajic[4]
[1]Olivia Newton John Cancer Research Institute, Heidelberg, Australia, [2]La Trobe University, Melbourne, Australia, [3]Peter MacCallum Cancer Centre, Melbourne, Australia, [4]Garvan Institute of Medical Research, Sydney, Australia

Many immunogenic tumours characterized by accumulation of immunosuppressive myeloid cells remain refractory to immune checkpoint blockade, including immune cell excluded pancreatic ductal adenocarcinomas (PDAC) or high-grade serous ovarian cancer (HGSOC).

Here, we report that genetic absence of the myeloid cell-specific Src-family kinase Hck in host mice reduces (1) primary tumour burden following orthotopic injection of PDAC-cells (Kras/p53 mutant KPC cells); and (2) liver metastasis following intra-splenic injection of KPC cells, or ascites and tumour formation in the omentum following intra-peritoneal injection of HGSOC-cells (p53/Brca2 mutant ID8 or HGS1 cells). Compared to Hck wild-type (wt) hosts, tumours in Hck-KO hosts are characterized by less abundant M2-like macrophages and MDSCs, and an influx of activated CD8+ and NK effector cells. Indeed, the prolonged survival of PDAC or HGSC tumour-bearing Hck-KO hosts is reverted following CD8 or NK ablation, and the increased influx of effector cells in Hck-KO hosts depends on IL12 and CXCR3. Furthermore, KPC liver metastases in Hck-KO hosts have less dense extracellular matrix and reduced abundance of cancer-associated fibroblasts with impaired immune suppressive and matrix remodelling gene signatures.

Hck-KO hosts conferred improved response of KPC tumours to standard-of-care chemotherapy (gemcitabine) and showed prolonged survival compared to Hck-wt hosts. Likewise, treatment of KPC tumour-bearing Hck-KO hosts with anti-PD1, anti-CTLA4 or agonistic CD40 antibodies, resulted in a virtual absence of liver metastases, associated with extended survival compared to treatment-naïve or checkpoint blockade-treated Hck-wt hosts. Importantly, therapeutic administration of an Hck inhibitor tool compound improves survival of Hck-wt hosts treated with gemcitabine or immune checkpoint blockade, and reduces the growth of patient-derived xenografts in “humanized” mice reconstituted with human myeloid cells.

Collectively, our results suggest that therapeutic targeting of HCK activity enables or enhances responses to immunotherapy by simultaneous stimulation of immune cell activity and inhibition of the immune suppressive tumour microenvironment.

Oral Abstracts

Session 4: Cancer Immunology and Signalling

TBA

Briony Forbes
Flinders University, Adelaide

Oral Abstracts

Session 4: Cancer Immunology and Signalling

Anti-fungal modulation of the immune-suppressive tumour microenvironment overcomes immunotherapy resistance in pancreatic ductal adenocarcinoma

Dr Sean Porazinski[1,2], Dr Jennifer Man[1], Dr Diego Chacon-Fajardo[1,2], Dr Howard Yim[3], Professor Emad El-Omar[3], A/Prof Anthony Joshua[1,2], A/Prof Marina Pajic[1,2]
[1]Garvan Institute of Medical Research, Darlinghurst, Australia, [2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney, Australia, [3]Microbiome Research Centre, St George and Sutherland Clinical School, UNSW, Sydney, Australia

Pancreatic cancer (PC) has 5-year survival rates of 11% and is projected to become the second leading cause of cancer-related deaths by 2030. Poor outcomes result from early metastasis and a lack of effectual therapies for advanced disease. Our previous large-scale genomics studies revealed PC is molecularly highly varied. This heterogeneity, lack of effective therapies and high mortality rate make PC a prime arena to advance personalised medicine strategies, where individual cancers are selected for optimal therapy depending on molecular subtype. Utilising our significant experience of molecular-guided anti-cancer therapies and stromal biology expertise, we are taking drug-repurposing approaches to test agents with effects on stromal biology in combination with standard-of-care chemotherapies and immunotherapy, to improve clinical outcomes.

Itraconazole is an FDA-approved anti-fungal with potential anti-cancer effects, although its efficacy in PC remains relatively unexplored. Our *in vivo* findings indicate that itraconazole, in combination with standard-of-care chemotherapy gemcitabine/nab-paclitaxel, significantly delays disease progression in both mouse and patient-derived xenograft models of PC, with itraconazole diminishing the aggressive mesenchymal phenotype normally observed within the tumour cell compartment. Single cell RNA-seq analyses of tumours from the KPC orthotopic model of PC suggest itraconazole treatment can also affect tumour-stroma crosstalk to inhibit immunosuppressive aspects of the stroma and pro-tumourigenic, pro-metastatic signalling. We further observe that itraconazole repolarises tumour-associated macrophages towards an M1-like phenotype, inhibits cancer-associated fibroblast activity and ECM deposition, and improves T cell infiltration into tumours. Excitingly, combining itraconazole with anti-PD1/-CTLA4 immunotherapy provides significant survival advantages in the KPC orthotopic model of PC. Finally, using a model of metastatic PC, we show that itraconazole hinders metastatic colonisation in the liver. This work aims to identify subtypes of PC responsive to itraconazole allowing optimisation and translation of tailored therapies combining itraconazole and the latest clinically-utilised chemotherapies and immunotherapies.

Oral Abstracts

Session 4: Cancer Immunology and Signalling

GD2-CAR-T cell therapy for aggressive primary brain tumours

Michael Brown
Centre for Cancer Biology

Oral Abstracts

Session 5: Spatial Transcriptomics in Cancer

How will single cell and spatial analysis help with cancer medicine? A case study of triple-negative breast cancer

Jasmine Plummer[1], Omotoso Ayodele[2], Destiny Burnett[2], Priscila Coelho[2], Judith Hurley[2], Carmen Gomez[2], Nadezhda Nikulina[3], HaYeun Ji[3], Felipe Dezem-Segato[1], Maycon Marcao[1], AC3[4], Sophia George[2]
[1] Center for Spatial OMICS, St Jude Children's Research Hospital, Memphis TN [2] Sylvester Comprehensive Cancer Center, University of Miami Health Systems, Miami FL [3] Akoya Biosciences, The Spatial Biology Company, Marlborough, Massachusetts, USA [4] African Caribbean Cancer Consortium

The US Black population consists of both US-born Black and immigrant Black populations from the Caribbean and Africa. Black individuals disproportionately develop aggressive cancers and are treatment refractory or resistant, thus leading to premature deaths. In women, breast cancer is more common among US Black women, and the most common non-viral driven cancer in African and Caribbean countries. Black women develop this disease younger than other ancestral groups and have a higher incidence of aggressive pathologies, such as metaplastic and triple-negative breast cancer. To better understand how Black womens' tumors are different from other ancestries, we created a spatial atlas of triple negative breast cancer across US Black and Caribbean Black individuals. Using ultra-highplexed protein spatial phenotyping, we detected of novel immune cell populations associated with a given African ancestry. Evaluating tumor microenvironment, allowed us to identify unique stromal cell neighborhood not previously seen in single cell data. Using a machine learning approach, we evaluated the stemness of tumors based on their ancestry. This tool was able to decipher the more aggressive tumors by their stemness and associate it with their ancestry and aggressive disease biology. This talk highlights measures used to interpret ancestral genomic differences at the cellular level with the hope that through the characterization of tissue composition and the proportion of cell sub-populations, new biology will be revealed allowing for more accurate clinical stratification of cancer.

Oral Abstracts

Session 5: Spatial Transcriptomics in Cancer

Systems immunology of breast cancer

Alex Swarbrick
Garvan Institute, Sydney

Oral Abstracts

Session 5: Spatial Transcriptomics in Cancer

Decomposing gene expression using Gene Over-Representation Projection Pursuit

Kristen Feher
SAiGENCI, Adelaide

Spatial technologies have exploded on the 'omics scene and they promise to be a gamechanger in understanding the spatial organisation of inter-cell signalling and its contribution to differences between healthy and diseased tissue architecture. The field is highly active in assessing the most useful spatial methods for extracting biological signal, which includes importing vast amounts of knowledge gained from scRNAseq data, e.g. cell population labels and marker genes. Using a Nanostring NSCLC CosMx dataset, I will introduce GORPP (Gene Over-Represenation Projection Pursuit), a novel method decomposes the major signals of a gene expression matrix such that cells aren't forced to be of a discrete type, but rather describes proximity to a number of landmarks. The method output automatically results in marker genes associated with each landmark. GORPP can be performed hierarchically and can naturally accommodate the concept of cellular plasticity. From a technical point of view, it can be used to critically assess the performance of spatial transcriptomic panels and point to improvements in panel design. Additionally, cell population labels and marker genes derived from scRNAseq data can be overlaid with the results of GORPP to assess the overlap of the different technologies. The cells' proximity to landmarks are transferred to the spatial domain in order to quantify how each landmark drives the overall tissue architecture. Finally, a neighbourhood analysis is applied to the landmarks in order to elucidate the possible inter-cell signalling processes.

Oral Abstracts

Session 5: Spatial Transcriptomics in Cancer

Temporal mass spectrometry proteomics and advanced microscopy to resolve matrisomal drivers of pancreatic cancer

Dr Brooke Pereira[1,2], Miss Shona Ritchie[1,2], Dr Cecilia Chambers[1,2], Miss Katie Gordon[1,2], Dr Morghan Lucas[1,2], Dr Astrid Magenau[1,2], Dr Kendelle Murphy[1,2], Dr Sean Warren[1,2], Dr Max Nobis[1,2], Dr Romain Rouet[1,2], Dr Sunny Wu[1,2], Dr Julia Yin[1,2], Dr Hao-Wen Sim[1], Prof Lorraine Chantrill[1,3], Prof Sean Grimmond[4], Prof Anthony Gill[1,5], Prof Jeff Evans[6], A/Prof Takako Sasaki[7], Prof Tri Phan[1,2], A/Prof Alex Swarbrick[1,2], Prof Marina Pajic[1,2], Prof Jennifer Morton[6,8], Prof Benjamin Parker[4], Dr David Herrmann[1,2], A/Prof Thomas Cox[1,2], Prof Paul Timpson[1,2]
[1]Garvan Institute Of Medical Research, Darlinghurst, Australia, [2]School of Clinical Medicine, University of New South Wales, Sydney, Australia, [3]Illawarra Cancer Care Centre, Wollongong Hospital , Wollongong, Australia, [4]The University of Melbourne, Parkville, Australia, [5]NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, St. Leonards, Australia, [6]Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom, [7]Department of Biochemistry, Oita University, Oita, Japan, [8]Beatson Institute, Cancer Research UK, Glasgow, United Kingdom

Pancreatic cancer (PC) is highly lethal, with a five-year survival rate of ~11% (1). PC is characterised by increasing fibrosis. We have shown that targeting fibrosis can improve chemotherapy efficacy and impair metastasis in pre-clinical models (2-4). As such, we aimed to use proteomics to dissect the matrix signatures of pancreatic tumours from the highly-metastatic KPC (Pdx1-Cre; LSL-K-rasG12D/+; LSL-p53R172H/+) and poorly-metastatic KPflC (Pdx1-Cre; LSL-K-rasG12D/+; LSL-p53fl/+) mouse models, with an aim to identify new metastatic drivers in this deadly disease.

We collected pancreatic tissue from KPflC, KPC and wildtype controls at early (~50 days), mid (~90 days) and late-stage disease (~200 days) and enriched them for matrix proteins using ISDoT de-cellularisation (5). These specimens were then analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

LC-MS/MS revealed an increased abundance of Nidogen-2 (NID2) in KPC tumours compared to KPflC. Western blotting and RT-qPCR show that NID2 is enhanced in KPC cancer-associated fibroblasts (CAFs). 3D organotypic matrices generated with NID2 CRISPR interference (CRISPRi) CAFs had reduced fibrosis, shown via second harmonic generation (SHG) multiphoton imaging and Picrosirius Red/birefringence analysis. 3D organotypic invasion assays revealed that depletion of NID2 significantly impeded the 3D invasion of cancer cells.

Subcutaneous and orthotopic co-seeding experiments using NID2 CRISPRi CAFs with cancer cells showed that NID2 depletion significantly impeded tumour growth and fibrosis. Intravital imaging with Quantum Dots and SHG revealed improved vascular patency in live NID2-depleted tumours, with improved response to gemcitabine/Abraxane chemotherapy. Strikingly, in orthotopic models, mice bearing NID2 CRISPRi tumours had significantly reduced liver metastasis and increased survival, revealing NID2 as a new stromal co-target in this aggressive disease.

References
1. Siegel et al., CA, 2023
2. Vennin et al., Science Translational Medicine, 2017
3. Vennin et al., Nature Communications, 2019
4. Murphy et al., Science Advances, 2021
5. Mayorca-Guiliani et al. Nature Medicine, 2017

Oral Abstracts

Session 6: Novel Therapeutics and Therapy Resistance

Considering the Tumour Immune MicroEnvironment of children's brain cancer: Is it TIME for better models?

Zahra Abbas[1,2], Omar Elaskalani[1], Hilary Hii[1], Meegan Howlett[1,2], Merridee Wouters[1], Jenny Truong[1], Iley Johnson[1], Annabel Short[1,2], Timo Lassmann[2,3], Terrance Johns[1,2], Joost Lesterhuis[1,2], Raelene Endersby[1,2]
[1] *Telethon Kids Cancer Centre, Telethon Kids Institute, Perth WA, Australia*
[2] *Centre for Child Health Research, University of Western Australia, Perth WA, Australia*
[3] *Computational Biology, Precision Health, Telethon Kids Institute, Perth WA, Australia*

Brain cancer is one of the deadliest human cancers and causes more childhood deaths than any other disease in Australia. Yet, children with cancer have a fraction of the treatment options than adults do and wait longer to access new drugs. The lack of advancements in childhood brain cancer treatment had previously been due to deficiencies in knowledge about the underlying biological causes. However, paediatric neuro-oncology has undergone an exciting and dramatic transformation during the past 20 years, driven by advances in genomic technology, international collaboration, and the generosity of families willing to share tissue samples for research. With donated tissue specimens, we employ a direct brain-to-brain workflow that has led to the successful development of multiple new patient-derived orthotopic xenograft (PDOX) mouse models of rare childhood brain cancers. Translation of novel agents into clinical trials for paediatric brain cancer depends heavily on treatment efficacy data generated using such models. While the use of small animals remains fundamental in cancer research, there are vast biological differences between children and adults. However, preclinical pipelines in paediatric cancer research have largely overlooked these age-related differences in metabolism, growth factor signalling, and immunology. To address this, we have developed improved preclinical models by implanting brain cancer cells into newborn mice to replicate cancer growing in a child's brain. Remarkably, tumours grew faster and the immune microenvironment was different compared to when the same cells were implanted into adult brain. We have characterised the immune microenvironment of our new model using flow cytometry, immunohistochemistry, and single cell RNA sequencing. With this information, we are now exploring how to exploit these tumour-immune interactions in the paediatric setting to identify optimal immunotherapeutics for childhood cancer.

Oral Abstracts

Session 6: Novel Therapeutics and Therapy Resistance

CDK9 inhibition disables multiple oncogenic transcriptional and epigenetic pathways in prostate cancer

Razia Rahman[1,2], Muhammed H. Rahaman[3], Adrienne R. Hanson[1], Nicholas Choo[4], Jianling Xie[1], Scott L. Townley[1], Ramin Hassankhani[3], Saiful Islam[3], Susanne Ramm[5,6], Kaylene J. Simpson[5,6,7], Gail P. Risbridger[4,6,8,9], Giles Best[1], Margaret M. Centenera[10,11], Steven P. Balk[12], Ganessan Kichenadasse[1,13], Renea A. Taylor[4,6,8,9], Lisa M. Butler[10,11], Wayne D. Tilley[11,14], Simon J. Conn[1], Mitchell G. Lawrence[4,6,8,9], Shudong Wang[3] and Luke A. Selth[1,2,11]

1. *Flinders Health and Medical Research Institute and Flinders Centre for Innovation in Cancer, College of Medicine and Public Health, Flinders University, Bedford Park, SA 5042, Australia.*
2. *Freemasons Centre for Male Health and Wellbeing, Flinders University, Bedford Park, SA 5042, Australia.*
3. *Drug Discovery and Development, Clinical and Health Sciences, University of South Australia, Adelaide, SA 5000, Australia.*
4. *Monash Partners Comprehensive Cancer Consortium, Monash Biomedicine Discovery Institute Cancer Program, Prostate Cancer Research Group, Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia.*
5. *Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre, Melbourne 3000, Australia.*
6. *The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville 3010 Australia.*
7. *Department of Biochemistry and Pharmacology, University of Melbourne, Parkville 3010, Australia.*
8. *Peter MacCallum Cancer Centre, Melbourne, VIC, Australia.*
9. *Melbourne Urological Research Alliance (MURAL), Monash Biomedicine Discovery Institute Cancer Program, Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia.*
10. *South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia.*
11. *Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide 5000, Australia.*
12. *Beth Israel Deaconess Medical Center, Boston, Massachusetts.*
13. *Medical Oncology, Flinders Medical Centre, Adelaide 5042, Australia.*
14. *Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, The University of Adelaide, Adelaide 5005, Australia.*

Cyclin-dependent kinase 9 (CDK9) stimulates oncogenic transcriptional pathways in cancer. Here, we evaluated the activity of an orally bioavailable CDK9 inhibitor, CDKI-73, in prostate cancer, a disease characterized by aberrant activity of multiple transcriptional regulators. CDKI-73 inhibited proliferation and caused cell death in diverse *in vitro* models of androgen receptor (AR)-driven and AR-independent models of prostate cancer. The activity of CDKI-73 was validated in more clinically-relevant systems, including xenografts, patient-derived tumor explants and patient-derived organoid models of therapy-resistant disease. Mechanistically, CDKI-73-mediated inhibition of RNA polymerase II serine 2 phosphorylation resulted in reduced expression of BCL-2 anti-apoptotic factors and defects in overall transcription. Moreover, transcriptomic and epigenomic approaches revealed that CDKI-73 suppressed signaling pathways regulated by AR, MYC and BRD4, key drivers of dysregulated transcription in prostate cancer, and led to reprogramming of cancer-associated super-enhancers. These latter findings prompted evaluation of CDKI-73 with the BRD4 inhibitor AZD5153, a combination that was synergistic in patient-derived organoids and cell lines that collectively modelled a broad spectrum of aggressive, therapy-resistant phenotypes. Collectively, our work provides new insights into CDK9's oncogenic activity and reveals CDKI-73 as a promising therapeutic agent for advanced prostate cancer.

Oral Abstracts

Session 6: Novel Therapeutics and Therapy Resistance

Unravelling the role of "Don't eat me signal" CD47 in regulating cellular and metabolic plasticity

Nirmal Robinson
Centre for Cancer Biology, University of South Australia

Glioblastoma (GBM) is an aggressive type of brain cancer with a very low survival (11-15 months) despite intensive therapeutic regimen consisting of surgery, radiotherapy, and chemotherapy. GBM has been untreatable, because they invade into other regions of the brain, become resistant to currently available therapies and escape elimination by the immune system.

We have identified that GBM cells produce a 'don't eat me' signal known as CD47 to escape from immune cells and is particularly increased in the region where cancer cells invade into other parts of the brain, which also lacks immune cells. Therefore, we hypothesised that CD47 could also regulate cellular mechanisms that help GBM to proliferate and migrate. Through CRISPR/Cas9 targeted gene knock out studies, we have shown that CD47 regulates cell proliferation and migration. In depth molecular analysis combined with metabolomics have revealed metabolic pathways regulated by CD47. Importantly, loss of CD47 exposed some key vulnerabilities of GBM which offers potential novel therapeutic strategies to treat GBM.

Oral Abstracts

Session 6: Novel Therapeutics and Therapy Resistance

Advancing treatment options for triple negative breast cancer through NF-kappa B inhibition

Dr Mark Bunting[1], Dr Belinda Cornes[1], A. Prof. Philip Gregory[2], Prof. Christopher Sweeney[1]
[1]SAiGENCI, The University of Adelaide, Adelaide, Australia, [2]Centre for Cancer Biology, University of South Australia, Adelaide, Australia

Triple negative breast cancer (TNBC) is amongst the most lethal breast cancer types. Patients with TNBC are typically younger, diagnosed with more advanced tumors, and have five-year survival rates of 77% versus 85-94% for estrogen receptor positive and HER2 positive breast cancers. Standard-of-care TNBC treatment consists of chemotherapy, surgery, radiotherapy, and for some patients, PD-1 inhibition. Despite substantial treatment, high rates of recurrence after local therapy are frequently observed. Constitutive activation of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kappa B) is hypothesised to contribute to the aggressive underlying biology of TNBC which exhibits stem cell-like properties, epithelial-mesenchymal transition (EMT) features, high metastatic potential, chemo-resistance, and limited response to immunotherapy. Preliminary data from our lab indicate loss of both Tristetraprolin (ZFP36/TTP) and Runt-related transcription factor 1 (RUNX1), known negative regulators of NF-kappa B, may lead to poorer survival outcomes of women with TNBC. Using bioinformatic analysis of patient data, *in vitro* cell line, and immunocompetent pre-clinical animals models of TNBC, we are investigating the TTP-RUNX1-NF-kappa B axis in TNBC tumorigenesis. Bioinformatic analysis of TCGA patient data who exhibit low tumour expression of TTP and RUNX1 reveals dysregulation of NF-kappa B- and TTP-target genes compared with patients retaining TTP and RUNX1 expression. Examples being COX-2, EGFR, inhibitors of apoptosis, and inflammatory cytokines. Combination treatment with chemotherapy, PD-1 inhibition, and NF-kappa B inhibition using a new formulation of dimethylaminoparthenolide will be conducted to assess the efficacy of blocking NF-kappa B hyperactivation in aggressive TNBC models. Modulation of pro-inflammatory and immunosuppressive factors, regulators of EMT, chemo-resistance, and anti-tumour immunity in the tumour microenvironment following treatment will be determined. Combining NF-kappa B- and PD- 1-inhibition in TNBC is both a logical and potentially high-yield approach given NF-kappa B activation may not only drive tumor progression but also enhance resistance to chemotherapy and immunotherapy.

Oral Abstracts

Session 6: Novel Therapeutics and Therapy Resistance

Targeting phosphorylation of specific proteins - A novel approach to overcome chemoresistance in TNBC

Ana Lonic[1], Freya Gehling[1], Winona Onglao[1,2], Terry Lim Kan Sian[3], Elizabeth Nguyen[3], Tracy Nero[4,5], Larissa Doughty[4,5], Michael Parker[4,5,6], Roger Daly[3], Yeesim Khew-Goodall[1,2]
[1]Centre For Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia, [2]School of Biological Sciences and Medical School, University of Adelaide, Adelaide, Australia, [3]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia, [4]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia, [5]Australian Cancer Research Foundation Facility for Innovative Cancer Drug Discovery, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia, [6]Australian Cancer Research Foundation Rational Drug Discovery Centre, St Vincent's Institute of Medical Research, Melbourne, Australia

Triple negative breast cancer (TNBC) is the most aggressive form of breast cancer that is more likely to recur and metastasise than the other forms. Due to a scarcity of reliable targeted therapies, when standard of care chemotherapy fails, the median survival period for this patient cohort is ~30 months. The heterogeneous nature of TNBC and lack of reliable actionable biomarkers to predict chemoresistance has hindered the development of new targeted therapies. We have identified a novel phosphorylation site on protein kinase C delta (herein termed p-PKCδ) that confers resistance to multiple chemotherapeutics in TNBC cell lines and is evident in 25-30% of TNBC patients. This specific phosphorylation site, whilst located within the kinase domain, is distinct from the catalytic site.

An *in-silico* screen identified drug-like compounds with potential to hinder the specific phosphorylation of PKCδ. In cell screening showed that compounds that reduce p-PKCδ levels also restore chemosensitivity in chemoresistant cells that had high p-PKCδ. Using differential phospho-proteomics, we have also identified candidate p-PKCδ-specific substrates, ie. those that are only phosphorylated by p-PKCδ but not PKCδ lacking the specific phosphorylation. Restoration of chemosensitivity following knockdown of these substrates, in chemoresistant cells with high p-PKCδ, suggests a role for the p-PKCδ substrates in mediating chemoresistance downstream of p-PKCδ. Together these data suggest that chemoresistance can be blocked in patients with high p-PKCδ by blocking either the specific phosphorylation site on PKCδ or phosphorylation of its substrates.

The identified p-PKCδ-specific substrates have roles in protein trafficking pathways, suggesting the mechanism of chemoresistance downstream of p-PKCδ involves alterations in trafficking. These results highlight the important role of p-PKCδ in chemoresistance in a cohort of TNBC patients and demonstrate a novel therapeutic avenue for overcoming resistance.

Oral Abstracts

Session 7: Systems Biology and Cancer

WWP2, an unexpected therapeutic target for multiorgan fibrosis

Enrico Petretto
Duke-National University of Singapore, Singapore

Pathological cardiac fibrosis is a final common pathology in inherited and acquired heart diseases that causes cardiac electrical and pump failure. Here, we use systems genetics to identify a pro-fibrotic gene network in the diseased heart and show that this network is regulated by the E3 ubiquitin ligase WWP2, specifically by the WWP2-N terminal isoform. Transgenic mice lacking the N-terminal region of the WWP2 protein show improved cardiac function and reduced myocardial fibrosis in response to pressure overload or myocardial infarction. In primary cardiac fibroblasts, WWP2 positively regulates the expression of pro-fibrotic markers and extracellular matrix genes. TGFβ1 stimulation promotes nuclear translocation of the WWP2 isoforms containing the N-terminal region and their interaction with SMAD2. WWP2 mediates the TGFβ1-induced nucleocytoplasmic shuttling and transcriptional activity of SMAD2. Moreover, we show that its myeloid-specific deletion reduces cardiac fibrosis. Using the same model, we establish the functional heterogeneity of macrophages and define an early pro-fibrogenic phase driven by Ccl5-expressing Ly6chigh monocytes. Among other cardiac macrophage subtypes, WWP2 dysfunction primarily affects the Ccl5-dependent infiltration and activation of Ly6chigh monocytes, which causes reduced myofibroblast trans-differentiation. WWP2 interacts with IRF7, promoting its non-degradative monoubiquitination, nuclear translocation and transcriptional activity, including upstream Ccl5. Thus, WWP2 plays also a role as a key regulator of IRF7-mediated Ccl5/Ly6chigh monocyte axis in heart fibrosis. These findings suggest WWP2 as a novel anti-inflammatory and antifibrotic target for therapeutic development.

Oral Abstracts

Session 7: Systems Biology and Cancer

Understanding the Neuroblastoma Transcriptome: Identifying molecular drivers for better tumour stratification

Dr Katherine Pillman[1,2], Ms Aayushi Notra[1], A/Prof Quenten Schwarz[1], Prof Greg Goodall[1,2], Prof Yeesim Khew-Goodall[1,2]
[1]Centre For Cancer Biology, an Alliance between SA Pathology and The University of South Australia, Adelaide, Australia, [2]School of Biological Sciences, The University of Adelaide, Adelaide, Australia

At present, neuroblastoma patients are stratified by risk and while low-risk patients often require no treatment, intermediate and high-risk patients undergo multiple highly invasive, sometimes multimodal therapies. Despite this, the 5-year survival rate remains <50%. With little advance in treatment options over the last decade, there is an urgent need to both improve tumour stratification to avoid the devastating consequences of under and over-treatment and develop tumour subtype-specific models to facilitate drug development and testing.

Neuroblastoma is a highly heterogeneous disease that occurs due to a block in the normal differentiation process of the adrenal gland during embryonic development. Remarkably, the most common genomic DNA alterations affect gene expression levels, involving transcription factors and gene copy number alterations.

We aim to uncover varying drivers of neuroblastoma tumours through the identification of molecular drivers (transcription factors, microRNAs, lncRNAs, alternative splicing factors) and a thorough understanding of the causes of heterogeneity in the neuroblastoma transcriptome, both within and between tumours.

To characterise normal development, we have conducted multi-omic profiling of *in vitro* developmental models, profiling gene expression at single-cell and bulk-seq resolutions, microRNAs, circRNAs, lncRNAs and alternative splicing. Leveraging computational modelling techniques including single-cell resolution in silico transcription factor perturbation analyses, we have pinpointed potential molecular drivers of normal differentiation, refining these candidates through interrogation of vivo data from normal development and tumours at single-cell and bulk-seq resolutions.

Our discoveries will serve as a foundation for developing laboratory models tailored to specific tumour subtypes, facilitating drug development and testing.

Oral Abstracts

Session 7: Systems Biology and Cancer

Studying ovarian cancer subclones and their microenvironments using spatial transcriptomics

Elena Denisenko1,†, Leanne de Kock1,2, Adeline Tan3, Aaron B. Beasley4, Maria Beilin5, Matthew E. Jones1, Rui Hou1, Dáithí Ó Muirí1, Sanela Bilic5, G. Raj K. A. Mohan5,6, Stuart Salfinger7, Simon Fox1, Khaing Hmon1, Yen Yeow1, Youngmi Kim8, Rhea John8, Tami S. Gilderman8, Emily Killingbeck8, Elin S. Gray4, Paul A. Cohen5,9,10,†, Yu Yu9,11,12,†, Alistair R. R. Forrest1,†
1. Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, The University of Western Australia, Nedlands, Perth, WA 6009, Australia
2. Children's Hospital of Eastern Ontario Research Institute, Ottawa, Ontario, Canada (Current Affiliation)
3. Anatomical Pathology Department, Clinipath, Sonic Healthcare, Perth, WA 6017 Australia
4. Centre for Precision Health, Edith Cowan University, Joondalup, WA 6027, Australia
5. Department of Gynaecological Oncology, Bendat Family Comprehensive Cancer Centre, St John of God Subiaco Hospital, 12 Salvado Rd, Subiaco, WA 6008, Australia
6. School of Medicine, University of Notre Dame, Fremantle,WA,6160, Australia
7. Western Australian Gynae and Surgery, Perth, WA, Australia.
8. NanoString Technologies, Seattle, WA, USA
9. Division of Obstetrics and Gynaecology, Medical School, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
10. Institute for Health Research, The University of Notre Dame Australia, 32 Mouat Street Fremantle, WA 6160, Australia
11. Curtin Medical School, Curtin University, 410 Koorliny Way, Bentley, WA 6102, Australia
12. Curtin Health Innovation Research Institute, Curtin University B305, Bentley, WA 6102, Australia

High-grade serous ovarian carcinoma (HGSOC) is a polyclonal disease characterised by the presence of subclones with distinct cancer genotypes. This intratumoural heterogeneity is linked to recurrence, chemotherapy resistance, and overall poor prognosis. Here, we used spatial transcriptomics platforms (10x Genomics Visium and NanoString CosMx Spatial Molecular Imaging (SMI)) to examine genetic heterogeneity of HGSOC cells and their association with infiltrating populations in samples from patients treated with neoadjuvant chemotherapy. We found evidence of multiple tumour subclones with different copy number alterations co-existing within individual tumour sections. Examining gene expression differences between subclones we found evidence that their cell-to-cell communication networks may be rewired by differences in ligand and receptor expression levels. We hypothesise that this may modulate their interactions with stromal and immune cells and likely also leads to the creation of subclone specific autocrine loops.

Oral Abstracts

Session 7: Systems Biology and Cancer

ProsperousPlus: a one-stop and comprehensive platform for accurate protease-specific substrate cleavage prediction and machine-learning model construction

Dr Fuyi Li[1]
[1]*South Australian Immunogenomics Cancer Institute (saigenci), The University of Adelaide, Adelaide, Australia*

Proteases contribute to a broad spectrum of cellular functions. Given a relatively limited amount of experimental data, developing accurate sequence-based predictors of substrate cleavage sites facilitates a better understanding of protease functions and substrate specificity. While many protease-specific predictors of substrate cleavage sites were developed, these efforts are outpaced by the growth of the protease substrate cleavage data. In particular, since data for 100+ protease types are available and this number continues to grow, it becomes impractical to publish predictors for new protease types, and instead it might be better to provide a computational platform that helps users to quickly and efficiently build predictors that address their specific needs. To this end, we conceptualised, developed, tested, and released a versatile bioinformatics platform, ProsperousPlus, that empowers users, even those with no programming or little bioinformatics background, to build fast and accurate predictors of substrate cleavage sites. ProsperousPlus facilitates the use of the rapidly accumulating substrate cleavage data to train, empirically assess and deploy predictive models for user-selected substrate types. Benchmarking tests on test datasets show that our platform produces predictors that on average exceed the predictive performance of current state-of-the-art approaches. ProsperousPlus is available as a webserver and a stand-alone software package at http://prosperousplus.unimelb-biotools.cloud.edu.au/.

Oral Abstracts

Session 7: Systems Biology and Cancer

Genetic conundrums and complexities in familial haematological malignancies

Christopher N Hahn[1,2,3], Parvathy Venugopal[1,2], Claire C Homan[1,2], Peer Arts[1,2,3], Jiarna R Zerella[1,3], Kerry Phillips[4], David M Ross[3,5], Devendra K Hiwase[3,5,6], Nicola K Poplawski[4], Anna L Brown[1,2,3], Hamish S Scott[1,2,3,7].

1. *Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, Australia.*
2. *Department of Genetics and Molecular Pathology, SA Pathology, Adelaide, Australia.*
3. *Adelaide Medical School, University of Adelaide, Adelaide, Australia.*
4. *Adult Genetics Unit, Royal Adelaide Hospital, Adelaide, SA, Australia.*
5. *Royal Adelaide Hospital, Central Adelaide Local Health Network, Adelaide, Australia.*
6. *South Australian Health & Medical Research Institute (SAHMRI), Adelaide Australia.*
7. *ACRF Genomics Facility, Centre for Cancer Biology, Adelaide, Australia.*

The genomic era has been revolutionary in highlighting genetic causes and involvements in many diseases and their pathogenic processes. This has been true for haematological malignancies (HMs) with identification of acquired mutations in many genes that drive initiation, maintenance, progression or drug resistance to malignancy. In addition, germline mutations in over 20 genes are now known to predispose predominantly to HMs in the continuing evolving landscape of HM predisposition.

Since 2004, we have collected over 250 families with multiple HMs and/or early onset of HM in the Australian Familial Haematological Conditions Study (AFHCS). We have described genetic variants, phenotype expansions and molecular mechanisms in numerous cancer predisposition genes (CPGs) such as *GATA2, RUNX1, CEBPA, PAX5, SAMD9L, DDX41* and DNA damage repair genes such as *BRCA1/2, PALB2 and CHEK2*. A conundrum is that many patients do not display a family history of HM due to *de novo mutation*, variable penetrance, late onset and/or clinical notes that do not contain family histories of blood cancers. Hence, pathogenic germline variants may be missed in “sporadic” HMs while contributing up to 15-20% of cases.

Genetic profiling of somatic mutations in HM affected patients and unaffected family members reveals different patterns of co-mutated genes for different CPGs. We identified different propensities to clonal haematopoiesis in blood of unaffected carrier individuals, and the mutation types occurring suggest different mechanisms of leukaemogenesis.

A relatively common finding in inherited bone marrow failure syndromes, some of which predispose to HM, is somatic genetic rescue (SGR). We have found SGR in known and new HM predisposition genes that confound their discovery, diagnosis and prognosis, and will present examples of each to help fill the knowledge gap. SGR has the potential to ameliorate or delay clinical symptoms, and importantly offers potential for use of cell or gene therapy.

Oral Abstracts

Session 8: Cancer Metabolism

Metabolic regulation of tumour cell MHC-I antigen presentation

Keziah E Ting[1,2], Nicola E Caine[1], Rasan M Sathiqu[1], Kristin K Brown[1,2,3]
[1]*Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia*
[2]*Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC 3010, Australia*
[3]*Department of Biochemistry and Pharmacology, The University of Melbourne, Melbourne, VIC 3010, Australia*

Tumour development and progression are dependent on the ability of cancer cells to evade immune control. One of the major mechanisms contributing to immune evasion is the downregulation of tumour cell major histocompatibility complex-I (MHC-I) expression, which leads to a failure of cancer cells to present antigens for recognition by T cells. Given that MHC-I downregulation is associated with disease progression and immunotherapy resistance across diverse tumour types, identifying the fundamental mechanisms contributing to this phenomenon could pave the way to develop new treatment strategies.

Cells within the tumour microenvironment frequently encounter harsh metabolic conditions. Restricted availability of some nutrients (e.g. amino acids) and overabundance of others (e.g. lactate) contributes to the reprogramming of cellular metabolism, which is a recognised hallmark of cancer. A growing body of evidence highlights the critical role that changes in nutrient availability play in regulating T cell activity/function. However, the metabolic regulation of tumour cell MHC-I antigen presentation is unknown.

With this in mind, we have investigated the consequences of altered nutrient availability on tumour cell MHC-I antigen presentation and shown that amino acid withdrawal rapidly reduces basal and interferon-gamma-induced MHC-I expression across diverse cancer cell types. Mechanistically, the reduction in MHC-I expression following amino acid withdrawal is mediated by induction of the ribotoxic stress response (RSR), which initiates degradation of MHC-I transcripts in a manner dependent on the RNA binding protein ZFP36L1. Given that nutrient availability is frequently compromised in the tumour microenvironment, we believe that RSR-dependent regulation of MHC-I likely plays a significant role in reducing MHC-I expression to promote immune evasion. Importantly, the downregulation of tumour cell MHC-I expression in the context of amino acid deprivation is reversible and therefore targeting amino acid metabolism affords opportunities to restore MHC-I expression and immune clearance.

Oral Abstracts

Session 8: Cancer Metabolism

Inducing synthetic lethality in prostate and breast cancer via androgens and lipids

Julie-Ann Hulin, Jai Meyers, Dong Gui Hu, Ross McKinnon, Peter Mackenzie, Robyn Meech
Clinical Pharmacology, College of Medicine and Public Health, Flinders University, Bedford Park, SA.

Prostate cancer (PCa) shows increased expression of the androgen receptor (AR), is initially dependent on androgen signalling for growth/survival, and is typically treated with androgen deprivation therapy (ADT). However, this inevitably drives tumours to androgen independence. Second-line AR antagonists provide little survival benefit, and the selective pressure can promote conversion to aggressive neuroendocrine phenotypes. A subset of breast cancers (BCa) that express AR (luminal-AR) also initially respond to ADT but evidence for sustained effectiveness is limited. In these cancers, androgen and lipid pathways converge: increasing lipid synthesis is a core mechanism by which AR induces cell growth. The master regulators of lipogenesis are the sterol regulatory element binding proteins (SREBPs): while AR and SREBP cooperate in lipogenic signalling, we show that they also tightly constrain each other's activity via a reciprocal feedback loop to avoid toxicity associated with hyperactivation of either pathway. We have developed a novel combinatorial approach to assess the lethality of dual hyperactivation of AR and SREBP pathways and show that this produces cytotoxicity within 24 hours. Mechanistically, we have discovered that AR reduces SREBP levels by an indirect mechanism involving the novel regulators UDP-glycosyltransferase (UGT) 2B11 and 2B28, which induce nuclear SREBP turnover. Several UGTs are expressed in steroid target tissues and are responsible for conjugation of a diverse range of molecules including steroids, rendering them inactive and promoting their elimination. However, the paralogous UGT2B11/28 genes encode orphan members with previously poorly defined functions. Together, our data suggest a new understanding of both UGT function and AR and SREBP signalling in prostate and breast cancers, and pave the way for identification of novel therapeutic drug targets.

Oral Abstracts

Session 8: Cancer Metabolism

Acquired resistance to CDK4/6 inhibition leads to metabolic re-writing and protection against ferroptosis in prostate cancer

Joshua Hodgson[1], Dr. Han Lee[2], Dr. Ralf Schittenhelm[2], Professor Lisa Butler[1], Dr. Margaret Centenera[1]
[1]University of Adelaide/ SAiGENCI, Adelaide, Australia, [2]Monash Proteomics and Metabolomics facility, Monash University, Melbourne, Australia

Advanced prostate cancer is fatal and effective treatments are urgently needed. Genomic studies have identified the cell cycle as dysregulated in men with advanced prostate cancer, thus targeting the cell cycle machinery has therapeutic potential. Cell cycle inhibitors, including the CDK4/6 inhibitor ribociclib, are approved for the treatment of metastatic breast cancer and are in clinical trial for prostate cancer. These agents result in increased overall survival compared to conventional therapy alone for breast cancer patients. Despite this, a number of patients will develop resistance to these agents with no clear mechanism identified. To understand how adaptive resistance may arise in prostate cancer we developed ribociclib-resistant V16D prostate cancer cells through dose escalation passage to a clinically relevant dose of 500nM. MS/MS based label free quantitative proteomics identified ~800 differential abundant proteins in ribociclib-resistant cells compare to parental cells (FC>1.2, adj.p.val<0.05). Pathway analysis of differentially abundant proteins revealed enrichment of metabolic pathways including amino acid metabolism, TCA cycle and fatty acid metabolism. Upon investigation of these pathways, ribociclib-resistant cells displayed increased fatty acid oxidation and abundance of monounsaturated and polyunsaturated (PUFA) fatty acids. Our lab has previously demonstrated that increased PUFA oxidation is protective against ferroptosis, an iron-dependent, non-apoptotic form of cell death, and promotes prostate cancer cell survival. Proteomic analysis revealed altered abundance of ferroptotic regulators including increased abundance of glutathione peroxidase 4 (GPX4), the key ferroptotic suppressive protein. Targeting of GPX4 with the small molecule inhibitor ML210 lead to an increase in peroxidated lipid species, indicative of ferroptosis, and a decrease in cell proliferation in ribociclib-resistant cells compared to parental V16D cells. Collectively, this data suggests that acquired resistance to ribociclib leads to metabolic re-writing that eliminates peroxidated lipid species and protects from ferroptosis.

Oral Abstracts

Session 8: Cancer Metabolism

Chronic lymphocytic leukaemia cells have a unique lipid profile and survive *in vitro* when cocultured with adipocytes

Alana White[1], Dr Giles Best[1], Professor Bryone Kuss[1], Dr Lauren Thurgood[1]
[1]*Molecular Medicine and Genetic Pathology, College of Medicine and Public Health, Flinders University, Bedford Park, Australia*

Chronic lymphocytic leukaemia (CLL) accounts for approximately two thirds of all leukaemia cases in Australia. CLL is characterised by the expansion of mature, dysfunctional B lymphocytes in the peripheral blood, bone marrow, and lymph nodes. Managing patients with this incurable disease is challenging due to heterogeneity in the clinical course and prevalence of treatment resistance.

CLL cells receive pro-survival and proliferative signals from non-neoplastic cells in regions of the bone marrow and lymph nodes, which are collectively termed the tumour microenvironment (TME). One cellular component of the TME is lipid-rich adipocytes, which make up 50-70% of the adult marrow volume. However, the potential role of adipocytes in the pathogenesis of CLL has been largely overlooked. Studies in other cancers, including breast, prostate, and multiple myeloma, have shown that adipocytes and lipids promote proliferation and confer more aggressive disease and drug resistance.

Our studies have identified a potential role of adipocytes in the pathogenesis of CLL. Employing an *in vitro* coculture model, we observed a >20% increase in the proportion of viable primary CLL lymphocytes when cocultured with murine-derived adipocytes for 96 hours than in samples cultured in media alone. A lipidomic analysis comparing CLL cells with their healthy counterpart identified nineteen lipid species that were significantly differentially expressed, suggesting that these specific lipids may be involved in CLL cell survival.

Collectively, these findings are consistent with the role of adipocytes and lipids in cancer pathogenesis and indicate a previously undescribed role of adipocytes in CLL. The unique lipid profile exhibited by CLL cells is the first step in gaining a comprehensive understanding of the role of lipids in CLL pathogenesis. The development of novel treatment approaches aimed at starving CLL cells of their energy derived from lipids may represent an avenue to improve CLL patient outcomes.

Oral Abstracts

Session 9: Cancer Biology to Therapy

Uncovering disease mechanisms and targeted therapies in hepatosplenic T cell lymphoma

Ms Susann Schönefeldt*[1], Ms Myint Myat Khine Aung*[1], Ms Sophie Kraupp[1], Ms Tamara Wais[1], Mr Tobias Suske[1], Ms Safia Zahma[1], Ms Christina Wagner[1], Dr Thomas Eder[2], Professor Florian Grebien[2], Professor Marco Herling[3], Professor Richard Moriggl[1], Assistant Professor Heidi Neubauer[1]
[1]Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Austria, [2]Institute of Medical Biochemistry, University of Veterinary Medicine Vienna, Austria, [3]Department of Hematology, Cellular Therapy, and Hemostaseology, University of Leipzig, Germany

Hepatosplenic γδ T cell lymphoma (HSTL) is a rare but aggressive disease that primarily affects young adults with a median age of 34 years. HSTL manifests as a fatal infiltration of malignant γδ T cells predominantly into the spleen and liver. The disease progresses rapidly, with a median survival of only 13 months, and has a poor response to available chemotherapy-based treatment. Hence, new therapeutic strategies are urgently needed. Given the scarcity of patients and lack of pre-clinical models, studying HSTL disease mechanisms and exploring novel targeted therapies has been limited. The JAK-STAT pathway is frequently dysregulated in HSTL and STAT5B-N642H is the most common gain-of-function mutation in HSTL patients. Therefore, we aimed to generate a pre-clinical mouse model of HSTL to explore disease mechanisms and identify new therapeutic targets. We utilized our transgenic mouse model harboring human STAT5B-N642H in the hematopoietic compartment. Syngeneic γδ T cell transplants from these mice resulted in the rapid development of γδ T cell lymphoma (γδ TCL). We generated clonal γδ TCL cell lines using outgrowth cultures from this model. These mutant STAT5B-positive cells recapitulate features of human patient-derived HSTL cells, demonstrated by their hyperactivation and dependence on IL2-JAK-STAT5 signaling and their immunophenotypic profiles. Importantly, intravenous allografts of the γδ TCL cells into immunocompetent mice resulted in an aggressive HSTL-like disease with pronounced hepatosplenomegaly. Utilizing our HSTL models to study disease mechanisms, RNA-sequencing identified key genes dysregulated by oncogenic STAT5B relevant to HSTL disease. Finally, to assess novel targeted therapeutic options for HSTL, we subjected murine and human HSTL cells to a panel of JAK inhibitors, revealing sensitivity of the cells to blocking JAK signaling. Overall, our novel pre-clinical HSTL mouse model and insights into HSTL disease mechanisms and drug sensitivities have the potential to assist with developing new therapeutic options for HSTL patients.

Oral Abstracts

Session 9: Cancer Biology to Therapy

Tales From the Crypt: how RIPK3 kinase unleashes the zombie protein, MLKL, to kill cells by necroptosis

James M Murphy[1, 2]
[1] *Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia*
[2] *Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC Australia*

Background: In 2012, Mixed lineage kinase domain-like (MLKL), a catalytically-dead ("zombie") cousin of conventional protein kinases, termed a pseudokinase, was implicated as the key effector in the programmed necrosis (or necroptosis) cell death pathway. This pathway has been implicated in innate immunity, the pathogenesis of inflammatory diseases, and tissue injury arising from ischemia-reperfusion. As a result, an improved fundamental knowledge of MLKL's activation mechanism is of enormous interest as we and others look to target the pathway therapeutically.

We have dissected the chronology of events in this pathway using novel tools, structural biology, biochemistry, microscopy, proteomics, mouse disease models and analysis of patient biopsies, enabling us to define four regulated steps in MLKL activation, which we term checkpoints. (1) MLKL resides in a dormant complex with its activating kinase, RIPK3, until a cell receives a cue, such as exposure to a pathogen metabolite or a death receptor ligand. Subsequently, MLKL is recruited to a high molecular weight platform termed the necrosome, where (2) RIPK3 phosphorylates the MLKL pseudokinase domain to provoke its structural interconversion and release from the necrosome. MLKL phosphorylation drives its assembly into oligomers, which are (3) translocated to the plasma membrane where they accumulate into large assemblies that we term hotspots. (4) When a critical threshold is surpassed, MLKL's four-helix bundle domain permeabilizes membranes, which ultimately leads to a cell's demise. Physiologically, this process appears to predominate in barrier tissues, and accordingly, our data suggest that necroptosis is dysregulated in Inflammatory Bowel Disease, a known risk factor for bowel cancer.

Our studies have uncovered a series of regulated events that govern the necroptosis pathway, raising the prospect that these checkpoints might be pharmacologically targetable for the treatment of inflammatory diseases, such as IBD.

Oral Abstracts

Session 9: Cancer Biology to Therapy

Overcoming Bcl-2 inhibitor resistance in acute myeloid leukemia

Assoc Prof Jason Powell[1], Dr Alexander Lewis[1], Mrs Victoria Pope[1], Mr Gus Nwosu[1], Prof Stuart Pitson[1]
[1]*Center for Cancer Biology, UniSA and SA Pathology, Adelaide, Australia*

The BH3 mimetic venetoclax is a highly selective oral inhibitor of the pro-survival protein Bcl-2 showing remarkable response rates for treatment of chronic lymphocytic leukemia. In acute myeloid leukemia (AML) venetoclax shows modest activity as a single agent but promising results when combined with chemotherapy or hypomethylating agents; however, intrinsic and acquired resistance to venetoclax has emerged as a major clinical problem in achieving deep and durable responses. The pro-survival Bcl-2 family member Mcl-1 on which AML cells are highly dependent on for survival, has been shown to drive venetoclax resistance in AML. We now report a novel mechanism whereby the accumulation of ceramide by inhibition of sphingosine kinase can target Mcl-1, and overcome venetoclax resistance. Enhancing cellular ceramide does this by inducing an apoptotic integrated stress response (ISR) through protein kinase R-mediated activation of the master transcription factor ATF4. This leads to transcription of the BH3-only protein, Noxa, and degradation of the pro-survival Mcl-1 protein. Targeting this novel ISR pathway in combination with venetoclax synergistically killed primary AML blasts, including those with venetoclax-resistant mutations, as well as leukemia-initiating cells, and reduced leukemic burden in patient-derived AML xenografts. Collectively, these findings provide mechanistic insight into the anti-cancer effects of ceramide and pre-clinical evidence for new approaches to augment Bcl-2 inhibition in the therapy of AML and other cancers with high Mcl-1 dependency.

Oral Abstracts

Session 9: Cancer Biology to Therapy

Preventing Resistance to EGFR Tyrosine Kinase Inhibitors in EGFR-mutant Non-Small Cell Lung Cancer

Dr Kimberley Clark[1], Dr Katherine Morel[1], Dr Mark Bunting[1], Prof Christopher Sweeney[1]
[1]*SAiGENCI, The University of Adelaide, Adelaide, Australia*

Lung cancer is the leading cause of cancer deaths worldwide, with over 8600 people estimated to die from this disease in Australia each year. Non-small cell lung cancer (NSCLC) is the most common lung cancer type, with up to 40% of tumours from patients with NSCLC harbouring mutations in the epidermal growth factor receptor (EGFR) gene. EGFR-directed therapy with tyrosine kinase inhibitors (TKIs) provides a substantial clinical benefit for patients that have disease with EGFR mutations. However, resistance to EGFR inhibition commonly occurs and patients experience disease progression and die from NSCLC. Activation of the Nuclear factor-kappa B (NF-κB) signalling pathway either prior to or in response to EGFR inhibition is a driver of tumour cell survival and NSCLC resistance to EGFR TKI. Yet, there are currently no NF-κB inhibitors approved for clinical use and the mechanisms by which NF-κB activation occurs are incompletely understood. This study aims to develop the understanding of the drivers of NF-κB hyperactivation following resistance to third generation TKI, Osimtertinib, using state-of-the art gene editing techniques of human and mouse lung cancer cells and preclinical models of disease. Analysis of publicly available patient data shows an association between NF-κB dysregulation and worse overall survival in patients with EGFR-mutant NSCLC. Furthermore, we have demonstrated that use of the NF-κB inhibitor, Dimethylaminoparthenolide (DMAPT), in combination with Osimertinib, leads to an increased survival time in a preclinical subcutaneous xenograft mouse model of EGFR-mutant NSCLC, when compared with Osimertinib alone. Ultimately, this project will contribute to the improved overall survivorship and optimised cancer care for patients with EGFR-mutant NSCLC.

Oral Abstracts

Session 9: Cancer Biology to Therapy

A novel role for intercellular adhesion molecule (ICAM)-1 on breast cancer cells

Ms Anahita Fouladzadeh[1], Ms Emma Thompson[1], Ms Michaelia Cockshell[1], Professor Michael Samuel[1,2], Professor Claudine Bonder[1,2]
[1]*Centre For Cancer Biology, University of South Australia and SA Pathology, Adelaide, AUSTRALIA,*
[2]*Adelaide Medical School, University of Adelaide, Adelaide, AUSTRALIA*

Growth and metastasis of the most aggressive and intractable subgroup of breast cancers, triple negative breast cancer (TNBC), is supported by a network of blood vasculature.

Tumour vasculature is built via angiogenesis (the proliferation of endothelial cells (ECs) from existing vasculature) and vasculogenic mimicry (VM, cancer cell-formed vascular-like structures). TNBCs have a high content of VM and while these patients are at high risk of succumbing to the disease. The contribution of VM to TNBC is still poorly understood and is the focus of this work.

Anti-tumour immunity is facilitated via leukocyte entry into the cancerous mass; a process regulated via complementary adhesion molecules on ECs and the leukocyte subsets. Intercellular adhesion molecule-1 (ICAM-1/CD54) is expressed by ECs and captures circulating leukocytes via their expression of integrins (e.g. LFA-1 or Mac-1). Whether VM structures express adhesion molecules to mediate leukocyte recruitment is unknown.

Data suggest that VM-competent TNBC cell lines (e.g. MDA-MB-231) express high levels of ICAM-1 and accordingly, suppressing expression of ICAM-1 via targeted siRNA significantly inhibits the adhesion of CD14+ monocytes and CD3+ T lymphocytes. In a syngeneic mouse model of TNBC, 4T1.13 breast cancer cells (without or with ICAM-1) injected into the mammary fat pad of BALB/c mice revealed a role for ICAM-1 in tumour growth. Moreover, loss of ICAM-1 on 4T1.13 cancer cells reduced tumour growth and was associated with a larger number of anti-tumourigenic CD8+ T cells in the 4T1.13-ICAM1-KO tumours. Further analysis identified a role for ICAM-1 in the tumour microenvironment of TNBC via regulation of pro-tumourigenic cytokines (e.g. IL-6), chemokines (e.g. CCL5) and proteases (e.g. MMP9).

These data suggest that VM-competent cancer cells express ICAM-1 to modify the tumour microenvironment via cellular and non-cellular mechanisms. Thus targeting ICAM-1 may be a promising strategy to improve the survival of patients with TNBC.

Oral Abstracts

Session 10: Tumour Microenvironment

Interplay between Inflammation, Anti-tumor Immunity and Tissue Tension

Valerie Weaver and colleagues
Center for Bioengineering and Tissue Regeneration, Departments of Surgery, Bioengineering and Therapeutic Sciences, Radiation Oncology, University of California, San Francisco

Solid tumors develop a fibrotic stromal microenvironment that is characterized by a remodeled, cross-linked extracellular matrix (ECM) that becomes progressively stiffened. The Weaver group has been exploring the molecular origins of tumor fibrosis and ECM stiffening and examining its impact on tumor development, progression and treatment response. Using genetically engineered mouse models (GEMMs) our studies revealed that infiltrating macrophages secrete TGFb that stimulates tissue resident fibroblasts to synthesize, deposit and remodel ECM and further drives stromal stiffening by inducing lysyl oxidase (LOX) and lysyl hydroxylase 2 (LH2)-dependent collagen crosslinking. My group performed culture experiments using two and three dimensional organoids with tuned ECM stiffness and transgenic and syngeneic mouse models and human PDX xenografts and demonstrated that a stiffened fibrotic ECM disrupts tissue organization, promotes cell growth and survival and drives invasion and tumor cell dissemination to promote malignant transformation and metastasis by inducing an epithelial to mesenchymal transition. We further determined that this fibrotic stromal simultaneously modifies anti-tumor immunity and compromises checkpoint inhibitor therapy response by reprogramming the tumor infiltrating macrophages towards a pro-tumorigenic ECM synthetic phenotype. Upon investigating molecular mechanisms regulating this macrophage phenotype we determined that it is the exposure to the stiffened ECM that fosters the metabolic reprograming of the infiltrating myeloid cells towards an immunosuppressive, fibrosis-promoting, wound-healing macrophage phenotype (TAM). Further analysis revealed that the stiffened ECM drives the TAM phenotype by altering cellular TGFβ SMAD signaling. We found that these tension-reprogrammed TAMs produce even larger quantities of TGFβ, a cytokine that is a hallmark of tolerized macrophages, which stimulates the tumor resident fibroblasts to remodel the ECM. We found that these tolerized TAMs are unable to mount toll-like receptor (TLR)-dependent pro-inflammatory responses to damage-associated molecular patterns (DAMPs) in the tumor microenvironment (TME), thereby preventing the activation of anti-tumor immunity. Moreover, these tension-tolerized TAMs synthesize large quantities of proline that significantly reduces tumor tissue levels of arginine and increase levels of secreted ornithine that compromises CD8 T cell viability and function. The net result is these tension-reprogrammed TAMs accelerate tumor aggression and metastasis and ultimately compromise immune checkpoint response. To address this pro-tumor immune pathology we discovered that a critical cellular coenzyme, Coenzyme A (CoA), can reverse this tension-tolerized TAM state. We determined that metabolically supplementing GEMM mice with mammary tumors with a combination of CoA with a TLR4 agonist MPLA, can transform these immunologically “cold” tumors into “hot” tumors, thereby slowing tumor growth and metastasis. The findings illustrate, for the first time, that a “metabolic adjuvant” supplementation is not only able to restore the anti-tumor responsiveness of these macrophages but can also prevent tumor aggression and metastasis. During my presentation I will discuss the interplay between tumor fibrosis, tissue tension and innate and acquired immunity. I will present data to show how these ECM properties promote tumor metastasis by dysregulating anti-tumor immunity and I will end with a discussion on plausible strategies that could be rapidly employed to improve cancer treatment.

Oral Abstracts

Session 10: Tumour Microenvironment

Mechanobiology of cytotoxic lymphocyte-mediated solid tumour rejection

Maté Biro

University of New South Wales, Sydney

Cytotoxic lymphocytes can migrate rapidly and with striking versatility in a continuous search for cells to subdue. Adoptive cell transfer immunotherapies attempt to harness the capacity of T cells and natural killer (NK) cells to effectively locate, engage and kill cancer targets, yet they have thus far largely proved unsuccessful when targeting solid malignancies due to insufficient tumour infiltration. Moreover, the mechanisms and cellular forces that underpin the coordinated movements and interactions of killer immune cells and tumour cells are incompletely understood. Here, we investigate the intercellular signalling and mechanical forces that these killer immune cells employ to effectively infiltrate and attack solid tumour cells. Using an integrative and multidisciplinary method encompassing advanced live-cell microscopy, image analysis, biophysics and modelling, we are uncovering the intricate mechanobiology of cytotoxic lymphocyte-mediated tumour rejection.

Oral Abstracts

Session 10: Tumour Microenvironment

Compressive stress promotes mammary tumour progression via Piezo1-CaMKII-RhoA-ROCK mechanotransduction signalling

Dr Sarah Boyle[1], Associate Professor David Gallego-Ortega[2], Associate Professor Kate Poole[3], Professor Michael Samuel[4]
[1]Centre for Cancer Biology, Adelaide, Australia, [2]University of Technology Sydney & Garvan Institute of Medical Research, Sydney, Australia, [3]University of New South Wales, Sydney, Australia, [4]Centre for Cancer Biology & Basil Hetzel Institute for Translational Health Research, Adelaide, Australia

External mechanical force is exerted upon tissues and cells throughout development and homeostasis. These tissue-level forces are transduced by the extracellular matrix (ECM) and initiate mechanotransduction signalling pathways, enhancing actin polymerisation and myosin contractility, thereby generating dynamic intracellular forces. Forces generated by the actin cytoskeleton counteract extracellularly exerted forces in a process termed mechano-reciprocity, which is fundamental to the maintenance of tissue integrity. The main inducer of cytoskeletal tension is myosin II, and its regulatory subunit myosin regulatory light chain-2 (Mlc2) can be directly activated by the Rho-ROCK signalling pathway. High extracellular matrix density, a feature of disease states such as cancer, activates mechanotransduction pathways that promote tumour progression. Therefore, investigating mechano-reciprocity and its regulation in homeostasis is key to understanding how this process becomes dysregulated in disease.

We have found that acute compressive force applied to murine mammary cancer cells and epithelial tissues can activate the Rho-ROCK signalling pathway, elevating RhoA-GTP levels and increasing actomyosin contractility and tension over and above that encountered at early stages of mammary cancer. We have also found that this is mediated via the mechanosensitive ion channel Piezo1 and downstream calcium signalling. Compression induces calcium ion influx and activation of calmodulin-dependent protein kinase II (CaMKII), leading to Rho-ROCK pathway activation, and this is suppressed by silencing Piezo1, calcium chelation, or inhibiting CaMKII.

The compressive stress-induced Piezo1-CaMKII-Rho-ROCK cascade increases cancer cell proliferation, enhances EMT, and ultimately promotes tumour growth. Our results therefore strongly suggest that Rho-ROCK-mediated mechanotransduction in cancer, induced by compressive stress from mammary tumour growth within a constricted space, may play a role in early tumour progression.

Oral Abstracts

Session 10: Tumour Microenvironment

A novel first-in-class anti-fibrotic blunts tumour desmoplasia, rewires stromal signalling and augments gemcitabine response and survival in pancreatic cancer

A/Prof Thomas Cox[1,2]
[1]Cancer Ecosystems Program, The Garvan Institute of Medical Research and The Kinghorn Cancer Centre, Darlinghurst, Australia, [2]School of Clinical Medicine, St Vincent's Healthcare Clinical Campus, UNSW Medicine and Health, UNSW Sydney, Sydney, Australia

The lysyl oxidase family represents a promising target in stromal targeting of solid tumours due to the importance of this family in crosslinking and stabilizing fibrillar collagens and its known role in tumour desmoplasia.

Using small-molecule drug-design approaches, we generated and validated PXS- 5505, a first-in-class highly selective, potent, mechanistic pan-lysyl oxidase inhibitor.

We demonstrate *in vitro* and *in vivo* that pan-lysyl oxidase inhibition decreases chemotherapy-induced pancreatic tumour desmoplasia and stiffness, reduces cancer cell invasion and metastasis, improves tumour perfusion, and enhances the efficacy of chemotherapy in the autochthonous genetically engineered KPC model, while also demonstrating anti-fibrotic effects in human patient-derived xenograft models of pancreatic cancer. Mechanistically, the decreases in tumour stiffness brought about by PXS-5505 reduces CAF activation within the tumour microenvironment and decreases STAT3 signalling in cancer cells to augment chemotherapy efficacy and prolong survival.

PXS-5505 is orally bioavailable, safe and effective at inhibiting lysyl oxidase activity in tissues, and has cleared Phase I safety trials thereby enabling Phase II trials. Our findings present the rationale for progression of a pan-lysyl oxidase inhibitor aimed at eliciting a reduction in stromal matrix to potentiate chemotherapy in pancreatic ductal adenocarcinoma.

Poster Abstracts

P1

Targeting subcellular JNK activity in pancreatic cancer

Antonia Cadell[1], Dr Brooke Pereira[1,2], Associate Professor Marina Pajic[1,2], Professor Paul Timpson[1,2], Associate Professor David Croucher[1,2]
[1]The Kinghorn Cancer Center, Garvan Institute of Medical Research, Darlinghurst, Australia, [2]St Vincent's Hospital Clinical School, UNSW Sydney , Australia

c-Jun N-terminal Kinase (JNK) is a potent oncogene in numerous cancers, including breast and pancreatic cancer. Although JNK can promote metastasis, it is also vital for tumour suppression and apoptotic signalling. Our research into the roles of JNK in triple negative breast cancer has unveiled the mechanism underlying these pleiotropic functions to be distinct, subcellular pools of JNK activity. Specifically, JNK activity was predominantly localised to the nucleus in normal breast tissue, but significantly elevated in the cytoplasm of triple-negative tumours. This prompted us to investigate whether similar patterns of subcellular JNK activity are observed and potentially targetable in metastatic Pancreatic Ductal Adenocarcinoma.

Our analysis of the ICGC patient cohort demonstrated that cytoplasmic JNK activity was constitutively elevated in pancreatic cancer, similar to the levels observed within TNBC tumours. Using inducible subcellular JNK inhibitors we carried out functional assays which revealed that inhibiting JNK in either the nucleus or the cytoplasm inhibited the proliferation of cells growing within a three-dimensional, organotypic collagen matrix. This was further characterised *in vivo* using a subcutaneous xenograft model which showed that inhibition of both forms of subcellular JNK significantly extended survival and slowed tumour growth. These results were also recapitulated within an orthotopic model, in which metastasis to the liver along with incidence of ascites were significantly reduced when JNK was inhibited in either the nucleus or cytoplasm.

This data suggests that while the dual JNK network states found within breast tissue differ from those seen in pancreatic cancer, JNK remains attractive therapeutic target. To investigate this further we have carried out RNA sequencing on xenograft tumours to understand the relationship and possible translocation of JNK between the nucleus and cytoplasm in PDAC.

Poster Abstracts

P2

Rationalising the inclusion of HDAC inhibitors with standard-of-care chemotherapy for high-risk neuroblastoma

Miss Monica Phimmachanh[1], Dr Jeremy Han[1], Mr King Ho Leong[1], Dr Sharissa Latham[1], Associate Professor David Croucher[1]
[1]Garvan Institute of Medical Research, Sydney, Australia

High-risk neuroblastoma is an aggressive, highly chemoresistant childhood tumour. These patients will receive intensive, multi-modal therapy, although relapse with treatment resistant disease occurs in up to 50% of cases.

We recently utilised mathematical modelling and longitudinal single-cell imaging to demonstrate that a non-genetic form of chemoresistance can arise in neuroblastoma through the impact of gene expression noise upon the stochastic nature of apoptotic signalling (Hastings*, Latham*, 2023, Science Advances). Within treatment naïve neuroblastomas, priming with the histone deacetylase (HDAC) inhibitor Vorinostat could overcome this chemoresistance and sensitise tumours to treatment with specific standard-of-care chemotherapies.

In order to further rationalise the inclusion of HDAC inhibitors with a wider range of standard-of-care chemotherapy treatments for high-risk neuroblastoma patients, we have now undertaken a functional analysis of a panel FDA-approved HDAC inhibitors. By using established high content imaging and applying multi-omics approaches, this analysis has demonstrated that HDAC inhibitors with differing specificity are capable of eliciting a diverse range of cell behaviour in neuroblastoma tumours, which impacts the manner in which they should be deployed in a clinical setting.

A key observation from this study was that the HDAC inhibitors Belinostat and Vorinostat did not directly induce apoptosis, but readily primed the cells to allow for sensitisation to standard-of-care chemotherapies. These differing functional outcomes were also associated with unique histone acetylation patterns and mechanistically coherent transcriptional changes as determined by RNAseq analysis.

These mechanistic insights are now being leveraged to design rationalised treatment regimens that combine these HDAC inhibitors with standard-of-care chemotherapies. These optimal combinations are currently being tested within patient derived xenograft models, in order to identify approaches capable of improving survival outcomes for high-risk neuroblastoma patients.

Poster Abstracts

P3

CDK9 inhibition as a therapeutic strategy in advanced prostate cancer

Miss Razia Rahman[1], Muhammed Rahaman[2], Adrienne R. Hanson[1,3], Jianling Xie[1,3], Scott L. Townley[1,3], Nicholas Choo[4,5], Kaylene J. Simpson[6,7,8], Susanne Ramm[7,8], Ganessan Kichenadasse[1], Simon J. Conn[1], Gail P. Risbridger[4,5], Renea A. Taylor[4,5], Mitchell G. Lawrence[4,5], Wayne Tilley[9,10], Margaret M. Centenera[10,11,12], Lisa M. Butler[10,11,12], Shudong Wang[2], Luke A. Selth[1,3]
[1]Flinders University, Adelaide, Australia, [2]University of South Australia, Adelaide, Australia, [3]Freemasons Centre for Male Health and Wellbeing, Flinders University, Adelaide, Australia, [4]Biomedicine Discovery Institute, Cancer Program, Monash University, Melbourne, Australia, [5]Prostate Cancer Research Program, Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia, [6]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia, [7]Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre, Melbourne, Australia, [8]Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, Australia, [9]Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, Australia, [10]Freemasons Centre for Male Health and Wellbeing, Adelaide Medical School, University of Adelaide, Adelaide, Australia, [11]South Australian Health and Medical Research Institute, Adelaide, Australia, [12]South Australian immunoGENomics Cancer Institute (SAiGENCI), University of Adelaide, Adelaide, Australia

Prostate cancer is the most frequently diagnosed non-skin cancer and a leading cause of cancer-related death in men worldwide. The key driver of prostate cancer is the androgen receptor (AR). Thus, the primary treatment strategy for metastatic prostate cancer involves blocking AR activity. While most men initially respond to AR-targeted therapies, they are not curative and patients inevitably progress to a lethal disease state termed castration-resistant prostate cancer (CRPC). Therefore, the development of new and effective treatment options for CRPC is essential to improve patient outcomes.

Targeted therapies employing small-molecule inhibitors have recently emerged as potential cancer therapeutics. One such plausible therapeutic target is Cyclin-Dependent Kinase 9 (CDK9), which regulates transcriptional elongation by phosphorylating RNA polymerase II. Dysregulated transcription due to elevated CDK9 activity has been observed in various haematological and solid cancers and CDK9 inhibitors are being intensively explored in clinical trials. Here we describe the efficacy of a novel orally bioavailable CDK9 inhibitor, CDKI-73, in prostate cancer. CDKI-73 potently inhibits proliferation and causes cell death by apoptosis in prostate cancer cell lines representing distinct CRPC subtypes. CDKI-73 was also evaluated in more clinically relevant systems, including patient-derived organoids, patient-derived tumour explants and mouse xenografts, and was found to be highly effective. Mechanistically, CDKI-73 reduced RNA polymerase II phosphorylation, affecting the transcription of anti-apoptotic genes and other cancer drivers (e.g. MYC) that are essential for cancer cell proliferation and survival. Additionally, CDKI-73 reduced AR phosphorylation resulting in reduced AR activity. Transcriptomic profiling revealed that CDKI-73 also downregulates signalling pathways driven by other oncogenic transcription factors, MYC and BRD4. Combining CDKI-73 with a BRD4 inhibitor exhibited synergistic anti-cancer activity in cell line and patient-derived organoid models of aggressive cancer. Collectively, our work provides new insights into CDK9's oncogenic activity and reveals CDKI-73 as a promising therapeutic agent for prostate cancer.

Poster Abstracts

P4

Desmoglein-2 expression by multiple myeloma is an independent predictor of poor prognosis that can be rapidly identified by flow cytometry.

Dr Barbara McClure[1,2], Dr Charlotte Downes[2], A/Prof Lisa Ebert[1], A/Prof Kate Vandyke[2,4], Dr Zahied Johan[1], Dr Lih Tan, Dr Giles Best[3], Dr Kay K Myo Min[1], Prof Andrew Zannettino[3], Prof Stuart Pitson[1], A/Prof Craig Wallington-Gates[3,5,6], Prof Claudine Bonder[1]
[1]Centre for Cancer Biology, University of South Australia and SA Pathology, Australia, [2]Adelaide Medical School, Faculty of Health and Medical Science, University of Adelaide, Australia, [3]Flinders Centre for Innovation in Cancer, Flinders University, Australia, [4]Precision Medicine Theme, South Australian Health and Medical Research Institute, Adelaide, Australia, [5]College of Medicine and Public Health, Flinders University, Adelaide, Australia, [6]Flinders Medical Centre, Adelaide, Australia

Multiple myeloma (MM) is the second most common haematological malignancy and is an incurable disease of neoplastic plasma cells (PC). Newly-diagnosed MM patients currently undergo lengthy genetic testing to match chromosomal mutations with the most potent drug/s to decelerate disease progression. However this approach to disease stratification currently results in only 17% of MM patients surviving 10-years post diagnosis. Faster detection and earlier intervention would unequivocally improve outcomes. Here, we show that the cell surface protein desmoglein-2 (DSG2) is overexpressed (gene and protein) in approximately 20% of bone marrow biopsies from newly-diagnosed MM patients. Importantly, DSG2 expression was strongly predictive of poor clinical outcome, with patients expressing high levels of DSG2 exhibiting an almost 3-fold increased risk of death. Preliminary mouse studies also suggest that targeting DSG2 attenuates MM growth and disease progression. As a prognostic factor, DSG2 is independent of (i) genetic subtype, (ii) routinely measured biomarkers of MM activity (e.g. paraprotein) and (iii) therapy received. DSG2 expression on diagnostic MM bone marrow PC is currently being prospectively assessed to determine the feasibility of rapid DSG2 flow cytometry as a prognostic biomarker for MM. To date, MM patients where elevated DSG2 is detected correlate with reduced progression free survival. The mechanism by which DSG2 promotes high-risk MM disease is largely unknown. To investigate this we have developed DSG2 CRISPR knockout MM cell lines and the impact of DSG2 related functions including modulation of secreted cytokines and additional adhesion surface markers have been explored. Functional studies have revealed a non-redundant role for DSG2 in adhesion of MM PC to endothelial cells. Together, our studies suggest DSG2 to be a cell surface biomarker that can be readily detected by flow cytometry to rapidly predict disease trajectory at the time of diagnosis.

Poster Abstracts

P5

Monitoring AKT activity and targeting in live tissue and disease contexts revealed by the novel Akt-FRET biosensor mouse.

Dr James R.W. Conway[2,3,4], Dr Sean C. Warren[2,3], Young-Kyung Lee[2], Andrew T. McCulloch[2], Dr Astrid Magenau[2,3], Victoria Lee[2], Xanthe L. Metcalf[2], Janett Stoehr[2], Dr Katharina Haigh[8], Lea Abdulkhalek[2], Cristian S. Guaman[2], Daniel A. Reed[2], Dr Kendelle J. Murphy[2,3], Dr Brooke A. Pereira[2,3], Pauline Melenec[2], Dr Cecilia R. Chambers[2], Dr Sharissa L. Latham[2,3], Helen Lenthall[2], A/Prof Elissa K. Deenick[2,3], Dr Yuanqing Ma[2,3], Prof Tri Phan[2,3], Prof Elgene Lim[2,3], Prof Anthony M. Joshua[2,3], Stacey Walters[2], Prof Shane T. Grey[2,3], A/Prof Yan-Chuan Shi[2,3], Dr Lei Zhang[2,3], Prof Herbert Herzog[2,3], A/Prof David Croucher[2,3], Prof Andy Philp[9,10], Dr David Herrmann[2,3], Prof Owen J. Sansom[5,6], Prof Jennifer P. Morton[5,6], A/Prof Antonella Papa[7], Prof Jody J. Haigh[8], Dr Max Nobis[1,2,3], Prof Paul Timpson[2,3]
[1]VIB-KU Leuven Center For Cancer Biology, Leuven, Belgium, [2]Garvan Institute of Medical Research & The Kinghorn Cancer Centre, Sydney, Australia, [3]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney, Australia, [4]Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, Finland, [5]Cancer Research UK Beatson Institute, Glasgow, UK, [6]Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, UK, [7]Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia, [8]Research Institute in Oncology and Hematology, Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada, [9]Centre for Healthy Ageing, Centenary Institute, Sydney, Australia, [10]Charles Perkins Centre, Faculty of Medicine and Health, University of Sydney, Sydney, Australia

AKT (protein kinase B) is a key regulator in a variety of cellular processes such as glucose metabolism, cell survival, proliferation and cell migration. Its activity is aberrantly upregulated in a plethora of cancers, metabolic and immune disorders, including but not limited to breast, pancreatic and prostate cancer. More specific, time-resolved monitoring of key drivers of metabolism and proliferation in tissue specific contexts can be achieved *in vivo* with the use of FRET-biosensor mice to track protein activity and the effect of therapeutic intervention.

Here, we describe the generation and characterization of a FRET-biosensor mouse to examine AKT activity in *in vivo* settings in a variety of tissues and cancers by the application of optical windows. Elevated levels of AKT activity were observed in the pancreatic cancer models driven by mutant KRasG12D/+ and KRasG12D/+;p53R172H/+, including PTEN loss driven PDAC and AKT activity mapped over the course of disease progression. Whole body PTENG129E/+ mutation or loss (PTENfloxed/+) mice were also crossed to the Akt-FRET biosensor mouse and AKT activity measured in several cancers such as lymphomas, adrenal, mammary and prostate cancer. Cell lines and organoid cultures established from these tumours retained the Akt-FRET reporter expression and inhibition of AKT was mapped in vitro over time in 2D and 3D contexts. AKT activity was also effectively inhibited by administration of a Pi3K inhibitor and pharmacodynamics mapped live *in vivo* in a breast cancer setting. Metabolic challenge in mice bearing optical windows over the pancreas (RIP-Cre), white fat or brown fat was moreover successfully imaged live *in vivo* in Akt-FRET mice following ip administration of glucose or insulin.
In conclusion, the described Akt-FRET biosensor mouse can be applied to a wide range of metabolic, immune and cancer settings and used successfully in characterizing disease etiology and monitoring treatment outcomes.

Poster Abstracts

P6

Inter-patient heterogeneity across patient-derived glioblastoma explant organoids

Kaitlin Scheer[1], Ms Erica Yeo[1], Dr Chloe Shard[1], Dr Helen Palethorpe[1], Mr Conor Ryan[1], Ms Sakthi Lenin[1], Dr Melinda Tea[1], Dr Santosh Poonnoose[2], Dr Minh-Son To[3], Dr Rebecca Ormsby[2], Stuart Pitson[1], Dr Lisa Ebert[1], Dr Guillermo Gomez[1]
[1]Centre for Cancer Biology, SA Pathology and the University of South of Australia, Adelaide, Australia, [2]Flinders Health and Medical Research Institute, College of Medicine & Public Health, Flinders University, Adelaide, Australia, [3]Flinders Medical Centre, Adelaide, Australia

The generation of patient-derived glioblastoma explant organoids (GBOs) is a recent advancement in patient-focused research. Much like their parent tumours, GBOs exhibit heterogeneity in growth rate and cellular composition. Following a protocol established by Jacob and colleagues in 2020, we set out to establish a biobank of GBOs and sought to determine the critical factors influencing GBO viability and growth in culture. Tissue quality was found to be the most important factor dictating generation of viable GBOs. Mostly necrotic samples, or those where the original tumour had only foci of proliferating tumour cells made for less successful GBO cultures, while highly cellular samples containing microvascular proliferation had good viability. GBOs established from dissection of patient glioblastoma tumours were tracked using a custom Fiji macro, which allowed rapid analysis of GBO size and shape over weeks of culture. Culture viability was best indicated by GBO roundness within 1 week of culture. Viable GBO cultures demonstrated large variations in growth rate, which we were further able to compare with the proliferation rates of matched cell lines and tumour progression in patient MRIs. Assessment of GBOs by H&E and immunofluorescence staining confirmed tissue viability regardless of growth rate, with immune and endothelial cells retained in most samples after several weeks of culture. The extent of non-tumour cell retention varied between cultures, although slower growing GBOs were more likely to retain in-tact vessel structures. Analysis of conditioned media from thawed GBOs demonstrated metabolic activity in all biobanked cultures, and growth tracking generally showed similar rates to those observed prior to biobanking. Slow growth limits expansion but not the usefulness of these cultures, and indeed the variability between patients and retention of non-tumour cells make GBOs a valuable resource for *in vitro* study of glioblastoma heterogeneity.

Poster Abstracts

P7

Investigating a novel matrisomal target in pancreatic cancer to reduce fibrosis and improve standard-of-care chemotherapy

Miss Jessie Zhu[1,2], Dr Cecilia Chambers[1,2], Miss Shona Ritchie[1,2], Miss Morghan Lucas[1,2], Mr Daniel Reed[1,2], Miss Alice Tran[1,2], Dr Kendelle Murphy[1,2], Dr Diego Chacon-Fajardo[1,2], A/Prof Benjamin Parker[3], A/Prof Marina Pajic[1,2], Prof Jennifer Morton[4], A/Prof Thomas Cox[1,2], Dr Brooke Pereira[1,2], Dr DavidHerrmann[1,2], Prof Paul Timpson[1,2]
[1]Garvan Institute of Medical Research, Sydney, Australia, [2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney , Australia, [3]Department of Anatomy & Physiology, The University of Melbourne, Melbourne, Australia, [4]Cancer Research UK, Beatson Institute, Glasgow, United Kingdom

Pancreatic cancer (PC) is a highly fatal cancer with a dismal 5-year survival rate of 11%. A key driver of this poor prognosis is the highly fibrotic response of the PC tumour microenvironment which forms a stiff, impenetrable barrier that shields tumours from therapeutic intervention whilst promoting metastatic progression. It is therefore of significant importance to explore novel therapies to reduce this fibrotic response in PC. Here, we aim to investigate previously under-explored, clinically relevant matrisomal proteins and target them in conjunction with standard-of-care chemotherapy as a novel therapeutic strategy to improve treatment outcomes for PC patients.

We have employed a proteomic approach to specifically interrogate the matrisomal changes in well-established genetically engineered PC mouse models: the KPflC (Pdx1-Cre; KrasG12D/+; p53fl/+) and KPC (Pdx1-Cre; KrasG12D/+; p53R172H/+) mouse models. Pancreatic tissues from early, mid and late-stage disease were isolated from KPflC, KPC and age-matched wildtype mice and de-cellularised, prior to quantification of protein abundance via liquid chromatography tandem mass spectrometry.

Here, we identified the enzyme, PLOD1, to be significantly enriched in PC compared to normal pancreas, which was validated via expression analysis in murine and patient tissue libraries. We have generated PLOD1 knockdown cancer cells and matched cancer-associated fibroblasts to further assess whether PLOD1 inhibition (i) has anti-fibrotic efficacy and (ii) can improve chemotherapy performance using 3D *in vitro* assays (cell-derived matrices, organotypic assay) and *in vivo* PC models.

The fibrotic tumour microenvironment in PC is a known driver of disease progression and drug resistance. Proteomic characterisation has helped us map the PC matrisome over disease progression and will assist in the assessment of novel anti-fibrotic therapies in combination with standard-of-care chemotherapy in PC.

Poster Abstracts

P8

Advanced 3D cell culture platform for recapitulating intestine tissues

Dr Chia-Chi Chien[1], Miss Chia-Lin Chien[2], Miss Theodora Almond[3], Miss Ilka Priebe[3], Dr Chun-Hsien Chen[2], Professor Rajvinder Singh[4], Dr Kim Fung[3]
[1]Australian Centre for Disease Preparedness, Commonwealth Scientific and Industrial Research Organisation, East Geelong, Australia, [2]OminiWell Pty Ltd, Adelaide, Australia, [3]Molecular Diagnostics Solutions, Health and Biosecurity, Commonwealth Scientific and Industrial Research Organisation, Adelaide, Australia, [4]Department of Gastroenterology, Lyell McEwin Hospital, Adelaide, Australia

Conventional two-dimensional (2D) cell culture does not recapitulate complex three-dimensional (3D) tissue architecture and does not enable the study of complex interactions between cells and extracellular matrix. The goal of 3D cell culture is to bridge the gap between 2D cell culture and animal experiments. It provides an authentic tissues/organs microenvironment for predicting the outcomes of drug testing which ultimately will reduce the need for animal experiments. Currently, the development of intestinal organoids is one such 3D breakthrough. The intestinal organoids are stem cells derived into a functional intestinal epithelium in 3D structures with the features of cell composition, regional specification, and intestinal architecture *in vitro.* However, forming organoids usually results in different sizes, which makes quantification difficult. Also, due to the nature of the sphere- and cyst-like geometry, the luminal side of the intestine for transepithelial study is limited and inaccessible. Therefore, the practice of microinjection is required for such a study which is difficult to manipulate and labour-intensive.

To simplify the process of intestine transepithelial study, we developed an advanced 3D cell culture platform (named BioTwin Chip) which can be used for intestine transepithelial study. Based on the spatial design of the fluidic channel, cells are guided to locations where these cells form even-sized microtissues or organoids. Using this platform, we demonstrated that the stem cells derived from human intestinal tissue formed organoids. In addition, both mono-cell culture of Caco-2 and a multi-cellular culture of Caco-2 and HT-29 had barrier functions.

In summary, we have been able to grow patient-derived intestine organoids and intestine cell lines into 3D tissue structures that have apical basal polarity and barrier functions in a "one-step" cell seeding process using the BioTwin Chip. It is a useful model for further study including studying the interactions between microbiome, pathogens, and their host.

Poster Abstracts

P9

Understanding the Dynamics of MEKK1 auto-regulation within Cellular Signalling Responses

Mr Alex Bohles[1], Mr Boaz Ng[2], Dr David Croucher[2,3], Dr Peter Mace[1]
[1]Biochemistry Department, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand, [2]The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, Australia, [3]St. Vincent's Hospital Clinal School, University of New South Wales, Sydney, Australia

Mitogen activated protein kinase signalling pathways play an essential role in the cell's response to a diverse range of stimuli and are often dysregulated in cancer. MEK Kinase 1 (MEKK1), a mitogen activated pathway kinase kinase kinase protein that regulates both c-Jun amino-terminal kinase (JNK) and extracellular signal-regulated protein kinase (ERK) pathways, is commonly mutated in luminal breast cancers leading to upregulated proliferation of tumors. MEKK1 is made up of a catalytic C-terminal kinase domain and a large N-terminal regulatory region consisting of a SWI2/SNF2 and MuDR (SWIM) domain, Really Interesting New Gene (RING) ubiquitin-ligase domain, and tumour overexpressed gene domain. Previous research suggests interactivity between the RING and kinase domains is essential in how MEKK1 responds to stimuli, but there is a clear gap in understanding MEKK1 auto-regulation mechanisms and how these alter downstream signaling responses.

To better understand auto-regulation of MEKK1, we have experimentally determined a closed conformation structure of the SWIM and RING domains through X-ray crystallography. It was found that the SWIM domain is a well-ordered domain that directly interacts with the RING domain on the same surface the RING-E2 ubiquitin conjugating enzyme interaction occurs. Through ubiquitin assays and mutagenesis of a key residue in the SWIM-RING interface, it was discovered that the interaction between the two domains provides a way to regulate RING function. This suggests the SWIM domain provides a secondary function other than acting as a substrate receptor for ubiquitination, signifying a wider role in MEKK1-mediated ubiquitination. Current experiments focus on the biological implications of MEKK1 auto-regulation in a cell signalling context, specifically looking at how mutagenesis of key residues alter MEKK1 auto-regulation and regulation of downstream signalling pathways. In turn, this will help our understanding of distinct signalling dynamics of MEKK1 at a protein level.

Poster Abstracts

P10

Dual epithelial and stromal targeting in triple negative breast cancer using ROCK2 inhibition

Daniel Reed[1], Dr Kendelle Murphy[1], Dr Man Nobis[1], Dr Brooke Pereira[1], Dr Astrid Magenau, Dr Cecilia Chambers[1], Anna Howell[1], Dr Sunny Wu[1], Julia Chen[1], Kate Harvey[1], Denise Attwater, A/Prof David Gallego-Ortega[2], Prof Alex Swarbrick[1], A/Prof Thomas Cox[1], Prof Anthony Gill[3], Prof Sandra O'Toole[1], A/Prof Liz Caldon[1], Prof Elgene Lim[1], Prof Paul Timpson[1], Dr David Herrmann[1]
[1]Garvan Institute of Medical Research, Sydney, Australia, [2]School of Biomedical Engineering, University of Technology Sydney, Sydney, Australia, [3]NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia, [4]Centre for Cancer Biology, University of South Australia, Adelaide, Australia

Breast Cancer (BC) initiation and progression is accompanied by extensive extracellular matrix (ECM) deposition and remodelling. Tissue fibrosis can limit drug delivery to the tumour fuelling treatment resistance, as well as enabling cancer cell invasion and metastasis. In TNBC, the predominant treatment strategy is systemic taxane-based chemotherapy; however, this approach often shows only modest efficacy and can further advance the fibrotic response. Therefore, the use of anti-fibrotic therapies has gained significant momentum for improving therapy efficacy and extending patient survival.
Our laboratory and others have shown that targeting fibrosis and the Rho-associated protein kinase 1/2 (ROCK1/2) signalling pathway in a range of cancer types can impair cancer spread and improve response to standard-of-care chemotherapy; however, poor specificity and drug toxicity of ROCK inhibitors limits successful translation into the clinic. Here, we assess efficacy of a novel, highly specific and clinically relevant ROCK2 inhibitor (ROCK2i).

Using publicly available BC patient cohorts, we show high ROCK2 expression significantly correlates with poorer patient survival. In collagen contraction and matrix production assays utilizing CAFs isolated from the PyMT mouse model and from human TNBC patients, we show that ROCK2i significantly decreases the ability of these CAFs to contract and remodel a collagen matrix and to produce a pro-tumorigenic ECM.

In vivo orthotopic experiments assessed the ability of ROCK2i to reduce tissue fibrosis and improve the response to standard-of-care chemotherapy. However, we were unable to show increased efficacy of chemotherapy when combined with ROCK2i in both chemo-resistant PyMT and chemo-sensitive MDA-MB-231 models.

Further investigation revealed that tumour fibrosis is increased following 3 cycles of chemotherapy. We now postulate that timing ROCK2i in line with this increase in fibrosis may provide an alternate treatment strategy to improve chemotherapy efficacy in TNBC, which we currently assess in the MDA-MB-231 model and highly fibrotic TNBC PDX models.

Poster Abstracts

P11

Lighting the fire: Turning up the heat in the prostate cancer immune microenvironment

Mr Sam Rollin[1], Mr Scott Townley[1,2], Ms Adrienne Hanson[1,2], Associate Professor Michael Michael[1], Associate Professor Luke Selth[1,2]
[1]Flinders Health and Medical Research Institute, Flinders University, Australia, [2]Freemasons Centre for Male Health and Wellbeing, Flinders University, Australia

Approximately 12% of Australian men develop prostate cancer in their lifetime, and for those who progress to metastatic disease no curative treatment exists. The primary means to extend patient survival is androgen deprivation therapy which switches off the key driver of proliferation, the androgen receptor. Unfortunately, androgen deprivation therapy is not curative and has significant side-effects, meaning alternative therapies are urgently required. Immunotherapies have revolutionised treatment of other cancers but have failed to have a significant impact in prostate cancer, primarily due to its “cold” tumour immune microenvironment. Our research has been investigating a unique means of reactivating prostate cancer immune activity through potent stimulation of the androgen receptor. This paradoxical approach to treatment is based on evidence that high doses of androgens effectively restrict prostate cancer growth, although the mechanisms underlying this phenomenon are poorly understood. We have found that potent stimulation of the androgen receptor activates critical anti-tumour immune pathways, which may convert the cancer cell to an immune-active state. More specifically, in therapy-sensitive and therapy-resistant models, androgen therapy promotes elevated expression of antigen-presenting MHC-I molecules, which are vital for detection by anti-cancer immune cells as demonstrated by qPCR and flow cytometry. Transcriptional profiling revealed that androgens also increase expression of key immuno-chemical signals (i.e., interferons, cytokines, and chemokines), and the interferon pathway indicating broader anti-tumour immune activity. Collectively, activation of these pathways results in T-cell activity in a murine prostate cancer model. We postulate that these effects could increase prostate tumour immune activity by facilitating greater recruitment and activation of anti-cancer immune cells. In ongoing work, we are evaluating the combination of androgen therapy and immunotherapy in a syngeneic immune-competent mouse model or prostate cancer. Our research is an important step towards making immunotherapy a more effective option for prostate cancer patients.

Poster Abstracts

P12

Generation and validation of anti-linker monoclonal antibodies for the surface detection of scFv-based CARs

Puiyi Tiffany Pang[1]
[1]*New England Biolabs, Nottinghill, Australia*

Chimeric Antigen Receptor (CAR)-T cell therapy is a highly innovative form of immunotherapy that has proven to be successful in the treatment of B cell malignancies and multiple myeloma. There is a need in multiple phases of the CAR-T development pipeline for highly specific detection reagents that can be leveraged to monitor the expression of CARs on the cell surface. Many commercially available CAR detection reagents, however, either lack specificity or are not versatile in their ability to detect CARs of differing antigen specificity. Here, we report on the generation and validation of rabbit monoclonal antibodies raised against two linker sequences that are commonly integrated into single-chain variable fragment (scFv)- based CARs. Methods: The monoclonal antibodies, E7O2V and E3U7Q, were generated by rabbits immunized with peptide sequences most commonly used to construct the linker region of scFv based CARs, Gly4Ser and Whitlow, respectively. E7O2V and E3U7Q were validated for specificity and versatility using flow cytometric analysis of non-transduced versus CAR-transduced cell lines and primary human T cells. Results: Flow cytometric analysis of live Jurkat cells and primary human T cells transduced with CAR constructs revealed that E7O2V and E3U7Q could detect surface expressed CARs containing the appropriate linker sequence, independently of scFv specificity. No specific staining was observed on non-transduced cells. Conclusions: In a flow cytometry assay, E7O2V & E3U7Q specifically detect surface expressed scFv-based CARs containing either a Gly4Ser linker or a Whitlow linker, respectively. Furthermore, these monoclonal antibodies are versatile in that they can also detect their respective linker sequence independently of scFv specificity. The potential exists to leverage these antibodies for CAR-T cell enrichment and for incorporation into multiparametric flow cytometry panels used to phenotype CAR-T cells during the discovery, manufacturing, & clinical phases of the development pipeline.

Poster Abstracts

P13

Single Cell Scoring of Molecular Phenotypes

Ms Malvika Kharbanda[1,2], Dr Dharmesh Bhuva[1,2], Prof. Melissa Davis[1,2]
[1]Saigenci, Adelaide, Australia , [2]WEHI, Melbourne, Australia

Molecular biology indicates that, just like fingerprints, every individual has a unique DNA sequence, and each cell of the individual has identical genetic material. However, the different molecular phenotypes in our body arise due to variable gene expression. Depending on their spatial and temporal states, different cells express distinct genes or groups of genes (gene sets) and suppress others to achieve the desired function. We can now quantify the gene expression of a single cell, that can be used to identify its phenotype and thereby define the cell's state. Most existing computational cell type phenotyping approaches for single cells focus on discerning cell type identity alone and thereby often ignore finer phenotypes.

Numerous computational methods have been developed to infer cell identity and a few methods have been developed to assess molecular phenotypes. Lack of extensive benchmarking has made it difficult to apply these methods to infer useful biology. In this thesis I develop a computational benchmarking study wherein I use cell type identification as a higher order molecular phenotyping technique to evaluate different methods. As part of the benchmark, I evaluate five methods across five scRNA-seq datasets and five sequencing technologies. The benchmarks assesses the ability of these methods to identify single cell types and the computational time required for the analyses. I also show that a simple rank-based method that is computationally efficient has similar performance to complex methods and is therefore suitable for studies atlas-scale data. These methods will be helpful in studying cancer systems by identifying cancer cells that are resistant or responsive to treatments. We can use these methods to identify rare cell types or subpopulations of cells. Precise dissection of a patient's cellular heterogeneity will help them in getting individualised treatment plans, based on how unique characteristics dictate the disease response and progression.

Poster Abstracts

P14

An orthotopic syngeneic mouse model of bortezomib-resistant multiple myeloma

Manjun Li[1], Melissa K. Bennett[1], John Toubia[1], Victoria S. Pope[1], Melinda N. Tea[1], Sarah Tamang[1], Michael S. Samuel[1], Paul H. Anderson[2], Briony L. Gliddon[1], Jason A. Powell[1] and Stuart M. Pitson[1]
[1] *Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia*
[2] *Clinical and Health Sciences, University of South Australia, Adelaide, Australia*

Bortezomib has significant benefit in multiple myeloma (MM) therapy. However, MM remains an incurable disease with resistance to bortezomib invariably developing, and the lack of subsequent effective treatments currently available. This emphasises the need for advanced models for pre-clinical evaluation of new therapeutic approaches for bortezomib-resistant MM. Currently available models are either based on flank xenografts in immunocompromised mice where the critical interactions of MM cells with the bone marrow microenvironment are absent or are derived from transgenic mouse models do not possess the level of genetic diversity seen in spontaneously occurring disease. While orthotopic, syngeneic proteasome inhibitor-resistant mouse models of myeloma have been derived from Vk*Myc or Bcl-xL/Myc mice, these transgenic mouse models best represent disease driven by Myc activation, which, although common, is not observed in many MM patients. Here, we provide a solution to this issue by generating and characterising an orthotopic syngeneic bortezomib-resistant MM mouse model based on the most well-characterized syngeneic MM mouse model derived from spontaneous MM forming C57BL/KaLwRij mice. We employed the murine 5TGM1 MM cell line derived from these mice, that when engrafted back into young C57BL/KaLwRij mice is known to accurately replicate many facets of human MM, including lytic bone disease and serum paraprotein production. Using bortezomib-resistant 5TGM1 cells, we have generated and extensively characterized a robust syngeneic mouse model of bortezomib-resistant MM that is well suited to the evaluation of new therapeutic approaches for proteasome inhibitor-resistant MM.

Poster Abstracts

P15

Mouse models of venetoclax-resistant acute myeloid leukemia for pre-clinical evaluation of new therapeutic approaches

Gus Nwosu[1,2], Victoria Pope[1], Paul Moretti[1], John Toubia[1,3], Alexander Lewis[1,2], Melinda Tea[1], Richard D'Andrea[1], Stuart Pitson[1], Jason Powell[1]
[1]Centre for Cancer Biology, University of South Australia, Adelaide, Australia, [2]Clinical and Health Sciences, University of South Australia, Adelaide, Australia, [3]Australian Cancer Research Foundation Genomics Facility, Adelaide, Australia

Acute myeloid leukemia (AML) is an aggressive and genetically heterogeneous disease with extremely poor patient outcomes. Chemotherapy is the current standard of care for AML however, this treatment is ineffective for achieving deep, durable patient remissions and thus, more effective therapies are urgently needed. Venetoclax is a potent and well tolerated Bcl-2 inhibitor with efficacy for the treatment of chemotherapy unfit AML patients. However, most patients that respond to venetoclax eventually relapse; thus, acquired resistance remains a barrier to the long-term efficacy of venetoclax. Pre-clinical models that recapitulate clinical biomarkers of venetoclax-resistance are a powerful tool for the discovery of novel resistance mechanisms and the validation of novel drug treatments to enhance venetoclax efficacy. Here we describe the generation and characterisation of two cell line models of venetoclax resistant (VR) AML, VR MV411 and VR MOLM-13 that each exhibit extremely high resistance to venetoclax (202 and 445-fold increased resistance to venetoclax, respectively, compared to the parental cells). We identify common and unique gene expression signatures across both cell lines suggestive of shared as well as discrete adaptive venetoclax resistance mechanisms, including dysregulation of Bcl-2 family members and upregulation of clinically relevant cell surface expressed targets. Additionally, we show that the VR MV411 cell line acquired the clinically relevant TP53 R248W mutation, and notably these cells demonstrate *in vivo* resistance to venetoclax and are suitable for *in vivo* assessments of novel therapeutic combinations. Together, these findings demonstrate the heterogeneity of adaptations to venetoclax, highlight the need for genetically diverse models of venetoclax resistance, and showcase the VR MV411 cell line as a powerful addition to the armament of tools for the pre-clinical study of venetoclax resistance.

Poster Abstracts

P16

ROCK activation promotes tumour progression in the intestine.

Dr Zahied Johan[1], Natasha Pyne[1], Dr Sarah Boyle[1], Prof Michael Samuel[1]
[1]*Centre for Cancer Biology, Adelaide, Australia*

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers and can be deadly if not detected early. While CRC is characterised by a sequence of genetic events, disease progression is particularly influenced by the inflammatory status of the tissue microenvironment. Activation of Rho-associated protein kinase (ROCK) has been reported in human cancers and our lab demonstrated that conditional activation of ROCK causes tumour-promoting changes within the extracellular matrix (ECM) in cancers of the skin (Kular et al. Dev Cell) and breast (Boyle et al. Nat Cell Biol). We conditionally activated ROCK within the intestinal epithelium of a chemical carcinogenesis model – using a tumour initiator, Azoxymethane (AOM) administered systemically and an inflammatory agent, dextran sodium sulfate (DSS) administered in drinking water, which acts as a tumour promoter. This AOM/DSS protocol causes the formation of benign intestinal polyps that progress to early carcinomas in the large intestine and is an established model of sporadic CRC. ROCK activation in this model accelerated tumour formation and resulted in more and larger tumours compared to control mice. These tumours had increased cellular proliferation and an altered microenvironmental profile. Within the stroma of ROCK-activated tumours, we observed increased populations of fibroblast and macrophage, along with higher level of Collagen I, an ECM protein. This data suggests that ROCK activation promotes tumour progression in this sporadic CRC model by partly generating a tumour-permissive environment. To further investigate ROCK-mediated tumour progression, we have established tumour-derived organoids (tumouroid) from mouse to be co-cultured with immune cells, for the analysis of secreted factors to reveal key ROCK-regulated inflammatory drivers of tumour progression.

Poster Abstracts

P17

Understanding the role of ROCK signalling pathway in modulating the functional characteristics of the tumour extra-cellular matrix

Dr Chun-Hsien Chen[1,2], Mr Edward Jack Buckley[1,2], Prof Claudine Bonder[1,2,3], Prof Michael S. Samuel[1,2,3]
[1]Centre for Cancer Biology, an alliance between SA Pathology and University of South Australia, Adelaide, Adelaide, [2]University of South Australia, Adelaide, Adelaide, [3]University of Adelaide, Adelaide, Adelaide

The tumour microenvironment (TME) comprises both cellular and non-cellular components. The non-cellular portion of the TME is the extracellular matrix (ECM) mainly produced by cancer-associated fibroblasts (CAF) that is a key regulator of tumour and stromal cell behaviour. It provides architectural and mechanical support, anchorage for cell adhesion, storage of water and growth factors, and induces intracellular signalling pathways. Collagens are the most abundant protein of the ECM and their dysregulation is linked to tumour desmoplasia, cancer cell survival, enhanced migration/invasion and metastasis. ROCK signalling hastens progression of various cancer types via regulation of the TME. We have found that activating ROCK in mammary tumour cells of the MMTV-PyMT cancer model increases the levels of key ECM proteins, and that this promotes tumour growth. However, questions about the role of the ROCK-regulated ECM in tumour progression remain, including how ECM architecture changes in ROCK-activated tumours, and how the changes its structure and composition influence cells within the tumour. To answer these questions, we conditionally activated ROCK in the MMTV-PyMT model and analysed ECM organisation in de-cellularised tumours. We also investigated the effects of ROCK-regulated ECM on mammary cancer cells by seeding cells on cell-derived matrix (CDM) generated by CAFs. We found that ROCK-activation in mammary tumours increases the amount of ECM collagen, collagen fibre thickness, and architectural complexity relative to control tumour ECM. *Ex vivo*, ROCK activation in tumour cells upregulated collagen, fibronectin and tenascin C protein levels and enhanced structural complexity in CDM produced by associated CAFs, termed ROCK-educated. Primary mammary tumour cells cultured on CDM of ROCK-educated CAFs showed enhanced migration and proliferation. In summary, ROCK activity in cancer cells modulates the composition and structural configuration of the tumour ECM in a way that may enhance functional characteristics of cancer cells that are required for tumour progression.

Poster Abstracts

P18

Investigating the Role of Rho-ROCK Signalling in Breast Cancer Metastasis

Ms Moganalaxmi Reckdharajkumar[1,2], Dr Sarah T. Boyle[1], Prof Gregory J. Goodall[1,2], Prof Michael S. Samuel[1,2]
[1]Centre for Cancer Biology, Adelaide, Australia, [2]University of Adelaide , Adelaide, Australia

Breast cancer is the most frequently diagnosed cancer in Australia and worldwide, accounting for over 20,000 diagnoses in Australia in 2022. Furthermore, breast cancer is currently the second leading cause of cancer-related deaths, responsible for 14% of all cancer deaths in Australian women. Most breast cancer-related deaths are due to metastasis, and the 5-year survival rate for women with metastatic breast cancer is only 20%. The Rho-ROCK (Rho-associated protein kinase) signalling axis plays an important role in several physiological and embryonic developmental processes and aberrant activation of ROCK is associated with tumour progression and metastasis in several malignancies. Our laboratory has previously established, using the PyMT mouse model of mammary cancer, that conditional activation of ROCK in mammary tumour cells triggers a paracrine signalling mechanism that recruits and reprograms fibroblasts in the tumour microenvironment to a tumour-promoting form. These reprogrammed fibroblasts upregulate their production of extracellular matrix (ECM) components to create a tumour-permissive microenvironment, thereby significantly increasing primary mammary tumour burden compared to that observed in control mice (expressing a kinase-dead (KD) version of ROCK).

To investigate the role of this pathway in metastatic disease, we tested whether ROCK activation in primary mammary tumour cells (MTCs) affected metastatic lung tumour burden in wild-type mice, injected with MTCs via the tail vein to model metastatic colonisation of the lung. Intriguingly however, we discovered that counter to our observation in the primary tumours, conditional activation of ROCK in MTCs in metastatic lung tumours suppressed fibroblast numbers despite a significant increase in the levels of tumour-promoting ECM components including collagen and periostin. We therefore hypothesise that the fibroblasts in lung metastases are a distinct population recruited by ROCK activation in tumour cells and are investigating the mechanisms underlying the observed divergent roles of ROCK in primary vs. metastatic mammary cancer.

Poster Abstracts

P19

Antibody Drug Conjugates and Pyroptosis: Using Fire to Fuel Anti-Tumour Immunity

Dr Nicole Wittwer[1,2], Dr Alexander H Staudacher[1,2], Dr Vasilios Liapis[1,2], Professor Michael P Brown[1,2,3]
[1]Centre For Cancer Biology, SA Pathology and University of South Australia, Adelaide, Australia, [2]Adelaide Medical School, University of Adelaide, Adelaide, Australia, [3]Cancer Clinical Trials Unit, Royal Adelaide Hospital, Adelaide, Australia

Emerging evidence suggests that the mechanism of chemotherapy induced cell death may influence the anti-tumour immune response in cancer patients. Unlike immunologically silent apoptosis, pyroptosis is a lytic and inflammatory form of programmed cell death characterised by pore-formation in the cell membrane and release of pro-inflammatory factors. Gasdermin E (GSDME), a gene commonly associated with hereditary hearing loss, has recently gained greater notoriety after cleavage of GSDME by certain chemotherapeutics as well as granzyme B was shown to elicit pyroptosis.

In a pre-clinical study, we investigated the immunomodulatory effects of a mesothelin targeting antibody drug conjugate (ADC) in mouse models of breast cancer. We demonstrated that the ADC controlled tumour growth and stimulated anti-cancer immune responses, through the intra-tumoural maturation of dendritic cells, down-regulation of regulatory T-cells and increase of interferon-γ secreting T-cells. Investigation of the mechanism of action revealed that a tubulin-targeting cytotoxic payload in the ADC induced cleavage of GSDME and elicited pyroptotic cell death in GSDME-expressing cells.

To determine the importance of GSDME-mediated pyroptosis for effectiveness of the ADC therapy, we used CRISPR/Cas9 to knockdown GSDME and compared *in vivo* responses to ADC therapy. These studies showed that, in the absence of tumoural GSDME, the ADC showed reduced anti-tumour effectiveness as a monotherapy but, when combined with Fms-like tyrosine kinase-3 ligand (Flt3L), a cytokine that expands dendritic cells in both lymphoid and non-lymphoid tissues, tumour control was restored.

Given that GSDME expression is suppressed in many cancers, these findings hold clinical significance, suggesting that patients with GSDME-silenced tumours may benefit from a combination of ADC and Flt3L therapy. Together, these results show for the first time that an ADC can elicit pyroptosis and that this fiery cell death is critical for anti-tumour immunity and therapeutic response.

Poster Abstracts

P20

Understanding beta common cytokine pleiotropy through mimetic ligands

Dr Tim Hercus[1], Dr Winnie Kan[1], Dr Karen Cheung Tung Shing[2,3], Dr Tracy Nero[2,3], Dr Ta-Yi Yu[4], Mr Marc Exposit[4], Dr David Baker[4], Prof Michael Parker[2,3], Prof Angel Lopez[1,5]
[1]Cytokine Receptor Laboratory, Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, Australia, [2]Australian Cancer Research Foundation Rational Drug Discovery Centre, St. Vincent's Institute of Medical Research, Melbourne, Australia, [3]Department of Biochemistry and Molecular Biology and the ACRF Facility for Innovative Cancer Drug Discovery, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia, [4]Institute of Protein Design, University of Washington, Seattle, United States of America, [5]Department of Medicine, University of Adelaide, Adelaide, Australia

Cytokines are molecular messengers with diverse functional properties (pleiotropism) that regulate numerous cell types and play essential roles in many biological processes. They function through cell surface receptors that are activated by cytokine-mediated oligomerization. Understanding mechanisms driving assembly and activation of cytokine:receptor complexes is an important approach to understand cytokine pleiotropy. The pleiotropic biology of many cytokines has limited their clinical utility but the development of biased or partial cytokine agonists has emerged as a powerful pathway to harness the clinically desirable functions of cytokines for therapeutic purposes.

IL-3 is a member of the βc family of cytokines that stimulates survival, proliferation, differentiation and activation of haematopoietic cells by activating a heterodimeric receptor comprising a cytokine-specific subunit (IL3Rα) and a signalling subunit (βc) that is shared by the receptors for GM-CSF and IL-5. We have investigated structure/function properties of cytokine:receptor assemblies in the βc family and have determined structures for the IL-3 and GM-CSF cytokine:receptor complexes. This identified specific sites of interaction between receptor subunits and their cognate cytokines, site 1 in the receptor alpha chains and site 2 in βc, that the cytokines "bridge" to promote dimerization and activation of their receptors. The development of novel chemical entities that cross-link the receptor subunits with different topologies, may reveal distinct forms of signaling as we have recently found with differential forms of IL-3 receptor assemblies.

IL-3 offers therapeutic potential to expand haemopoietic progenitor cells in settings of leukopenia but is restricted by toxicity arising from activation of innate immune cells. We are using our structural and functional knowledge of the IL-3 receptor to guide the engineering of novel ligands with the goal of understanding the relationship between receptor dimerization and activation and ultimately to develop mimetic ligands that selectively activate some IL-3 receptor functions in haematopoietic progenitor cells.

Poster Abstracts

P21

Understanding the role of the Rho-ROCK pathway in modulating mammary tumour immunity

Edward J. Buckley, Natasha Kolesnikoff, M. Zahied Johan, Sarah T. Boyle and Michael S. Samuel
Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide SA 5000

It is well-accepted that a solid tumour is not simply a collection of cancer cells but is also composed of non-transformed cells that can be co-opted to support its growth and spread. We and others have established that tumour cells secrete paracrine factors into the extracellular space to form a tumour-permissive microenvironment via stromal cell reprogramming and remodelling of the extracellular matrix. Using a model in which Rho-associated kinase (ROCK) can be conditionally activated in mammary tumour epithelial cells, we found that ROCK activity enhances the production of a pattern-recognition molecule (PRM). Clinically, high levels of this molecule are associated with poor survival of patients with invasive breast cancer, and therefore investigating this novel signalling axis has the potential to inform future therapy approaches. Importantly, tumour cell-derived, ROCK-regulated PRM upregulated CD206 expression in macrophages *in vitro* (in the RAW 264.7 macrophage cell line), *ex vivo* (in mouse bone marrow-derived macrophages) and in vivo (in the PyMT mammary cancer model), indicative of polarisation to an M2, tumour-promoting phenotype. Taken together, these observations reveal for the first time, a potential tumour-promoting mechanism of the ROCK-PRM axis via reprogramming of stromal macrophages, with implications for cancer therapy.

Poster Abstracts

P22

A new understanding of the role of 14-3-3 proteins in lung cancer.

Jo Woodcock, Rhys Hamon, Carl Coolen, Xin Jiang, Clifford Young*, Angel Lopez and Stuart Pitson
*Centre for Cancer Biology, an alliance of SA Pathology and University of South Australia, and *Clinical Health Sciences, University of South Australia, Adelaide.*

Our research focusses on the family of 14-3-3 proteins and the role they play in the development of lung cancer. Studies have shown that one member of this seven-membered family, 14-3-3 zeta, is disproportionately increased in more than half of lung tumours and correlates with disease severity and patient survival, indicating that the 14-3-3s play a role in the biology of the cancer.

14-3-3 proteins form dimers that behave like a pair of 'helping hands', to cradle and support other important functioning proteins, enabling 14-3-3 dimers to control many biological functions in cells. Surprisingly little is known about the specific functions of different 14-3-3 dimer pairs or what effect increased 14-3-3 zeta abundance has on the function of other 14-3-3 dimers in lung cells. To address this knowledge gap we have taken several approaches. Firstly, we have determined at the molecular level just how 14-3-3 dimers hold together. Using this knowledge we have developed tools to dissect the binding capabilities of different 14-3-3 dimer pairs by proteomics, which has revealed distinct roles for specific dimer species. We are now tracking how these dimer specific functions are altered when the relative abundance of 14-3-3 zeta is increased to determine how this contributes to cancer development. To better understand the global effects associated with elevated expression of 14-3-3 zeta that occur in lung cancer, we have engineered cells to mimic the changes in 14-3-3 zeta abundance and used RNAseq to elucidate all the molecular changes that occur. This is the first time that the broader effects of 14-3-3 dimer dysregulation have been studied and revealed that many of the changes related to cancer progression correlate with differential changes in 14-3-3 dimer populations. This is an important discovery for the field and further emphasises the role played by 14-3-3 dysregulation in lung cancer.

Poster Abstracts

P23

Examining the efficacy of targeting mutant TET2 in AML

Leeann Desouza[1,2], Keith Lau[1], Victoria Pope[1], Daniel Thomas[3], Jason Powell[1], Stuart Pitson[1]
[1]Centre for Cancer Biology, University of South Australia, [2]Clinical and Health Sciences, University of South Australia, Adelaide, SA, Australia, [3]Adelaide Medical School, University of Adelaide, South Australia and Precision Medicine, South Australian Health and Medical Research Institute, Adelaide, Australia.

Acute Myeloid Leukaemia (AML) is the most common haematological malignancy seen in older populations, accounting for 31% of all adult cases with a 5-year survival rate of 28%. Currently, standard induction chemotherapy is effective in achieving remission, however, 60% of AML patients relapse making this a major hurdle in improving overall survival. Initial mutations responsible for establishing pre-leukemic conditions occur most frequently in genes involved in epigenetic regulation such as TET2. Ten-eleven translocation dioxygenase (TET) proteins are involved in active DNA demethylation, an epigenetic mechanism which regulates gene expression through the conversion of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC). Loss of function TET2 mutations are commonly seen in AML resulting in genomic hypermethylation and downregulation of tumour suppressor genes. Sequencing of matched diagnostic and relapsed AML patient samples indicates that TET2-mutant clones present at diagnosis are always maintained at relapse. This data suggests TET2 is a strong epigenetic driver and represents a tractable target to prevent relapse for this molecular subclass of AML. Since TET activity is required for cell survival and homeostasis, we have taken a non-canonical approach to target TET enzymes in TET2 mutant AML, exploiting the therapeutic window between wild type TET2 normal hematopoietic stem cells and TET2 mutant AML. To address this, we have generated TET2 knockout AML cell lines using CRISPR/Cas9 and treated with TET inhibitor, TETi76. Combinational therapies with cytarabine in vitro have shown increased susceptibility in TET2 knockout cells in comparison to wildtype. Thus, enhancing existing therapies by directly targeting TET2-mutant LSCs at diagnosis may have the potential to reduce the likelihood of relapse, which remains a major challenge in improving survival outcomes.


CSL

Hosted by


Centre for Cancer Biology


SAiGENCI
SOUTH AUSTRALIAN
IMMUNOGENOMICS
CANCER INSTITUTE


Flinders
University