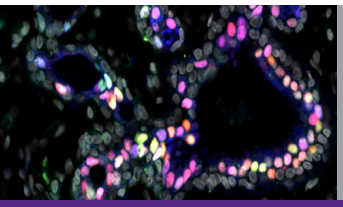
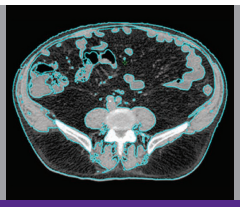
Oncology Abstracts

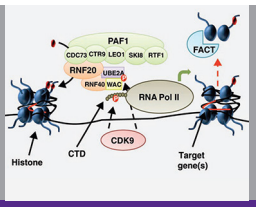
March 2019 Volume 1
ISSN 2631-4657 (online)

7th International Pacific Rim (PacRim) Breast and Prostate Cancer Meeting

17–20 March 2019, Barossa Valley, South Australia







published by
bioscientifica

Online version available at
www.oncology-abstracts.org

Oncology Abstracts

Volume 1
March 2019

7th International Pacific Rim (PacRim) Breast and Prostate Cancer Meeting

17–20 March 2019, Barossa Valley, South Australia

Abstract book

Meeting Co-Convenors

Wayne Tilley (University of Adelaide, Australia)
Elgene Lim (Garvan Institute of Medical Research, Australia)

Program Advisory Committee

Gert Attard
Stephen Birrell
Myles Brown
Lisa Butler
Jason Carroll
Judith Clements
Suzanne Conzen
Felix Feng
Ed Gelmann
Geoffrey Greene
Theresa Hickey
Lisa Horvath
Anthony Joshua
Karen Knudsen
Elgene Lim
Geoff Lindeman
Pete Nelson
Steffi Oesterreich
Carlo Palmieri
Stephen Plymate
David Quinn
Ganesh Raj
Juergen Reichardt
Paul Rennie
Gail Risbridger
Carol Sartorius
Luke Selth
Janet Stanford
Alex Swarbrick
Chris Sweeney
Renea Taylor
Wayne Tilley
Amina Zoubeidi

Ron Ross Award Chairperson

Juergen Reichardt

Local Organizing Committee

Wayne Tilley
Elizabeth Kuczek
Theresa Hickey
Luke Selth

Meeting Coordinator

Elizabeth Kuczek

SPONSORS

We are extremely grateful to our generous sponsors whose support is essential for the continued success of the *International PacRim Breast and Prostate Cancer Meeting* series.

Platinum Sponsor

The Hospital Research Foundation

Gold Sponsors

Janssen
The University of Adelaide
Dame Roma Mitchell Cancer Research Laboratories

Silver Sponsors

Pfizer Oncology
H3 Biomedicine
Bioscientifica
Lilly
Amgen
Roche
Queensland University of Technology
Connie Johnson Breast Cancer Research
National Breast Cancer Foundation
Cancer Research UK
Monash University

Bronze Sponsors

Novartis
Specialised Therapeutics Australia
AstraZeneca
Australian National University
Endocrine-Related Cancer
Besins Healthcare

Full details are available at www.pacrimmeeting.com.

Preface

The *7th International Pacific Rim (PacRim) Breast and Prostate Cancer Meeting* was held in the Baraossa Valley, South Australia from 17–21 March 2019. The goal of this unique international "think-tank" meeting, which was attended by experts in the field of breast and prostate cancer, including leading scientists and clinicians and representatives of biotechnology and pharmaceutical companies, is to improve the understanding, management and prevention of breast and prostate cancer. The focus of the 7th meeting was *Breast and prostate cancer – more similar than different*. The lively extended discussions of each session (each session being introduced by four or five experts using the unique and often provocative *PacRim* short-talk format) addressed this overarching theme of the meeting, with a view to leveraging similarities between these two cancers to develop new therapeutic targets and predictive biomarkers to improve disease outcomes.

Highlights of the *7th PacRim Meeting* included the *5th Ron Ross Award and Oration* (Funmi Olopade, University of Chicago, who gave an outstanding presentation on the molecular genetics of breast cancer progression and the causes of aggressive breast cancer in young women, especially those of African ancestry), and the inaugural *Rob Sutherland Award* (Luke Selth, University of Adelaide; eight excellent finalists presented for the inaugural award; see www.pacrimmeeting.com for details). These prestigious awards recognize the outstanding contributions of two foundation members of *PacRim* to breast and prostate cancer research. Additional highlights included the extended discussions focussed on the contemporary session topics and the great debate, which featured leading international researchers who addressed the question of "Are humans the only model?" in an insightful and very entertaining manner. Poster awards were made to higher degree students and early career researchers and trainees presenting at the meeting. The poster sessions, enthusiastically attended by all *PacRim* meeting participants, stimulated discussion that lasted well beyond the sessions and resulted in valuable new collaborations. The abstracts from the meeting are the feature of this volume of Oncology Abstracts. For details of all presentations and awards, and previous meetings, and sponsors, see the meeting website: www.pacrimmeeting.com.

PacRim meetings have facilitated the establishment of international networks across Australia, Canada, the Americas and the broader Asia-Pacific region to foster collaborative research studies and international funding opportunities, transfer of new cutting-edge technologies and exchange of expertise and personnel. The International *PacRim* meeting series aims to encourage cooperation among organizations and individuals for the purpose of advancing the progress of breast cancer research. A particular aim going forward is to bring leading researchers together to meet in areas of the world that are under-represented in breast and prostate cancer research. This is currently particularly relevant to the Global South and to East Asia, where we plan to hold future meetings.

The meeting organisers hope that publication of the poster abstracts from the *7th PacRim Meeting* will highlight the quality of research presented and will stimulate interest in attending and supporting future meetings.

Wayne Tilley, University of Adelaide and Elgene Lim, Garvan Institute (Co-convenors, *7th PacRim Meeting*).

CONTENTS

7th PacRim Meeting Poster Presentations

Poster Presentations

P001

S phase dysregulation occurs following resistance to CDK4/6 inhibition ER+ breast cancer

Sarah Alexandrou[1,2], Heloisa Helena Milioli[1,2], Neil Portman[1], Christine Lee[1], Kristine Fernandez[1], David Blake[3], Elgene Lim[1,2] & C Elizabeth Caldon[1,2]
[1]Cancer Division, Garvan Institute of Medical Research, New South Wales 2010, Australia; [2]St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, New South Wales 2010, Australia; [3]Cyclacel Pharmaceuticals Inc., Dundee DD1 5JJ, UK.

Endocrine resistant estrogen receptor positive (ER+) breast cancers are dependent upon cyclin-dependent kinases (CDK) 4/6 for proliferation, making them highly suitable for CDK4/6 inhibitor treatment. Despite initial efficacy, acquired resistance to CDK4/6 inhibitors is emerging and is now a major consideration in pre-clinical and clinical drug development. Current models of CDK4/6 inhibitor resistance do not mimic the clinical scenario where CDK4/6 inhibition will occur in the context of endocrine therapy and/or resistance. We aimed to characterise the mechanisms of resistance, identify clinically targetable pathways and evaluate novel therapeutic strategies for endocrine therapy and CDK4/6 inhibitor resistant ER+ breast cancer. To identify mechanisms of resistance we generated a palbociclib resistant (PalbR) MCF-7 breast cancer cell line. We show that PalbR cells have disrupted cell dynamics that result in an elongation of the S phase of the cell cycle. Prolonging S phase leads to a reduction in the CDK inhibitor proteins $p21^{Cip1}$ and $p27^{Kip1}$, and an activation of CDK2. CDK2 is therapeutically targetable using the CDK2/9 inhibitor CYC065 (Cyclacel, Phase I). CDK2 inhibition in combination with palbociclib enhanced growth inhibition and promoted the induction of senescence in PalbR cells. To complement this model, we have developed a panel of *in vitro* models that mimic the clinical treatment of patients. Here we combine palbociclib with an endocrine therapy; tamoxifen or fulvestrant, and show that palbociclib synergises with endocrine therapy to inhibit proliferation. In parallel we have generated an *in vivo* ER+ patient-derived xenograft model resistant to chronic fulvestrant+ palbociclib treatment that has downregulation of cell cycle associated transcripts. Resistance to palbociclib occurs via cell cycle dysregulation of S phase, supressing CDK inhibitors. Our novel panel of resistant models provides a framework to identify mechanisms of acquired resistance, and a vehicle for testing clinically relevant therapies that could counteract this resistance.

DOI: 10.1530/oncolabs.1.P001

P002

BMP4 is a bonafide breast cancer metastasis suppressor

BL Eckhardt[1], A Redfern[2], EK Sloan[3], Y Cao[4], BS Parker[5], N Ueno[1] & RL Anderson[6]
[1]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA; [2]School of Medicine, University of Western Australia, Perth, Australia; [3]Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Australia; [4]Department of Pathology, The University of Melbourne, Parkville, Australia; [5]La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Australia; [6]Olivia Newton-John Cancer Research Institute, Heidelberg, Australia.

Metastasis is the major cause of death in breast cancer patients, largely due to the poor efficacy of existing therapies. Here we report that bone morphogenetic protein-4 (BMP4) blocks metastasis in animal models of breast cancer and predicts improved survival in patients. In preclinical models of spontaneous metastasis, we demonstrate that BMP4 acts as an autocrine mediator to modulate a range of known metastasis regulating genes, including *SMAD7*, via activation of canonical BMP-SMAD signaling. Restored *BMP4* expression in metastatic mammary tumour lines blocks metastasis and increases survival by sensitizing cancer cells to anoikis, thereby reducing the number of circulating tumor cells. Knockdown of its downstream mediator *SMAD7*, reverses the protection against metastasis afforded by BMP4. Silencing of *BMP4* in poorly metastatic lines enhanced their metastatic capacity in mice. Finally, administration of recombinant BMP4 markedly reduces spontaneous metastasis to lung and bone. Collectively, these findings demonstrate that BMP4 can modulate the metastatic potential of breast cancer, without impacting on primary tumor growth. As such, we propose that BMP4 is a *bonafi*de breast cancer metastasis suppressor. A high throughput screen for small molecules that mimic the activity of BMP4 is underway. In a cohort of 535 breast cancer samples, we show that BMP4 and SMAD7 are prognostic for improved recurrence-free survival and overall survival in breast cancer patients, indicating the importance of canonical BMP4 signaling in the suppression of metastasis and highlighting new avenues for therapy against metastatic disease.

DOI: 10.1530/oncolabs.1.P002

P003

Using coordination chemistry and nanotechnology to develop a brand new class of therapeutics

Marcel B Bally[1,3,4,5], Michael Abrams[5], Tom R Redelmier[5], Roger Gilabert-Oriol[1], Devon Heroux[1], Kent Chen[1] & Ada WY Leung[1,2,5]
[1]Department of Experimental Therapeutics, BC Cancer-Vancouver Research Centre, Vancouver, British Columbia, Canada; [2]Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada; [3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; [4]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada; [5]Cuprous Pharmaceuticals Inc., Vancouver, British Columbia, Canada.

We have recently discovered that metals which coordinate with selected compounds can be prepared inside liposomes. This technology, which we refer to as Metaplex™ technology, is enabling development of a brand new class of therapeutics. Previously the development of metal drug complexes (CDCs) has been hindered because of their very poor aqueous solubility. As an example, diethyldithiocarbamate (DDC) is the primary metabolite of disulfiram, an approved drug for the treatment of alcoholism that is being repurposed for cancer. The anticancer activity of DDC is dependent on complexation with copper to form copper bis-diethyldithiocarbamate ($Cu(DDC)_2$), a highly insoluble complex that has not been possible to develop for indications requiring parenteral administration. This issue has been resolved by preparing $Cu(DDC)_2$ in the presence of pre-formed liposomes. DDC reacts with copper; a reaction that can be observed through a colour change as the solution goes from a light blue to dark brown. In the absence of liposomes the complex precipitates out of solution. In the presence of liposomes, the compound remains in solution. This method has now been successfully applied to other compounds with metal coordination sites including the anti-parasitic drug clioquinol, the natural product quercetin, the semi-synthetic flavonoid flavopiridol and the novel RNA polymerase/G-Quartet targeted agent CX-5461. Our method provides a simple, transformative solution enabling the development of water insoluble compounds as viable candidate anticancer drugs. Our team is now using Metaplex™ technology with the goal of developing agents capable of inducing immunogenic cell death *in vivo*. ICD is a form of cell death where dying tumour cells emit signals known as damage-associated molecular patterns (DAMPs), which ultimately stimulate an adaptive immune response and potentially long-term protection against tumour growth. Metaplex™ technology can be used to generate nanomedicines capable of having direct anti-proliferative activity as well as an ability to generate a lasting immune response against tumours.

DOI: 10.1530/oncolabs.1.P003

P004

A GWAS identified functional variation in PSA (KLK3) gene that confers lower risk is also associated with more aggressive disease and lower survival in men with prostate cancer

Srilakshmi Srinivasan[1,2], Thomas Kryza[1,2], Nathalie Bock[1,2], Carson Stephens[1,2], Ying Dong[1], Janaththani Panchadsaram[1,2], Leire Moya[1,2], Joan Röhl[1,2], Joanna L Perry-Keene[3], Katie Buzacott[3], Tokhir Dadaev[4], Mark N Brook[4], Hans Lilja[5], Amanda Spurdle[6], Hannu Koistinen[7], Ulf-Håkan Stenman[7], Zsofia Kote-Jarai[4,8], Rosalind Eeles[4,8], The Practical Consortium[9], The Australian Prostate Cancer BioResource[2], Judith Clements[1,2] & Jyotsna Batra[1,2]
[1]Cancer Program, Institute of Health and Biomedical Innovation and School of Biomedical Sciences, Queensland University of Technology, Brisbane, Queensland, Australia; [2]Australian Prostate Cancer Research Centre – Queensland, Translational Research Institute, 37 Kent Street, Woolloongabba, Queensland, Australia; [3]Anatomical Pathology, Pathology Queensland, Queensland, Australia; [4]Department of Genetics and

Epidemiology, The Institute of Cancer Research, London, UK; [5]Departments of Laboratory Medicine, Surgery (Urology Service) and Medicine (Genitourinary Oncology), Memorial Sloan Kettering Cancer Center, New York, USA; [6]Molecular Cancer Epidemiology Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; [7]Department of Clinical Chemistry, Biomedicum Helsinki, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland; [8]Royal Marsden NHS Foundation Trust, London, UK; [9]Centre for Cancer Genetic Epidemiology, Cambridge, UK.

Objective
Prostate cancer susceptibility is influenced by common variants at multiple loci, however, the mechanisms by which these germline variants influence prostate cancer risk remain largely unknown. A single nucleotide polymorphism (SNP) rs17632542 in the PSA gene has been identified to be associated with prostate cancer risk using large scale genome-wide associate studies. This SNP was previously questioned for its association with prostate cancer due to its association with PSA levels as well. We aimed to verify that this SNP plays a functional role in mediating prostate cancer risk and progression.
Methods
We conducted in silico and functional analysis in several prostate cancer cell models and in clinical samples to identify the biological role of the rs17632542 SNP.
Results
The non-synonymous rs17632542 SNP (c.536T>C), in exon 4 of the PSA-encoding *KLK3* gene was associated with disease risk, and aggressiveness and survival in opposite directions. The prostate cancer associated rs17632542 SNP leads to amino acid change Ile to Thr at position 161, which lowers the proteolytic activity of PSA towards extracellular matrix proteins and diminishes the proliferation and migration of prostate cancer cells. In addition, we show that the 'Thr' PSA protein variant displayed significant functional differences in the tumour microenvironment and thus may play a multifunctional role in tumourigenesis and metastasis. The minor 'C' allele leads to lower levels of serum PSA-inhibitor complexes and is associated with higher free PSA levels. Furthermore, the c.536 T>C change leads to altered *KLK3* splicing and reduced mRNA levels of *KLK3* in an allele-specific manner.
Conclusions
Genetic correction of the rs17632542 variant with PSA levels; and/or the free-to-total PSA ratio may reduce the inaccuracies for prostate cancer diagnosis based on PSA levels alone.

DOI: 10.1530/oncolabs.1.P004

P005

Expression of the interleukin-3/receptor complex by breast cancer cells promotes vascular mimicry via a PI3K-dependent mechanism and is associated with poor outcome

Emma J Thompson[1], Camille Duluc[1], Emma F Barry[1], Gelareh Farshid[2,3], Kaylene J Simpson[4], Phillip A Gregory[1], Xiaochun Li[1], Stephen Madden[5], Cathy M Owczarek[6], Nick J Wilson[6], Gino Vairo[6], Andrew D Nash[6], Wendy V Ingman[3,7], Geoffrey J Lindeman[4,8], Elgene Lim[9], Yeesim Khew-Goodall[1], Angel F Lopez[1,3] & Claudine S Bonder[1,3]
[1]Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, South Australia, Australia; [2]BreastScreen SA and SA Pathology, Adelaide, South Australia, Australia; [3]Adelaide Medical School, Faculty of Health Sciences, University of Adelaide, Adelaide, South Australia, Australia; [4]Peter MacCallum Centre, Melbourne, Victoria, Australia; [5]Royal College of Surgeons in Ireland, Dublin, Ireland; [6]CSL Limited, Bio21 Institute, Parkville, Victoria, Australia; [7]The Robinson Research Institute, University of Adelaide, Adelaide, Australia ; [8]The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; [9]Garvan Institute of Medical Research and St.Vincent's Clinical School, University of New South Wales, Darlinghurst, New South Wales, Australia.

Abstract unavailable

DOI: 10.1530/oncolabs.1.P005

P006

ROCK educates cancer-associated fibroblasts via secreted Creld2 to create a tumour-promoting microenvironment

Sarah T Boyle, Marina Kochetkova & Michael S Samuel
Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia.

Abstract unavailable

DOI: 10.1530/oncolabs.1.P006

P007

Estrogen receptor positive luminal progenitors the cancer cell origin for Estrogen receptor positive breast cancer

Genevieve Dall[1,2], Serene Yeow[2], Jessica Vieusseux[2], Yashar Seyed-Razavi[1], Nathan Godde[5], Mandy Ludford-Menting[4], Sarah M Russell[4,6], Alan Ashworth[7], Robin Anderson[8,9], Kelly Phillips[8,10], Gail Risbridger[1], Mark Shackleton[3,8] & Kara Britt[2,8]
[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia; [2]Breast Cancer Risk and Prevention Laboratory, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Australia; [3]Cancer Development and Treatment, Monash University, Alfred Hospital, Melbourne, Australia; [4]Immune Signaling Laboratories, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Australia; [5]La Trobe Institute for Molecular Science, Department of Biochemistry and Genetics, La Trobe University, Melbourne, Australia; [6]Centre for Micro-Photonics, Swinburne University, Hawthorn, Australia; [7]Helen Diller Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California, USA; [8]The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia; [9]Metastasis Research Laboratory, Olivia Newton-John Cancer Research Institute, Heidelberg, Australia; [10]Division of Cancer Medicine, Peter MacCallum Cancer Centre, Melbourne, Australia.

Abstract unavailable

DOI: 10.1530/oncolabs.1.P007

P008

Mechanisms underlying uncontrolled genome doubling in breast cancer

Christine S Lee[1], Samuel Rogers[2,3], Kristine Fernandez[1], Sarah Alexandrou[1], Niantao Deng[1,9], C Marcelo Sergio[1], Abhijit Kulkarni[4], Lucy Gugasyan[4], Elizabeth A Musgrove[1], Andrew J Deans[5,6], Andrew Burgess[7,8] & C Elizabeth Caldon[1,9]
[1]The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, New South Wales 2010, Australia; [2]Children's Medical Research Institute, The University of Sydney, Westmead, New South Wales 2145, Australia; [3]Faculty of Medicine and Health, University of Sydney, New South Wales 2006, Australia; [4]Cytogenetics, Monash Pathology, Monash Medical Centre, Victoria 3168, Australia; [5]St. Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia; [6]Department of Medicine, University of Melbourne, Parkville, Victoria, 3010, Australia; [7]ANZAC Research Institute, Concord, New South Wales 2139, Australia; [8]The University of Sydney Concord Clinical School, Faculty of Medicine and Health Concord, New South Wales 2139, Australia; [9]St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney, New South Wales 2052, Australia.

Uncontrolled genome doubling is an underlying cause of cancer cell aneuploidy and genomic instability, but relatively few drivers have been identified for this process. Cyclin E1 and cyclin E2 are cell cycle regulators whose dysregulation in oncogenesis promotes both increased proliferation and genomic instability. Due to their roles in normal physiological endoreduplication of the genome for

specialised cell types, we hypothesised that cyclin E1 and cyclin E2 may be drivers of genome doubling in cancer. We show that cyclin E2, but not cyclin E1, promotes genomic instability through increased re-replication to drive genome doubling. Using chromatin extracts we show that cyclin E2 localises and recruits core proteins (MCM2, MCM7) to the pre-replication complexes (preRC) necessary to initiate DNA replication, leading to increased whole genome replication. By contrast, cyclin E1 overexpression does not increase whole genome replication but instead leads to the depletion of Cdt1, the preRC factor required for DNA replication initiation. We recapitulate genome instability via genome doubling with the overexpression of cyclin E2, and karyotypes of these cyclin E2 overexpressing cells have acquired chromosomes and large chromosomal rearrangements during genomic instability. An examination of public datasets showed that cyclin E2 (but not cyclin E1) correlates with high ploidy and genomic instability across breast cancers. Thus cyclin E2 is a likely contributor to chromosomal instability in the evolution of breast cancer via its role in inappropriate whole genome duplication.

DOI: 10.1530/oncolabs.1.P008

P009

Targeting stromal remodelling and cancer stem cell plasticity overcomes chemoresistance in metastatic triple negative breast cancer

Aurélie Cazet[1,*], Mun Hui[1,2,*], Benjamin Elsworth[1], Sunny Wu[1], Daniel Roden[1], Chia-Ling Chan[1], Sandra O'Toole[1,3], D Neil Watkins[1,4], Renea Taylor[5], Thomas Cox[1], Michael Samuel[6], Miguel Martín[7] & Alexander Swarbrick[1]

[1]The Kinghorn Cancer Centre and Cancer Research Division, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia; [2]The Chris O' Brien Lifehouse, Camperdown, New South Wales, Australia; [3]Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia; [4]St Vincent's Hospital, 2010 Darlinghurst, NSW, Australia; [5]Monash Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, Australia; [6]Tumour Microenvironment Laboratory, Centre for Cancer Biology, University of South Australia, Adelaide, Australia; [7]Hospital General Universitario Gregorio Marañón, Madrid, Spain.
*These authors contributed equally to this work.

The cellular and molecular basis of stromal cell recruitment, activation and crosstalk in carcinomas is poorly understood, limiting the development of targeted anti-stromal therapies. In mouse models of triple negative breast cancer (TNBC), Hedgehog ligand produced by neoplastic cells reprograms cancer-associated fibroblast (CAF) to provide a supportive niche for the acquisition of a chemo-resistant, cancer stem cell (CSC) phenotype *via* FGF5 expression and production of fibrillar collagen. Stromal treatment of patient-derived xenografts with smoothened inhibitors (SMOi) reverses this phenotype, downregulates CSC markers expression and sensitizes tumours to docetaxel, leading to markedly improved survival and reduced metastatic burden. These promising preclinical study results led us to establish the EDALINE Phase I trial of docetaxel chemotherapy in combination with the SMOi Sonidegib in patients with metastatic TNBC. Twelve patients who had previously failed on standard of care treatments with taxanes and/or anthracyclines were enrolled. 3 patients derived clinical benefit, with one experiencing a complete response. Importantly, markers of pathway activity correlated with response. These studies identify Hh signalling to CAFs as a novel mediator of cancer stem cell plasticity and represent the first clinical demonstration of therapeutic benefit derived from targeting cancer-associated fibroblasts in the metastatic setting. Interestingly, Hh signalling seems to follow a similar pattern of activation in the prostate and could represent an exciting relevant therapeutic target in prostate cancer.

DOI: 10.1530/oncolabs.1.P009

P010

Epi-transcriptomic alterations in ER-positive breast cancer

Sara Charmsaz, Sinéad Cocchiglia, Ben Doherty, Damir Varešlija, Arnold D Hill & Leonie S Young

Endocrine Oncology Research Group, Department of Surgery, Royal College of Surgeons, Dublin, Ireland.

Endocrine therapy including tamoxifen and aromatase inhibitors (AIs) are standard therapy for ER- positive breast cancer and despite its success a significant number of patients develop resistance to treatment. Transcriptional and epigenetic re-programing including DNA and RNA methylation develops with high frequency in response to therapy. Global DNA and RNA multi-omic studies have been utilized to understand the altered transcriptome of endocrine resistant breast cancer cell models and we have uncovered alterations in oncogenic, differentiation and kinase signalling pathways. RNA modifications including RNA-methylation play an important role in many biological processes. The RNA methyl mark N6-methyladenosine (m6A) is the main transcriptional modification event in RNA-methylation. Studies show that m6A modifications are catalysed through m6A 'writers' (METTL3, METTL14 and WTAP) and RNA-methylation is inhibited through m6A 'erasers' (FTO, ALKBH5). The role of RNA-machinery in breast cancer is largely unknown. Here we determine the role of RNA-methylation machinery in development of endocrine resistant metastatic breast cancer. The role of FTO and METTL3, the key players in RNA-methylation was investigated in a cohort of breast cancer patients where we observed that METTL3 ($P=0.0242$) is associated with prolonged disease-free-survival and FTO ($P=0.0182$) is associated with reduced disease-free-survival ($n=870$). Moreover, using gene expression analysis comparing patients with disease-recurrence versus those with no recorded relapse we have observed elevated levels of FTO and significant elevation of ALKBH5 ($P=0.0068$) in patients with subsequent disease-recurrence ($n=24$). Identifying FTO as a contributor to the metastatic progression we have analysed the role of FTO in models of endocrine resistant and sensitive breast cancer showing, elevated levels of FTO in endocrine resistant cells (LY2, LetR cells) in comparison to endocrine sensitive (MCF7 cells). Furthermore we have observed FTO to drive mammosphere formation, anchorage independent growth and proliferation all of which are hallmarks of metastasis. In summary, this study identifies the m^6A mRNA methylation machinery as a potential therapeutic target for breast cancer progression that needs exploring.

DOI: 10.1530/oncolabs.1.P010

P011

The WinPro study: A window of opportunity study of endocrine therapy with and without prometrium in postmenopausal women with early stage hormone receptor-positive breast cancer

Julia Chen[1,2], Emma Carson[1,2], Davendra Segara[2], Andrew Parker[2], Sandra O'Toole[1], Alan Coates[3], Bruce Mann[4], Geoffrey Lindeman[5], Wayne Tilley[6] & Elgene Lim[1,2]

[1]Garvan Research Institute, Sydney, NSW, Australia; [2]St Vincent's Hospital, Sydney, NSW, Australia; [3]University of Sydney, NSW, Australia; [4]Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; [5]Walter+Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; [6]Dame Roma Mitchell Cancer Research Laboratory, Adelaide, South Australia, Australia.

There is bidirectional interplay between PR and ER in human breast cancers. There is evidence for a reprogramming of ER chromatin binding sites with 470 genes differentially regulated by dual treatment with estrogen plus progestogen compared to estrogen alone in breast cancer cell lines. Functionally, there was an additive anti-cancer effect with the addition of natural progesterone to endocrine therapy in preclinical breast cancer models. This is a phase II multi-site, randomised, open-label, three-arm, study in 200 postmenopausal women with early-stage ER+, PR+, HER2-negative breast cancer. Eligible patients will be randomised 1:1:1 to receive 14 days of intervention with either letrozole 2.5 mg PO daily, letrozole 2.5 mg + prometrium 300 mg PO daily or tamoxifen 20 mg + prometrium 300 mg PO daily, between diagnosis of breast cancer and definite surgery. The primary endpoint of this study is to determine geometric mean suppression of the centrally assessed proliferation marker Ki67 after two weeks of intervention, compared with baseline. Secondary endpoint is safety and tolerability of combination therapy. Translational endpoints including evaluating a gene set as a predictive biomarker for a reduction in Ki67, changes in the apoptotic markers Bcl-2 and Caspase 3 in the tumours following intervention and changes in ER, PR, AR, FoxA1, Cyclin D1 protein and mRNA expression in the tumours following intervention will also be assessed. The IMPACT study reported a geometric mean reduction in Ki67 after 2 weeks of preoperative tamoxifen of 59.5% and anastrazole of 76%. This allows estimation of power to detect differences between Arm 1 and either Arm 2 or Arm 3 with a p-value of 0.025. With a total trial recruitment of 200 and allowing 4% dropouts, this would

give 80% power to detect an improvement in Ki67 suppression from 76% in the letrozole alone control arm to 92% in either experimental arm.

DOI: 10.1530/oncolabs.1.P011

P012

Targeting AR in endocrine-resistant breast cancer

KeeMing Chia[1,2], Heloisa Milioli[1,2], Neil Portman[1,2], Geraldine Laven-Law[3], Rhiannon Coulson[1,2], Aliza Yong[1,2], Davendra Segara[2], Andrew Parker[2], Catherine E Caldon[1,2], Niantao Deng[1,2], Alexander Swarbrick[1,2], Wayne D Tilley[3], Theresa E Hickey[3] & Elgene Lim[1,2]

[1]Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia; [2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Darlinghurst, New South Wales, Australia; [3]Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia.

Introduction

Resistance to endocrine therapy is a major clinical problem in estrogen receptor positive (ER+) breast cancer. The androgen receptor (AR) is expressed in ~90% of all primary ER+ breast cancers and high expression of AR is associated with a better patient outcome in this tumours. However, uncertainty surrounding the role of AR in endocrine resistance is reflected in current clinical trials in which both AR agonists and antagonists are being investigated. Here, we sought to investigate the optimal approach in targeting AR in endocrine-resistant breast cancer.

Methods

The consequences of AR activation, using AR cognate ligand 5α-dihydrotestosterone (DHT) and selective AR modulator enobosarm, or AR antagonism using enzalutamide were evaluated on preclinical models of endocrine-resistance.

Results

Treatment with DHT and enobosarm inhibited the growth of MCF7 TamR and LTED cells but enzalutamide had no effect. AR activation was associated with attenuation of ER signaling in both models. DHT strongly inhibited the proliferation of endocrine-resistant PDX models. Enobosarm similarly suppressed the proliferation of HCI-005 PDX, and to a lesser extent in Gar15-13 PDX. Antagonizing AR with enzalutamide had no effect on growth of Gar15-13, consistent with our *in vitro* data. AR agonists reduced expression levels of ER and PR in HCI-005, and transcriptomic analysis of AR agonist-treated Gar15-13 identified significant negative enrichment of genes related to proliferation and estrogen response. These observations indicate that the growth-suppressive effects of AR activation *in vivo* were mediated through inhibition of ER signaling. Furthermore, we established a highly prognostic AR gene signature through RNA-sequencing analysis of Gar15-13 treated with DHT using the clinically-annotated METABRIC dataset.

Conclusion

We have demonstrated that activating AR is an effective therapeutic approach in endocrine-resistant breast cancer and that AR activity is tumor suppressive regardless of endocrine-therapy sensitivity.

DOI: 10.1530/oncolabs.1.P012

P013

Single cell transcriptome analysis reveals human prostate cancer cells upregulate retinoic acid signalling in response to androgen withdrawal

Ashlee K Clark[1,2], Hieu Nim[3,4], Natalie Lister[1,2], Mitchell G Lawrence[1,2,5], Shivakumar Keerthikumar[5,6,7], David L Goode[5,6,7], MURAL[1], Hong Wang[1,2], Melissa Papargiris[1,2], Andrew Ryan[8], Arun Azad[6,9], Mark Frydenberg[1,10], Gail P Risbridger[1,2,5] & Renea A Taylor[1,5,11]

[1]Monash Partners Comprehensive Cancer Consortium, Monash Biomedicine Discovery Institute Cancer Program, Prostate Cancer Research Group, Monash University, Clayton, Victoria 3800, Australia; [2]Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia; [3]Faculty of Information Technology, Monash University, Victoria 3800, Australia; [4]Australian Regenerative Medicine Institute, Monash University, Victoria 3800, Australia; [5]Prostate Cancer Research Program, Cancer Research Division, Peter MacCallum Cancer Centre, University of Melbourne, Melbourne, Victoria 3000, Australia; [6]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria 3010, Australia; [7]Computational Cancer Biology Program, Peter MacCallum Cancer Centre, Melbourne, Victoria 3000, Australia; [8]TissuPath, Mount Waverley, Victoria, 3149, Australia; [9]School of Clinical Sciences, Department of Medicine, Monash University, Victoria 3168, Australia; Prostate Cancer Research Program, Cancer Research Division, Peter MacCallum Cancer Centre, University of Melbourne, Melbourne, Victoria 3000, Australia; [10]Department of Surgery, Monash University, Melbourne, Victoria 3800, Australia; [11]Department of Physiology, Monash University, Clayton, Victoria 3800, Australia.

A current challenge in cancer therapeutics is incomplete response to treatment and emergence of therapy-resistant disease. Androgen deprivation therapy (ADT), the standard treatment for advanced prostate cancer, and effectively reduces the tumour burden in most patients. Yet, residual tumour cells that withstand ADT eventually develop lethal castration-resistance. Eliminating these castrate-tolerant cells, by combining ADT with other treatments, might delay or even prevent castration-resistant prostate cancer (CRPC). Clinical trial evidence supports this notion, with the CHAARTED and STAMPEDE studies showing that combining ADT with upfront docetaxel improves the overall survival of men with metastatic prostate cancer. These studies suggest that chemotherapeutics and other AR-targeted therapies target a population of cells that are not sensitive to castration alone. Therefore, we hypothesise that more effective co-targeting strategies could eliminate castrate-tolerant cells and improve outcomes for men with advanced prostate cancer. To identify signalling pathways that facilitate the survival of castrate-tolerant cells, we used prostate cancer patient-derived xenografts (PDXs) and single-cell transcriptomics. We show that a subpopulation of castrate-tolerant cells exist in multifocal regions of low, intermediate and high risk tumors, and can survive long-term castration. Castrate-tolerant cells significantly upregulate components of the retinoic acid signalling pathway, including *CRABP*2 (Cellular Retinoic Acid Binding Protein 2) and *RARRES*3 (Retinoic Acid Receptor Responder 3). Pre-clinical studies with PDX-derived organoids showed that inhibiting retinoic acid signalling stimulates the growth of castrate-tolerant cells and renders them sensitive to docetaxel treatment. Altogether, these data show that specific signalling pathways are up-regulated in castrate-tolerant cells, including retinoic acid signalling, providing rational co-targeting strategies to improve the efficacy of ADT and delay or prevent progression to CRPC.

DOI: 10.1530/oncolabs.1.P013

P014

Nuclear ErbB-2 activity modulates the interferon signaling pathway in breast cancer cells resistant to anti-ErbB-2 therapies

Rosalía I Cordo Russo[1], Santiago Madera[1], María F Chervo[1], Esmaeil Ebrahimie[2], Luke Selth[2], Violeta A Chiauzzi[1], Zoya Kikhtyak[2], Cecilia J Proietti[1], Roxana Schillaci[1], Eduardo H Charreau[2], Theresa E Hickey[2], Wayne D Tilley[2] & Patricia V Elizalde[1]

[1]Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires Argentina; [2]Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, AHMS, University of Adelaide, Adelaide, SA 5005, Australia.

Overexpression of ErbB-2, a member of ErbB family of receptor tyrosine kinases, occurs in 15–20% of breast cancers (BC) and is considered a major oncogenic driver. Despite clinical efficiency of ErbB-2-targeted therapies (e.g. trastuzumab), resistance to said drugs is a major issue. While ErbB-2 is mainly a cell membrane-bound receptor, it can migrate to the nucleus (NErbB-2) where it acts as a transcription factor or coactivator. We revealed that NErbB-2 is a major proliferation driver in trastuzumab-resistant BC. Here, we used JIMT-1 BC cells, which constitutively express NErbB-2 and are intrinsically trastuzumab-resistant, to explore the transcriptional consequences of NErbB-2 activity. RNAseq was performed on JIMT-1 cells transfected with and without a human ErbB-2 nuclear

localization domain mutant (hErbB-2ΔNLS), unable to translocate to the nucleus, which acts as a dominant negative inhibitor of endogenous NErbB-2 migration. Exclusion of ErbB-2 from the nucleus resulted in up-regulation of 280 genes and down-regulation of 33 genes. Functional analysis using String Database revealed that blockade of NErbB-2 presence increased expression of genes involved in type-I interferon and cytokine-mediated signaling pathways (FDR 7.52E-34 and 4.48E-32, respectively). Interferon beta (IFNβ) and lambda (IFNλ), key players in interferon signaling, were among top up-regulated genes. In independent validation experiments, blockade of NErbB-2 induced IFNβ and IFNλ mRNA expression in JIMT-1 and HCC-1569 trastuzumab-resistant cells. hErbB-2ΔNLS also induced TRIM22 and OAS-2 mRNA expression, two proteins activated by interferon signaling. JIMT-1 xenografts demonstrated that blockade of NErbB-2 localization by injection of hErbB-2ΔNLS significantly inhibits *in vivo* tumor growth. Interestingly, IFNβ and IFNλ mRNA levels were also up-regulated in hErbB-2ΔNLS-injected tumors. Moreover, treatment with IFNβ or IFNλ inhibited *in vitro* proliferation of JIMT-1 cells. Collectively, these findings reveal IFNβ and IFNλ as novel targets of NErbB-2 and suggest that NErbB-2 drives the growth of trastuzumab-resistant BC cells via transcriptional repression of interferons.

DOI: 10.1530/oncolabs.1.P014

P015

Combinatorial co-targeting by miRNAs: a subtle but strong regulator of epithelia-mesenchymal transitions

Joseph Cursons[1,2], Katherine A Pillman[3,4], Kaitlin G Scheer[3], Philip A Gregory[3,4], Momeneh Foroutan[1,5], Soroor Hediyeh-Zadeh[1], John Toubia[5], Edmund J Crampin[6,7,8], Gregory J Goodall[3,4], Cameron P Bracken[3,4] & Melissa J Davis[1,2,9]
[1]Bioinformatics Division, Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, VIC 3052, Australia; [2]Department of Medical Biology, University of Melbourne, Parkville, VIC 3010, Australia; [3]Centre for Cancer Biology, an Alliance of SA Pathology and University of South Australia, North Terrace, Adelaide, SA 5000, Australia; [4]Department of Medicine, University of Adelaide, Adelaide, SA 5005, Australia; [5]The University of Melbourne, Department of Surgery, St. Vincent's Hospital, Fitzroy, VIC 3065, Australia; [6]Systems Biology Laboratory, University of Melbourne, Parkville, VIC 3010, Australia; [7]ARC Centre of Excellence in Convergent Bio-Nano Science, Department of Biomedical Engineering, University of Melbourne, Parkville, VIC 3010, Australia; [8]School of Mathematics and Statistics, University of Melbourne, Parkville, VIC 3010, Australia; [9]Department of Biochemistry, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC 3010, Australia.

Epithelial-mesenchymal transition (EMT) and the reverse mesenchymal-epithelial transition (MET) are normal biological processes, however they are also thought to play a critical role in the progression and metastasis of cancers, including breast cancer. Cancer cells reactivate the gene expression programs of EMT and MET through a wide range of mechanisms, and better understanding of these regulatory processes will lead to the identification of therapeutically actionable targets. MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression, functioning in part by facilitating the degradation of target mRNA transcripts. MiRNAs have an established role in controlling EMT, and many studies have demonstrated the role of individual miRNAs using overexpression at levels greatly exceeding physiological abundance, which can in turn lead to off-target effects, and over-estimation of functional effects. Computationally, we place the TCGA breast cancer samples, and a collection of >60 breast cancer cell lines on a landscape defining epithelial and mesenchymal phenotypes, and use this as a tool to explore phenotypic transitions. Analysing a human mammary cell model of EMT with endogenous changes in miRNA expression, we found evidence that a set of miRNAs, including the miR-200 and miR-182/183 family members, cooperate in post-transcriptional regulation, both reinforcing and buffering transcriptional output. Investigating this, we demonstrate that combinatorial treatment could induce MET with miRNA concentrations much closer to endogenous levels and with less off-target effects. This discovery, that co-operative targeting by miRNAs is important for their physiological function, has opened the way for a more-refined understanding of post-transcriptional regulatory processes. Future work classifying miRNAs should consider such combinatorial effects, and combinatorial co-targeting represents a new strategy for tuning biological processes involved in cancer progression through small adjustments to this critical regulatory layer.

DOI: 10.1530/oncolabs.1.P015

P016

AR chromatin binding is reprogrammed in the absence of FOXA1 in ER- breast cancers

Iza Denis[1], Luke A Selth[1], Jessica LL Robinson[2,3], Hisham Mohammed[2,3], Thomas Carroll[2,3], Gordon D Brown[2,3], David E Neal[2,3], Jason S Carroll[2,3], Wayne D Tilley[1] & Theresa E Hickey[1]
[1]Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, AHMS, University of Adelaide, Adelaide, SA 5005, Australia; [2]Cancer Research UK Cambridge Institute, Robinson Way, Cambridge, CB2 0RE, UK; [3]Department of Oncology, University of Cambridge, Cambridge, CB2 0XZ, UK.

Introduction
75% of breast cancers (BCa) are driven by the estrogen receptor α (ER+). Tumours lacking ER (ER-) are more aggressive and have the poorest prognosis. The androgen receptor (AR) is also widely expressed in BCa (90% of primary tumours). FOXA1 is a pioneer factor required for oncogenic AR signalling in PCa but its role in AR signaling in ER-BCa is not clear. We previously showed that cell growth is increased when FOXA1 is overexpressed in AR-driven PCa and BCa cell lines, suggesting that FOXA1 enhances growth. To further address FOXA1's role in ER- BCa, we examined the consequence of FOXA1 loss on AR-chromatin interactions (cistrome) in a well characterized model of AR driven ER-BCa.
Hypothesis
AR cistrome is reprogrammed in the absence of FOXA1 in ER- BCa.
Methods
Genome-wide chromatin binding profiles for AR and specific AR interactors were performed using ChIP-seq in the ER-AR+ MDA-MB-453 BCa cell line. The AR protein interactome was interrogated using SILAC-RIME proteomic technique.
Results
Depletion of FOXA1 inhibited MDA-MB-453 cell proliferation, suggesting a requirement for FOXA1 to sustain cell growth. AR recruitment was increased at a large number of sites (73%) in the absence of FOXA1, suggesting that AR chromatin binding is reprogrammed when FOXA1 is missing. Proteomic analysis in the absence of FOXA1 revealed an increased interaction of AR with several proteins, including TFAP2A. Motif analysis indicated that the gained AR binding sites were enriched for TFAP2A binding motifs; co-IP analyses confirmed the interaction between AR and TFAP2A. Co-localisation of core TFAP2A, AR and FOXA1 binding events (20% overlap) was identified.
Conclusion
In the absence of FOXA1, AR cistrome is reprogrammed in ER-AR+ MDA-MB-453 cells. The gained genomic AR binding sites appear to be dependent on a novel factor, TFAP2A, which could to be critical for AR signaling.

DOI: 10.1530/oncolabs.1.P016

P017

Loss of FAM3B promotes prostate cancer progression by modulating glucose metabolism

Shanshan Bai[1], Subing Cao[1], Ladan Fazli[2], Lianjin Jin[1], Melody Baddoo[3], Eva Corey[4], Arun Sreekumar[5], Xuesen Dong[2], Erik K Flemington[3] & Yan Dong[1]
[1]Department of Structural and Cellular Biology, Tulane University School of Medicine, Tulane Cancer Center, New Orleans, Louisiana, USA; [2]Department of Urologic Sciences, Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia, Canada; [3]Department of Pathology, Tulane University School of Medicine, Tulane Cancer Center, New Orleans, Louisiana, USA; [4]Department of Urology, University of Washington, Seattle, Washington, USA; [5]Department of Molecular and Cellular Biology and Alkek Center for Molecular Discovery, Baylor College of Medicine, Houston, Texas, USA.

Abstract unavailable

DOI: 10.1530/oncolabs.1.P017

P018

Assessing alterations in organelle contacts during prostate cancer development

Emma Evergren[1] & Lisa Butler[2]
[1]Centre for Cancer Research and Cell Biology, Queen's University, Belfast, UK; [2]South Australian Health and Medical Research Institute, Adelaide, Australia.

Aggressive prostate cancer is characterized by altered lipid metabolism and metabolic stress. At a subcellular level these changes are localized to the endoplasmic reticulum (ER) and mitochondria. Traditionally the function of these organelles has been studied separately. A growing body of evidence shows that the interaction between mitochondria and ER at specialized membrane contact sites play a key role in regulating fundamental cellular processes such as lipid synthesis, mitochondrial metabolism, calcium signaling, apoptosis and oxidative stress. In this study we have undertaken the first high resolution imaging study of prostate tissue to evaluate changes in mitochondrial ER-associated membranes (MAMs). We have analyzed the number and size of MAMs in human patient samples with varying Gleason grade groups using transmission electron microscopy. Quantification of the number of mitochondria in close spatial proximity (0–25 nm) to the ER in intermediate grade cancer compared to normal tissue, showed a prominent increase in tight membrane contacts. In control cells 15% of mitochondria associated closely with the ER compared to 75% in the transformed cells. Furthermore, the size of the contact sites in transformed cells was larger compared to control and covered 15% of the mitochondria perimeter, suggesting a more efficient lipid and calcium transfer at these contact sites in transformed prostate epithelial cells. Based on these findings we believe that organelle proximity and contact sites represent pathological features of prostate cancer, which may correlate with metabolic reprogramming occurring during prostate cancer progression. Our current efforts focus on identifying the protein complexes that mediate the associations between organelles and evaluating whether the occurrence of MAMs impacts on treatment responses and the development of treatment resistance.

DOI: 10.1530/oncolabs.1.P018

P019

Array comparative genomic hybridisation of familial prostate cancer tumours identifies a recurrent copy number gain on chr19p13.3 encompassing the EEF2 gene

LM FitzGerald, K Raspin, JR Marthick, RC Malley, S Donovan & JL Dickinson
Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia.

In a bid to discover genomic features associated with prostate cancer (PrCa) development and progression, copy number variations (CNVs) have been studied in tumour samples. Early comparative genomic hybridisation (CGH) studies led to the identification of many chromosomal regions of loss and gain, with a small number of these shown to be consistent across studies and, significantly, some suggested to be associated with PrCa progression. More recently a small number of studies have applied dense genome-wide SNP array platforms to fresh-frozen prostate tumours to identify recurrent CNVs, however these platforms are not suitable for the more widely available formalin-fixed, paraffin-embedded (FFPE) tumour samples. With the aim of replicating or identifying novel recurrent prostate tumour CNVs and elucidating the underlying genes involved, we applied the Agilent Oligonucleotide array-based CGH (aCGH), with both genome-wide and custom probes, to 12 FFPE prostate tumour DNA samples from a single Tasmanian family, PcTas9. Analysis of these data revealed as little as two to tens of CNVs present in each tumour, the majority of which were gains. In addition, several recurrent CNVs were identified, the most common of which was present on chromosome 19p13.3 and contained only two genes, including EEF2. EEF2 has recently been shown to be overexpressed in various cancer types, including breast and PrCa where it has been suggested to be associated with Gleason score. Currently, we are performing EEF2 gene and protein expression studies in tumour samples from PcTas9 and additional Tasmanian familial and sporadic PrCa cases, and will investigate whether these are associated with Gleason score. Our findings to date suggest that increased EEF2 protein levels observed in prior prostate tumour studies may be due to a copy number gain on 19p13.3.

DOI: 10.1530/oncolabs.1.P019

P020

Circular RNAs add further diversity to AR isoform repertoire

Subing Cao[1], Tianfang Ma[1], Nathan Ungerleider[2], Claire Roberts[2], Lianjin Jin[1], Monica Concha[2], Xia Wang[2], Melody Baddoo[2], Ladan Fazli[3], Eva Corey[4], Oliver Sartor[5], Xuesen Dong[3], Erik Flemington[2,*] & Yan Dong[1]
[1]Department of Structural and Cellular Biology, Tulane University, New Orleans, Louisiana, USA; [2]Department of Pathology, Tulane University, New Orleans, Louisiana, USA; [3]Department of Urologic Sciences, Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia, Canada; [4]Department of Urology, University of Washington, Seattle, Washington, USA; [5]Department of Medicine, Tulane University, New Orleans, Louisiana, USA.
*Presenter

Circular RNAs (circRNAs) are a newly appreciated class of regulatory RNA species that play vital roles in various cell signaling and metabolic processes. Deregulated expression of circRNAs has been found to be associated with various human diseases including many types of cancer. Despite their growing links to cancer, there has been limited characterization of circRNAs in metastatic castration-resistant prostate cancer, the major cause of prostate cancer mortality. Here, initiated with a global analysis using a publically available exome capture RNA-seq dataset from 47 metastatic castration-resistant prostate cancer samples, we identified circRNAs generated from the key prostate cancer driver gene-androgen receptor (AR). We validated and characterized the top four most abundant AR circRNAs using RNase R RNA-seq. Expression of these AR circRNAs as upregulated in castration-resistant compared to hormone naïve patient-derived xenografts and was further increased in enzalutamide-resistant patient-derived xenografts. The upregulation of these AR circRNAs was not due to global increase of circRNA formation in these tumors. Instead, the levels of AR circRNAs correlated strongly with that of the linear AR transcripts (both full-length AR and AR splice variants) in clinical samples and patient-derived xenografts. In cultured cells, androgen supplementation led to a significant downregulation of these AR circRNAs as well as the linear AR transcripts, and the downregulation was attenuated by enzalutamide treatment. Using nuclear/cytoplasmic fractionation and the Basescope RNA in-situ hybridization assay, we demonstrated predominant cytoplasmic localization of these AR circRNAs, indicating likely cytoplasmic functions. CircRNAs have previously been shown to be secreted into the circulation and readily detectable in the plasma. With higher exoribonuclease resistance and RNA stability compared to the linear AR transcripts, these AR circRNAs may serve as a new species of circulating biomarker for metastatic castration-resistant prostate cancer patients.

DOI: 10.1530/oncolabs.1.P020

P021

Estrogen receptor alpha controls gene expression via translational offsetting

Julie Lorent[1], Richard J Rebello[2,3], Vincent van Hoef[1], Mitchell G Lawrence[2,3,4], Krzysztof J Szkop[1], Eric Kusnadi[2], Baila Samreen[1], Preetika Balanathan[3], Karin Scharmann[5,6], Itsuhiro Takizawa[3], Sebastian A Leidel[5,6,7], Gail P Risbridger[2,3,4], Ivan Topisirovic[8], Ola Larsson[1] & Luc Furic[2,3,4]
[1]Department of Oncology-Pathology, Karolinska Institutet, Science for Life Laboratory, Stockholm, Sweden; [2]Prostate Cancer Translational Research Laboratory, Peter MacCallum Cancer Centre, Melbourne, VIC, 3000, Australia; [3]Cancer Program, Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, 3800, Australia; [4]Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC, 3010, Australia; [5]Max Planck Institute for Molecular Biomedicine, Münster, Germany; [6]Cells-in-Motion Cluster of Excellence, University of Münster, Münster, Germany; [7]Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland; [8]Lady Davis Institute, Gerald Bronfman Department of Oncology and Departments of Biochemistry and Experimental Medicine, McGill University, Montreal, QC, H3T1E2, Canada.

Estrogen receptor alpha (ERα) activity is associated with increased cancer cell proliferation. Studies aiming to understand the impact of ERα on cancer-associated phenotypes have largely been limited to its transcriptional activity. Herein, we demonstrate that ERα coordinates its transcriptional output with selective modulation of mRNA translation. Importantly, translational perturbations caused by depletion of ERα largely manifest as 'translational offsetting' of the transcriptome, whereby amounts of translated mRNA and protein levels are maintained constant despite changes in mRNA abundance. Transcripts whose

levels, but not polysome- association, are reduced following ERα depletion lack features which limit translational efficiency including structured 5'UTRs and miRNA target sites. In contrast, mRNAs induced upon ERα depletion whose polysome- association remains unaltered are enriched in codons requiring U34-modified tRNAs for efficient decoding. Consistently, ERα regulates levels of U34-modification enzymes, whereas altered expression of U34-modification enzymes disrupts ERα dependent translational offsetting. Altogether, we unravel a hitherto unprecedented mechanism of ERα-dependent orchestration of transcriptional and translational programs, and highlight that translational offsetting may be a pervasive mechanism of proteome maintenance in hormone-dependent cancers.

DOI: 10.1530/oncolabs.1.P021

P022

Characterisation of developmental pathways that drive metastatic progression of breast cancer at single cell resolution

Fatima Valdes-Mora[1,2], Robert Salomon[3,4], Brian Gloss[5], Andrew MK Law[5], Kendelle Murphy[6], Daniel L Roden[2,3], Lesley Castillo[5,7], Yolanda Colino-Sanguino[1], Nona Farbehi[3], James Conway[6], Paul Timpson[2,6], Christopher J Ormandy[2,7] & David Gallego-Ortega[2,5]
[1]Histone Variant Group, Genomics and Epigenetics Division, Garvan Institute of Medical Research. Sydney, NSW Australia; [2]St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Sydney, NSW, Australia; [3]Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Sydney, NSW, Australia; [4]*Current*: Institute for Biomedical Materials and Devices, University of Technology Sydney, Sydney, NSW, Australia; [5]Tumour Development Group, The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, NSW, Australia; [6]Invasion and Metastasis Laboratory, The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, NSW, Australia; [7]Cancer Biology Laboratory, The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, NSW, Australia.

Tumour cell heterogeneity constitutes a challenge for cancer treatment and deeply impact the outcome of patients. A simultaneous overview of cancer cells and associated stromal cells is critical for the design of improved therapeutic regimes. Single-cell RNA-seq has emerged as a powerful method to unravel heterogeneity of complex biological systems; this has enabled *in vivo* characterization of cell type compositions through unsupervised sampling and modelling of transcriptional states in single cells. Here we used single-cell RNA-seq to elucidate the cellular composition and functional diversity of breast tumours during the induction of metastatic disease. We characterised with unprecedented definition, how the activation of developmental programs associated to pregnancy results in the acquisition of an aggressive phenotype. We use a transgenic model of alveolar cell differentiation to manipulate the lineage composition of the mammary epithelium in the MMTV-PyMT mouse mammary tumour model. We showed that cancer cells are classified in a structure comparable with the lineages of the epithelial mammary gland hierarchy, revealing high dynamics and plasticity of cancer cells during disease progression. This cancer progression program is orchestrated by alveolar cells, which in conjunction with cancer-associated fibroblasts and myeloid cells form a multi-cellular process that resembles an aberrant involution. Finally, we analysed the interactome of the tumour ecosystem to define a high-resolution landscape of the molecular pathways of cell-to-cell communication that underpins extra-cellular remodelling and inflammation associated to the aggressive involution mimicry. Our study recapitulates developmental mechanisms that have gone awry during carcinogenesis in a model of pregnancy-associated breast cancer, revealing breast heterogeneity and key molecular events that result in cancer progression. scRNA-seq technology is generating a paradigm-shift in our understanding of cancer biology, the simultaneous observation of the different cell species involved in metastatic programs will contribute to the development of novel drug combinations and more specific cancer therapies.

DOI: 10.1530/oncolabs.1.P022

P023

The Myoepithelium as a Risk Predictor in Ductal Carcinoma *In Situ* of the Breast

Gemma M Wilson[1], Barbara J Guild[1], Christine L Clarke[1], Nirmala Pathmanathan[2] & J Dinny Graham[1,2]
[1]Westmead Clinical School, The Westmead Institute for Medical Research, University of Sydney, Westmead, 2145 Australia; [2]Westmead Breast Cancer Institute, Westmead, 2145, Australia.

The establishment of mammographic screening programs has resulted in a striking increase in the incidence of ductal carcinoma *in situ* (DCIS), with DCIS accounting for approximately 20% of all new screen-detected breast cancers. An estimated 40% to 70% of DCIS lesions may progress to invasive disease if left untreated. This number is considerably reduced by treatment: surgical excision followed by radiation therapy is curative in over 95% of cases. However, of the DCIS cases that do recur, 50% recur as invasive breast cancer. The key distinguishing feature for DCIS is the presence of myoepithelial cells, which harbour tumour suppressor functions and confine the tumour cells within the duct. However, malignancy-associated changes to the myoepithelial cells lead to progressive loss of the myoepithelial layer, permitting microinvasion and metastasis of the tumour cells resulting in invasive cancer. Currently, the likelihood of recurrence or malignant progression of each DCIS case is unpredictable, thus surgery plus radiotherapy, is the current standard of care, resulting in substantial overtreatment. Emerging evidence has revealed that the levels and distribution of myoepithelial markers become progressively altered in DCIS compared to normal myoepithelium, and that these changes are correlated with recurrence or progression to invasive disease. To explore this, we assembled a cohort of low and high grade DCIS (with or without association with invasive breast cancer) with extensive clinical follow-up in which to characterise expression of a panel of markers by immunohistochemistry. The markers p63, CD10, smooth muscle myosin heavy chain and calponin, were universally strongly positive in myoepithelium of normal breast. Marker expression was significantly reduced in DCIS-associated myoepithelium, compared to normal breast tissue. Moreover, marker loss was associated with disease progression, with early data suggesting that marker loss predicts long-term outcome. Our study suggests utility of myoepithelial markers in clinical management and reducing over-treatment of DCIS.

DOI: 10.1530/oncolabs.1.P023

P024

Lasofoxifene is an effective inhibitor of breast cancer lung and liver metastasis in a mammary intraductal (MIND) xenograft model of mutant ERα+ breast cancer

Marianne Greene[1], Muriel Lainé[1], Ya-fang Chang[1], Linda Phung[1], Richard Hiipakka[1], Barry Komm[2] & Geoffrey L Greene[1]
[1]Ben May Department for Cancer Research, University of Chicago, Chicago Il, USA; [2]CSO, Sermonix Pharmaceuticals.

The standard of care for early postmenopausal ERα+ breast cancer patients is adjuvant endocrine therapy, with or without a CDK 4/6 inhibitor in the metastatic setting. However, many patients are resistant or experience recurrence 10 to 15 years after treatment. A subset (10–40%) of ERα+ therapy-resistant breast cancers express somatic ESR1 mutations. The two most common ERα mutations are Y537S and D538G, which confer ERα constitutive activity. Lasofoxifene is a SERM developed to treat vulvovaginal atrophy and osteoporosis. In this study, we tested the hypothesis that lasofoxifene would be an effective inhibitor of MCF7 tumor explants engineered to express Y537S or D538G ERα. We used the mammary intraductal mouse model (MIND) for our studies. Three MCF7 cell variants, MCF7 WT, MCF7 Y537S and MCF7 D538G were injected into the mammary ducts of NSG mice. Cells were labeled with a luciferase reporter to monitor tumor growth by *in vivo* imaging. Mice were treated with different doses of lasofoxifene, vehicle, or the SERD, fulvestrant. After 70 days of treatment, primary tumor growth, as measured by endpoint tumor weight, of MCF7 WT, D538G and Y537S explants was significantly inhibited versus vehicle by 10 mg/kg lasofoxifene and fulvestrant. Compared to fulvestrant, lasofoxifene was significantly more effective at 5 and 10 mg/kg for the MCF7 Y537S and D538G tumors. Notably, the two MCF7 mutants metastasized to the lung and liver, whereas WT MCF-7 cells were only very weakly metastatic by the end of the study. Lasofoxifene significantly inhibited the metastasis of both MCF7 Y537S and D538G to the lungs and liver at 5 and 10 mg/ml. In contrast, fulvestrant only inhibited metastasis of the MCF7 D538G mutant to both organs. These data suggest that lasofoxifene may be useful as a treatment for ERα+ metastatic breast cancers, including those that express constitutively active ERα mutations.

DOI: 10.1530/oncolabs.1.P024

P025

Estrogen receptor regulated miR-342 suppresses a pro-metastatic gene network

Rachael Lumb[1], Victoria K Arnet[1,2], Cameron N Johnstone[3], Katherine A Pillman[1], John Toubia[1], Emily Hackett-Jones[1], Caroline A Phillips[1], Pannapa Pinweha[1], Suraya Roslan[1], Xiaochun Li[1], Traude Beilharz[4], Yeesim Khew-Goodall[1], Gregory J Goodall[1,2], Robin L Anderson[3] & Philip A Gregory[1,2]
[1]Centre for Cancer Biology – An Alliance between SA Pathology and the University of South Australia, Adelaide, SA, Australia; [2]Discipline of Medicine, The University of Adelaide, Adelaide, SA, Australia; [3]Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, Australia; [4]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia.

Breast cancer is the most frequently diagnosed cancer in women and the second leading cause of female cancer related death. Despite significant advances in early detection, surgery and therapy, treatment remains a challenge if metastatic disease develops. Metastasis occurs with high frequency in triple negative (ER/PR/HER2 negative, TNBC) breast cancers, which are a heterogeneous group of cancers with poor clinical outcome. We used an integrated approach to identify miRNAs that influence breast cancer metastasis as well as indicate patient outcome. Through this we identified miR-342 which we found is: (1) strongly downregulated in mouse and human TNBC cell lines that are prone to metastasise, (2) sufficient to repress breast cancer metastasis in immune competent and xenograft mouse models, and (3) an independent prognostic marker of patient outcome in large patient cohorts. Using genome-wide Argonaute-CLIP analysis we identified 120 direct target genes of miR-342, including a high representation of E2F1-driven and actin dynamics pathways. We propose these pathways may represent new targets for treatment of metastatic TNBC.

DOI: 10.1530/oncolabs.1.P025

P026

A molecular portrait of epithelial-mesenchymal plasticity in prostate cancer progression

N Stylianou[1], ML Lehman[1,2], C Wang[1], AT Fard[1], A Rockstroh[1], L Fazli[2], L Jovanovic[1], M Ward[3], MC Sadowski[1], AS Kashyap[4], R Buttyan[2], ME Gleave[2], TF Westbrook[5], ED Williams[1], JH Gunter[1], CC Nelson[1] & BG Hollier[1]
[1]Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation, Faculty of Health, School of Biomedical Sciences, Queensland University of Technology, Princess Alexandra Hospital, Translational Research Institute, Brisbane, QLD, Australia; [2]Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, Canada; [3]Glycation and Diabetic Complications Group, Mater Research Institute, Translational Research Institute, School of Medicine, University of Queensland, Brisbane, QLD, Australia; [4]Tissue Repair and Regeneration Program, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia; [5]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA.

The propensity of cancer cells to transition between epithelial and mesenchymal phenotypic states via the epithelial-mesenchymal transition (EMT) program can regulate metastatic processes, cancer progression, and treatment resistance. Transcriptional investigations using reversible models of EMT, revealed the mesenchymal-to-epithelial reverting transition (MErT) to be enriched in clinical samples of metastatic castrate resistant prostate cancer (mCRPC). From this enrichment, a metastasis-derived gene signature was identified that predicted more rapid cancer relapse and reduced survival across multiple human carcinoma types. Additionally, the transcriptional profile of MErT is not a simple mirror image of EMT as tumour cells retain a transcriptional 'memory' following a reversible EMT. This memory was also enriched in mCRPC samples. Cumulatively, our studies reveal the transcriptional profile of epithelial-mesenchymal plasticity and highlight the unique transcriptional properties of MErT. Furthermore, our findings provide evidence to support the association of epithelial plasticity with poor clinical outcomes in multiple human carcinoma types.

DOI: 10.1530/oncolabs.1.P026

P027

Estrogen maintains mammographic density via heparanase mediated induction of SDC-1 and -4

Xuan Huang[1,2], Tony Blick[1,2], Thomas Lloyd[3], Vito Ferro[4], Theresa Hickey[5], Wayne Tilley[5], Larisa Haupt[2], [#]Erik W Thompson[1,2] & [#]Honor J Hugo[1,2,6]
[1]Institute of Health and Biomedical Innovation, Queensland University of Technology, Queensland, Australia; [2]Translational Research Institute, Queensland, Australia; [3]Radiology Department, Princess Alexandra Hospital, Queensland, Australia; [4]School of Chemistry and Molecular Biosciences, University of Queensland, Queensland, Australia; [5]Dame Rome Mitchell Cancer Research Laboratories, School of Medicine, University of Adelaide, Adelaide, Australia; [6]School of Biomedical Science, Queensland University of Technology, Queensland, Australia.
# Denotes equal shared last author.

Mammographic density (MD) is an independent risk factor for breast cancer, however what molecule or pathway within MD tissue contributes to this risk? High MD is characterized by an abundance of connective tissue stroma which is rich in heparan sulfate proteoglycans (HSPGs). Of these, SDC1 and SDC4 are upregulated in breast cancer, and we have observed a significantly higher abundance of these proteins in high vs low MD pair-wise comparisons from breast tissue from 8 individual women. Heparanase promotes HSPG-bound growth factor release via cleavage of HS chains leading to shedding and upregulation of SDC expression. Heparanase is upregulated by estrogen, as is MD, which decreases following menopause and tamoxifen therapy, and increases with HRT, implying MD and its associated breast cancer risk are modifiable. We sought to examine whether heparanase expression determines SDC1/4 protein expression and thus MD by directly modulating this enzyme in human mammary tissue grown *ex-vivo*. Patient-derived explants (PDEs) from prophylactic mastectomy material were supplemented with media containing estradiol or estradiol/tamoxifen, or the antagonistic heparanase mimetic PG545. RNA was collected for QRT-PCR, and remaining tissue used for IHC. Conditioned media was collected from the explants and tested via ELISA for shed SDC1 as an indicator of heparanase enzyme activity. Mammographic density change was measured using a single-sided MRI machine (NMR-MOUSE). Heparanase inhibition via PG545 in PDEs led to a decrease in shed SDC1 in explant conditioned media, a reduction in MMP-9 and SDC1 gene expression and a reduction in SDC1 protein in glandular tissue. PG545 treatment also led to a significant drop in NMR T1 values equating to a reduction in mammographic density. Hormone treatment led to an expected increase in TFF-1, but also a positive linear correlation between heparanase and SDC1 or heparanase and SDC4 expression. These effects were uncoupled by tamoxifen. The results of this study suggest that estrogen maintains MD via its positive effect on heparanase and subsequent syndecan 1 and 4 protein abundance. Agents designed to thwart this pathway *in vivo* have the potential to prevent breast cancer developing in women with high mammographic density.

DOI: 10.1530/oncolabs.1.P027

P028

Immune signalling is a key driver of breast density and breast cancer risk

Maddison Archer[1,2], Xuan Sun[1,2,3], Danielle J Glynn[1,2], Leigh J Hodson[1,2], Cecilia Huo[4], Kara Britt[5,6], Erik Thompson[4,7], Lucy Woolford[8], Andreas Evdokiou[1], Jeffrey W Pollard[9], Sarah A Robertson[2,3] & Wendy V Ingman[1,2]
[1]Discipline of Surgery, School of Medicine, The Queen Elizabeth Hospital, University of Adelaide, Woodville, Australia; [2]The Robinson Research Institute, University of Adelaide, Adelaide, Australia; [3]Discipline of Obstetrics and Gynaecology, School of Medicine, University of Adelaide, Adelaide, Australia; [4]The University of Melbourne Department of Surgery, St Vincent's Hospital Melbourne, Melbourne, Australia; [5]Metastasis Research Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia; [6]The Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia; [7]Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology and Translational Research Institute, Queensland, Australia; [8]School of Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia; [9]MRC and University of Edinburgh Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK.

High breast density is an independent risk factor for breast cancer. There is exciting potential for breast density to become a widespread health assessment tool, used to identify the women most at risk of breast cancer in order to intervene early and reduce that risk. However, a better understanding of causal biological mechanisms that lead to high breast density is required in order to develop therapeutic approaches. This project aimed to identify and investigate biological drivers of breast density. We have developed a grounding-breaking new approach to study breast density. Fresh breast tissue from surgical samples are cut into 1 cm slices and x-rayed. The x-ray image guides biopsies of high and low density regions from the same individual. The cellular and molecular components of these tissues are assessed under the microscope and statistically analysed as paired samples. This enables us to overcome the problem of heterogeneity within the breast, and the high variability between individuals. Using this approach, we demonstrated that regions of high density contained increased abundance of epithelial and stromal cells compared to regions of low density. Density was not associated with changes in hormone receptors or epithelial cell proliferation. Most striking however were differences in immune cells and immune signalling factors between paired samples. High density was associated with increased abundance of macrophages and pro-inflammatory protein C-C Motif Chemokine Ligand 2 (CCL2). To investigate whether immune signalling is a driver of high breast density, we engineered a genetically modified mouse model whereby the mammary gland specific MMTV promoter drives constitutive CCL2 expression. These studies revealed that CCL2-driven inflammation led to increased density and increased susceptibility to mammary cancer. This is the first study to demonstrate a causal role for immune system signalling in breast density and opens the door for new approaches to reducing breast cancer risk using anti-inflammatory drugs in women with dense breasts.

DOI: 10.1530/oncolabs.1.P028

P029

Translation of the ERα-PR crosstalk story to the clinic (the PIONEER study), and further preclinical exploration of the diverse role of progestins in ER-positive breast cancer

Sanjeev S Kumar[1,2], Rasmus Siersbaek[1], Sankari Nagarajan[1], Elena Provenzano[2], Carlos Caldas[1,2], Pan Pantziarka[3], Richard D Baird[2] & Jason Carroll[1]
[1]Cambridge Institute, CRUK, Cambridge, UK; [2]Cambridge Breast Cancer Research Unit, Addenbrooke's Hospital, Hills Rd, Cambridge UK; [3]AntiCancer Fund.

Background
Published preclinical findings from our lab (Cambridge Institute, CRUK) provided new insights into the functional 'cross-talk' between the oestrogen receptor alpha (ERα) and the progesterone receptor (PR) in breast cancer (Mohammed *et al.*, Nature, 2015). Addition of a PR agonist to anti-oestrogens directly modifies ERα chromatin binding and the transcriptional response in breast cancer cells, and is anti-proliferative *in vitro* and *in vivo*.
PIONEER Clinical Trial design
PIONEER is a three-arm, open label, multi-centre randomised phase II pre-surgical window trial evaluating effects of 15 days of preoperative therapy with Letrozole (LET), or LET plus Megestrol Acetate (MA, an off-patent semi-synthetic derivative of progesterone) 40 mg, or LET plus MA 160 mg in postmenopausal women with newly diagnosed, ER+ HER2- invasive primary breast cancer. >60 patients have been recruited in Cambridge, London (Guys and St Thomas' and St Bart's), Cornwall, Belfast, Bristol and Birmingham, with 3 other UK sites in active set up. The primary endpoint is % change in proliferation between baseline and day 15 tumour biopsies, measured by Ki67 immunohistochemical (IHC) assessment. Secondary endpoints include: expression of Aurora Kinase A, Caspase 3 and AR/PR/EMT markers by IHC; and safety endpoints. Exploratory endpoints include: transcription factor mapping (ChIP-seq) on paired fresh-frozen tumour samples. PIONEER will help determine if there is value in conducting a follow-on adjuvant study to investigate the longer term benefit of combining an aromatase inhibitor with MA, and if so, at what dose (40 mg vs 160 mg). Primary endpoint data and correlative ChIP-seq findings for an initial cohort of recruited patients will be presented.
Preclinical work
Other novel, more potent progestins are also being characterised in the lab, including Trimegestone (EC214), in cell lines, as well as ex vivo (PDX and primary tumour explants) and in vivo models. The role of progestins in the setting of the most common ESR1 mutations (Y537S and D538G) is also being explored preclinically using CRISPR-derived MCF7 and T47D mutant clones. Growth data in these cell lines after treatment with a panel of progestins will be presented, as well as ERα and PR ChIP-seq data, as well as plans for in vivo work with cell line xenograft models (in collaboration with Simak Ali).

DOI: 10.1530/oncolabs.1.P029

P030

Androgen receptor binding sites are highly mutated in prostate cancer

Tunç Morova[1,3], Mehmet Gönen[1,2], Kush Dalal[3], Attila Gursoy[2], Özlem Keskin[2] & Nathan A Lack[1,3]
[1]School of Medicine, Koç University, Istanbul, Turkey; [2]College of Engineering, Koç University, Istanbul, Turkey; [3]Vancouver Prostate Centre, University of British Columbia, Vancouver, Canada.

Androgen receptor (AR) signalling is essential to nearly all prostate cancer cells. Any alterations to AR-mediated transcription can have a profound effect on prostate carcinogenesis and tumour growth. While the AR protein has been extensively studied, little is know about mutations to the non-coding regions where AR binds to DNA. Using clinical whole genome sequencing, we demonstrate that AR binding sites have a dramatically increased rate of mutations that is greater than any other transcription factor and specific to only prostate cancer. Demonstrating this may be common to lineage-specific transcription factors, estrogen receptor binding sites also had an elevated rate of mutations in breast cancer. Based on the mutations observed at the binding site of AR and other related transcription factors, we propose that AR occupancy impairs access of base excision repair enzymes to endogenous DNA damage. To identify critical binding sites we systematically tested enhancer activity at every clinical AR binding site. From this we demonstrated that approximately 10% of the binding sites have enhancer activity. Combining these results with chromosomal confirmation capture approaches, we link specific somatic mutations at enhancer sites to alterations in gene regulation. Overall, this work demonstrates that non-coding mutations at AR binding sites can play a critical role in prostate cancer.

DOI: 10.1530/oncolabs.1.P030

P031

New combination therapies for castration-resistant prostate cancer

Mitchell G Lawrence[1,2], Laura H Porter[1], Daisuke Obinata[1], Shahneen Sandhu[3,4], Luke A Selth[4], Stephen Q Wong[2], Nicholas Choo[1], David Pook[1], Carmel J Pezaro[1], David L Goode[2,3], Ashlee K Clark[1], Melissa Papargiris[1], Roxanne Toivanen[1,2], Scott M Dehm[5], Melbourne Urological Research Alliance[1], CASCADE[2,3], kConfab[2,3], Heather Thorne[2,3], Wayne D Tilley[4], Richard B Pearson[2,3,6], Ross D Hannan[2,3,6,7], Declan G Murphy[3,8], Mark Frydenberg[1], Luc Furic[1,2,3], Renea A Taylor[1,2] & Gail P Risbridger[1,2,3]
[1]Monash Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, Australia; [2]Peter MacCallum Cancer Centre, Melbourne, Australia; [3]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia; [4]Dame Roma Mitchell Cancer Research Laboratories and Freemasons Foundation Centre for Men's Health, University of Adelaide, Adelaide, Australia; [5]Masonic Cancer Center & Departments of Laboratory Medicine and Pathology and Urology, University of Minnesota, Minneapolis, U.S.A.; [6]Department of Biochemistry and Molecular Biology, The University of Melbourne, Melbourne, Australia; [7]ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, Australian National University, Canberra, Australia; [8]Epworth Healthcare, Melbourne, Australia.

Abstract unavailable.

DOI: 10.1530/oncolabs.1.P031

P032

Clinical validation of circulating cytokines as markers of prognosis and response to docetaxel in men with metastatic castration resistant prostate cancer

KL Mahon[1,2,3,8], HM Lin[2,8], M Lee-Ng[10], D Cain[4], C Jacobs[4], MR Stockler[1,3,9], H Gurney[3,11], G Mallesara[12], K Briscoe[13], G Marx[14], C Higano[7], JS de Bono[5], KN Chi[6], SN Breit[10], DA Brown[10] & LG Horvath[1,2,3,8]
[1]Chris O'Brien Lifehouse, Sydney, Australia; [2]Garvan Institute of Medical Research, Sydney, Australia; [3]University of Sydney, Sydney, Australia; [4]Oncogenex Pharmaceuticals Inc, Bothell, WA, USA; [5]Royal Marsden Hospital and Institute of Cancer Research, London, UK; [6]University of British Columbia, BC Cancer Agency, Vancouver Prostate Centre, Vancouver, BC, Canada; [7]University of Washington, Fred Hutchinson Cancer Research Centre, Seattle, WA, USA; [8]University of NSW, Sydney, Australia; [9]National Health and Medical Research Council Clinical Trials Centre, Sydney, Australia; [10]St Vincent's Centre for Applied Medical Research, Sydney, Australia; [11]Westmead Hospital, Sydney, Australia; [12]Calvary Mater, Newcastle, Australia; [13]Coffs Harbour Hospital, Australia; [14]Northern Haematology and Oncology Group, Sydney, Australia.

Background
Elevated circulating macrophage inhibitory cytokine -1/growth differentiation factor 15 (MIC-1/GDF15), interleukins 4 (IL4) and 6 (IL6) levels were associated with poor prognosis and resistance to docetaxel chemotherapy in an exploratory cohort of men with metastatic castration resistant prostate cancer (mCRPC). To establish level 2 evidence of biomarker utility, these cytokines were tested in internal and external validation cohorts.
Methods
Internal validation cohort: Plasma samples taken at baseline (BL) and preC2 docetaxel ($n=120$). MIC-1/GDF15, IL-4 and IL-6 measured by ELISA assay.
External validation cohort: Serum samples taken at BL and/or preC3 docetaxel in 430 men with mCRPC on phase III SYNERGY study (docetaxel $\pm$ custirsen as 1st line chemotherapy in mCRPC with no OS benefit in the experimental arm). MIC-1/GDF15 measured by ELISA assay.
Associations between cytokine levels, PSA response, time to PSA progression and OS were assessed by non-parametric tests and Cox Regression survival analyses.
Results
Internal validation: At a median follow-up of 14 months, higher MIC-1/GDF15 levels at BL and preC2 were associated with shorter OS (BL; HR 1.2 95%CI 1.0–1.4; $P=0.03$ and preC2; HR 1.3 95%CI 1.1–1.5; $P=0.004$). Increase in MIC-1/GDF15 after chemotherapy correlated with lack of PSA response ($P<0.001$). IL4 and IL6 did not correlate with survival or demonstrate additional value.
External validation: At a median follow-up of 23 months, higher MIC-1/GDF15 levels at BL and preC3 predicted shorter OS (BL; HR 1.4 95%CI 1.2–1.6; $P<0.0001$ and preC3; HR 1.6 95%CI 1.3–1.8; $P<0.0001$). Higher pre C3 MIC-1/GDF15 levels were also associated with shorter time to PSA progression (HR 1.2 95% CI 1.0–1.4; $P=0.02$). Rise in MIC-1/GDF15 from BL to preC3 correlated with lack of 50% PSA fall at 12 weeks ($P<0.001$).
Conclusion
Adherence to a biomarker development pipeline provides level 2 evidence of the prognostic value of circulating MIC-1/GDF15 in men with mCRPC receiving docetaxel. A prospective biomarker led study is now necessary to establish clinical utility.

DOI: 10.1530/oncolabs.1.P032

P033

LobSig, a prognostic signature for ILC

Amy E McCart Reed[1], Samir Lal[1], Katia Nones[2], Jamie R Kutasovic[1], Craig P Coorey[1], Priyakshi Kalita de Croft[1], Andrew Dalley[1], Luyu Kuo[1], Leesa Wockner[2], Alan Robertson[3], Kaltin Ferguson[1], Colleen Niland[1], Mythily Sachchithananthan[1,6], Ana Cristina Vargas[1], Gregory Miller[1], Debra Black[1], Julie Johnson[1], Lynne E Reid[1], Renique Males[1], Irma Gresshoff[1], Alan Porter[4], Elizabeth Evans[4], Jodi M Saunus[1], Fares Al-Ejeh[2], John V Pearson[2], Georgia Chenevix-Trench[2], Lachlan Coin[3], Nicola Waddell[2], Sunil R Lakhani[1,5] & Peter T Simpson[1]
[1]UQ Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Herston, 4029, QLD, Australia; [2]QIMR Berghofer Medical Research Institute, Herston, Brisbane, Queensland 4006, Australia; [3]Institute for Molecular Bioscience, The University of Queensland, St Lucia, Brisbane, Queensland 4072, Australia; [4]Wesley Breast Clinic, Auchenflower, Queensland 4066, Australia; [5]Pathology Queensland, The Royal Brisbane & Women's Hospital, Herston, 4029, QLD, Australia; [6]Australian Breast Cancer Tissue Bank, Westmead, 2145, NSW, Australia.

Invasive lobular carcinoma (ILC) is the most common special type of breast cancer, and is characterized by functional loss of E-cadherin, resulting in cellular adhesion defects. ILC typically present as estrogen receptor positive, grade 2 breast cancers, with a good short-term prognosis. Several large-scale molecular profiling studies have now dissected the unique genomics of ILC. We have undertaken an integrative analysis of gene expression and DNA copy number to identify novel drivers, and prognostic biomarkers, using in-house ($n=25$), METABRIC ($n=125$) and TCGA ($n=145$) samples. Using in *silico* integrative analyses a 194-gene set was derived that is highly prognostic in ILC ($P=1.20\times10^{-5}$) – we named this metagene 'LobSig'. Network analysis identified few candidate pathways, though gene sets related to proliferation were identified, and a LobSig-high phenotype was associated with the TCGA proliferative group (c^2 $P<8.86\times10^{-4}$). Within a 10-year follow-up period, LobSig outperformed the Nottingham Prognostic Index, PAM50 risk-of-recurrence (Prosigna), OncotypeDx, and MapQuantDx (Genomic Grade Index) in a stepwise, multivariate Cox proportional hazards model, particularly in grade 2 cases ($\chi^2P=9.0\times10^{-6}$) which present most frequently and are difficult to prognosticate clinically. Importantly, LobSig status predicted outcome with 96.6% accuracy amongst cases classified as 'moderate risk' according to Nottingham Prognostic Index in the METABRIC cohort. LobSig is a clinically relevant prognostic signature which warrants future development.

DOI: 10.1530/oncolabs.1.P033

P034

Androgen receptor activation in Endocrine-Resistant ER-positive breast cancer

Heloisa Helena Milioli[1,2,*], Kee Ming Chia[1,2,*], Neil Portman[1,2], Aliza Yong[1,2], Gerald Tarulli[3], Luke Selth[3], Wayne Tilley[3,*], Theresa Hickey[3,*] & Elgene Lim[1,2,*]
[1]Garvan Institute of Medical Research, Sydney NSW Australia; [2]St Vincent's Clinical School, University of New South Wales, Sydney NSW Australia; [3]Dame Roma Mitchell Cancer Research Laboratories, The University of Adelaide, Adelaide SA Australia.
*These authors contributed equally to the work.

Steroid hormone receptors (SHR) play a major role in the normal breast development and breast cancer progression. Estrogen receptor (ER) is expressed in approximately 75% of breast cancers, and the majority of these tumours also express the androgen receptor (AR). While ER-directed therapies have been effective in the majority of patients, a significant subset develops resistance and requires alternative treatment approaches. In the endocrine-resistant setting, emerging insights into the role of androgen signalling have revived interest in AR-targeted therapies for novel pre-clinical studies and clinical trials. An understanding of AR activation in this setting is critical to improve the rational design of trials involving AR-directed therapies. In this study, we sought to characterize the AR downstream signalling pathway and assess the efficacy of AR-targeted agents in endocrine-resistant cell lines and patient-derived xenografts (PDXs) models. We performed RNA-Seq and ChIP-Seq analyses to interrogate the SHR dynamic at the genomic, transcriptomic and proteomic levels. Transcriptional profiling of AR-targeted tumours revealed gene signatures ($\sim$200 genes) associated with ER-regulated genes repertoire that predicts for better disease outcome (pval = 6e-10) in ER-positive breast cancer patients across METABRIC and ROCK public cohorts. AR agonists DHT and enobosarm (selective AR modulator) inhibited *in vitro* and *in vitro* tumour growth of endocrine-resistant MCF7 cells and two PDXs (Gar15-13 and HCI005) and demonstrated anti-proliferative (Hallmarks of G2M-checkpoint and E2F-targets) and anti-estrogenic (ER-targets) effects. These findings validate the utility of AR agonists in the treatment of endocrine-resistant ER-positive breast cancer, and further support the identification of biomarkers for novel AR-directed therapies and subsequent clinical trials.

DOI: 10.1530/oncolabs.1.P034

P035

Therapeutic targeting of Ezh2 enhances PD-1 blockade by induction of interferon gamma response

Anjali V Sheahan[1], Katherine L Morel[1], Deborah L Burkhart[1], Nadia Boufaied[2], Sukanya Panja[3], Carla Calagua[4], Ying Huang[1], Huihui Ye[4], Shana Y Trostel[5], Nichelle C Whitlock[5], Scott Wilkinson[5], Adam G Sowalsky[5], Adam S Kibel[6], Massimo Loda[1,7,8], Christopher J Sweeney[9], Antonina Mitrofanova[3], Stephanie K Dougan[10], David P Labbé[2], Brian M Olson[11] & Leigh Ellis[1,7,8]
[1]Department of Oncologic Pathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA; [2]Division of Urology, Department of Surgery, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; [3]Department of Health Informatics, Rutgers School of Health Professions, Rutgers Biomedical and Health Sciences, Newark, NJ, USA; [4]Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA, USA; [5]Laboratory of Genitourinary Cancer Pathogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; [6]Department of Urology, Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [7]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [8]The Broad Institute, Cambridge, MA, USA; [9]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA; [10]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA; [11]Department of Hematology and Medical Oncology, and Department of Urology, Emory University, Atlanta, GA, USA.

Prostate cancers are considered immunologically 'cold' tumors as they demonstrate poor response to check-point inhibitor therapy (CPI). Enrichment of interferon gamma (IFNγ) response genes, critical for innate and adaptive immune response to viral infections, have been demonstrated to indicate a positive response to CPI. Tumor IFNγ signaling acts as both an activator and inhibitor of effector T-cell response/trafficking via regulation of Th-1 chemokines (CXCL9/10), and immune checkpoints (PD-L1 and PD-1). Enhancer of zeste homolog-2 (EZH2) is a histone methyltransferase that mediates gene repression, is commonly over-expressed in prostate cancer and is known to negative regulate IFN response genes. With this, we hypothesized that inhibition of EZH2 would induce IFN gene response and potentiate prostate tumor response to CPI. EZH2 inhibition of 3D prostate tumor organoids significantly induced double-strand RNA and PD-L1 expression, and IFN response gene signatures. Generation of a novel EZH2 repression signature (EZH2_RS) was used to segregate prostate cancer patients, from three independent clinical cohorts, based on EZH2 activity. Correlation analysis confirmed that tumors with low EZH2 function had increased enrichment of IFNγ response and Th1 immune cell infiltration gene signatures, and PD-L1 gene expression. By employing a mixed lymphocytic reaction assay, we demonstrated that EZH2 inhibition significantly repressed splenocyte-mediated cytotoxic tumor elimination, which is rescued upon CPI. *In vivo,* the combination of EZH2 inhibition and CPI significantly slowed prostate tumor growth compared to control and single therapy arms. Collectively, our findings indicate EZH2 mediates prostate cancer immune evasion and its subsequent inhibition enables CPI response. This data provides strong rationale for further clinical development of this combination strategy for the treatment of prostate cancer.

DOI: 10.1530/oncolabs.1.P035

P036

Lipid elongation in prostate cancer: an androgen regulated process and a novel therapeutic target

Zeyad D Nassar[1,2,3], Margaret M Centenera[1,2,3], Jelle Machiels[4], Irene Zinonos[1,6], Adrienne Hanson[1], Katarzyna Bloch[4], Chui Yan Mah[1,2,3], Natalie K Ryan[1,3], Elizabeth D Williams[5], Andreas Evdokiou[1,6], Wayne D Tilley[1,2], Luke A Selth[1,2], Lisa M Butler[1,2,3] & Johannes V Swinnen[4]
[1]University of Adelaide Medical School, Adelaide, SA 5005, Australia; [2]Freemasons Foundation Centre for Men's Health, Adelaide, SA 5005, Australia; [3]South Australian Health and Medical Research Institute, Adelaide SA 5000, Australia; [4]KU Leuven- University of Leuven, LKI-Leuven Cancer Institute, Department of Oncology, Laboratory of Lipid Metabolism in Cancer, Leuven, Belgium; [5]Translational Research Institute, Queensland University of Technology, Brisbane QLD 4001, Australia; [6]Basil Hetzel Institute, Queen Elizabeth Hospital, Woodville South SA 5011, Australia.

Objective
Although initially effective, androgen deprivation therapy fails to achieve an enduring remission in patients with advanced prostate cancer (PCa) and the cells maintain active androgen receptor (AR) signalling. Hence, a detailed understanding of the AR-driven downstream processes that are required for tumour cell growth and survival, such as lipid metabolism, is essential to reveal new therapeutic targets. In this study, we aimed to evaluate the effect of androgens on the lipid profile of PCa cells, investigate the AR-dependent downstream pathways that mediate these changes, and evaluate their therapeutic potential as novel targets.
Methods
ESI-MS/MS-based lipidomics was used to assess lipid profiles in PCa cell lines, xenografts and patient-derived explants, and the effect of AR signalling on lipid profile. Chromatin immunoprecipitation (ChIP) and RT-PCR were used to validate AR regulation of key lipidomic enzymes, and their expression was modulated by siRNA and shRNA targeting. Tumour growth (orthotopic and subcutaneous) and metastasis was assessed in vivo using NOD/SCID mice.
Results
A complexity of changes in phospholipid profiles in response to androgen treatment was revealed. A consistent phenomenon of lipid elongation was observed for multiple phospholipid classes in response to androgen treatment, which was reversed by the antiandrogen, enzalutamide. Importantly, elongation of fatty acyl chains was also evident in clinical prostate tumors compared to non-malignant tissues. Potent and direct AR regulation of three enzymes that catalyze elongation (Elongation of Very Long Chain Fatty Acids) ELOVL2, 5 and 7 was demonstrated in prostate cancer cells, xenografts and clinical specimens. Targeting ELOVL5 (the most abundant ELOVL in clinical PCa) by siRNA or shRNA reversed the androgen-induced elongation phenotype, and significantly attenuated prostate cancer cell viability, adhesion, migration, 3D growth and in vivo tumor growth and metastasis.
Conclusions
These findings identify acyl chain elongation as a novel AR-regulated process, and an exciting new therapeutic target for prostate cancer.

DOI: 10.1530/oncolabs.1.P036

P037

Novel role of CBFβ as a regulator of breast cancer phenotype, progression and metastasis

A-M Money[1], J Schaverin[1], P Aubel[1], E Neist[1], T Owens[1], A Swarbrick[2], I Taniuchi[3], CJ Ormandy[2], PT Simpson[4] & MJ Naylor[1,2]
[1]School of Medical Sciences, University of Sydney, Sydney, NSW, Australia; [2]Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; [3]RIKEN Center for Integrative Medical Sciences, Yokohama City, Kanagawa, Japan; [4]Centre for Clinical Research, University of Queensland, Herston, Queensland, Australia.

Abstract unavailable.

DOI: 10.1530/oncolabs.1.P037

P038

Flicking the switch off, targeting MCL-1 in the treatment of breast and prostate cancer

L Castillo[1], S George[1], H-M Lin[1], N Yeung[1], A Mawson[1], AI Young[1], AM Law[1], J Hastings[1], D Gallego-Ortega[1,2], N Deng[1], D Croucher[1,2], A Swarbrick[1,2], L Horvath[3,4,5,6], CJ Ormandy[1,2] & SR Oakes[1,2]
[1]The Kinghorn Cancer Centre, Cancer Research Division, Garvan Institute of Medical Research, Darlinghurst, NSW, 2010, Australia; [2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Darlinghurst, NSW, 2010, Australia; [3]Chris O'Brien Lifehouse, Sydney, Australia; [4]Garvan Institute of Medical Research, Sydney, Australia; [5]University of NSW, Sydney, Australia; [6]University of Sydney, Sydney, Australia.

The ability to survive is essential for carcinogenesis. We previously showed that in to addition promoting cancer cell survival, Myeloid Cell Leukemia 1 (MCL-1)

regulates cancer cell invasion possibly via direct regulation of the cytoskeletal protein Cofilin and via modulation of the output of the SRC family kinase pathway. We also showed that dual inhibition of MCL-1 and SRC family kinases was effective in suppressing metastasis of breast cancer intraductal xenografts made from immortalized triple negative breast cancer cells. We are now exploring the potential of targeting MCL-1 alone or in combination with dasatinib and taxane-based cytotoxic therapy in clinically relevant models of metastatic breast and prostate cancer. Here we will report the findings from this study and the progress of a systems biology approach to predicting response.

DOI: 10.1530/oncolabs.1.P038

P039

Quest for the lost andromedin

Christopher Ong & Martin Gleave
Vancouver Prostate Centre, Vancouver, Canada.

The exquisite dependency of PCa on androgens for growth and survival was first recognized in the 1940's when Huggins and Hodges demonstrated the antitumour activity of hormonal manipulation in the treatment of PCa. Since then, androgen deprivation therapy has been the standard of care in the treatment of metastatic and locally advanced PCa. Drugs targeting the androgen/androgen receptor (AR) axis have been well-validated clinically and remain without a doubt the most effective class of therapies for treatment of advanced PCa. Despite the central role of AR pathway in PCa biology, the nature of these androgen-regulated genes that drive PCa growth/survival has been poorly elucidated. A first clue regarding the nature of androgen-regulated factors that mediate growth and survival came from the Cunha laboratory in the early 1970's who showed from tissue recombination studies that prostate development was dependent on reciprocal interactions between the epithelium and the mesenchyme of the urogenital sinus. Their discovery that hormonal effects on the epithelium were mediated by soluble secreted paracrine factors induced by androgens in mesenchymal/stromal cells naturally spawned the 'andromedin hypothesis' that the paracrine mediators may be secreted soluble *andro*gen-*medi*ated growth factors called andromedins. Andromedins are thought to diffuse from the stroma into the epithelial layers and orchestrate growth and differentiation of the prostate by binding to cognate epithelial receptors. Over the years, a number of growth factors have been implicated as andromedins such as FGF7, FGF10, and IGF1. However, since none of these are androgen-regulated, a true andromedin has remained elusive. In early 2000's, seminal work by Issacs found that the malignant transformation of normal prostatic epithelial cells is associated with a switch from a paracrine to an autocrine mechanism in androgen-stimulated growth. We have recently found that SEMA3C drives cancer growth by transactivating multiple receptor tyrosine kinases including EGFR, HER2 and MET via Plexin B1. Notably, we found that SEMA3C is a secreted, soluble autocrine growth factor in PCa and importantly combined with our findings that SEMA3C is transcriptionally induced by AR in a GATA2-dependent manner, these data together makes SEMA3C the first bona fide PCa andromedin to be identified. The identification of SEMA3C as an androgen-induced autocrine growth factor in PCa makes SEMA3C a promising new target for treatment of mCRPC.

DOI: 10.1530/oncolabs.1.P039

P040

Unravelling the role of cell plasticity in BrCa development and metastasis

Beatriz Perez San Juan[1,3], Soroor Zadeh[4,5], Vinod Ganju, Cristina Vargas, Sandra O'Toole, Elgene Lim[1,2,3], Melissa Davis[4,5] & Christine Chaffer
[1]The Kinghorn Cancer Centre & Cancer Research Division, Garvan Institute of Medical Research, Sydney, NSW, Australia; [2]St Vincent's Clinical School Faculty of Medicine, Sydney, NSW, Australia; [3]University of New South Wales, Sydney, NSW, Australia; [4]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; [5]The University of Melbourne, Melbourne, Victoria, Australia.

The plastic cancer cell model establishes that genetically identical cancer cells undergo bi-directional conversions between the highly aggressive tumour-initiating (TIC) state and the non-TIC cell state. We have identified subpopulations of breast cancer cells that readily switch from the non-TIC to TIC state, through activation of the EMT transcription factor ZEB-1. We have shown that non-TICs of basal BrCa are uniquely endowed with this plastic phenotype due to the cell's ability to maintain the chromatin at the ZEB-1 promoter in a poised state, ready for activation. This bivalent regulation confers non-TICs with the ability to convert toward more aggressive cellular states, acquiring metastatic and adaptive potential. Characterizing cellular plasticity in clinical samples, and the molecular networks underlying it, will allow us to better understand tumour progression, chemoresistance and recurrence. We are currently testing cell plasticity dynamics in human cell models and patient derived xenografts (PDXs), combining leading-edge genomic techniques (single cell transcriptomic analysis, RNA-seq and MINT-CHIP) with functional assays and *in vivo* models of tumorigenesis. By FACS (Fluorescence activated cell sorting) analysis we have observed matching profiles of TIC and non-TIC populations present in basal-like cell lines and triple negative BrCa PDXs. Using newly identified subpopulations we are currently defining the molecular network that controls non-TIC to TIC inter-conversions, as well as TICs evolution, by crosschecking epigenetic and transcriptomic data. Our preliminary results point to a role of the canonical and non-canonical Wnt/Notch signaling pathways, adhesion and immune system response signals in regulating the transition between cellular states. Through a clearer understanding of the mechanisms that drive non-TIC to TIC plasticity, we aim to discover novel therapeutic strategies that can target phenotypic switching to more aggressive cellular states, ultimately aiming to improve patient outcomes.

DOI: 10.1530/oncolabs.1.P040

P041

Extending genetic portraits of human prostate cancer

Richard Pestell
Pennsylvania Cancer and Regenerative Medicine Center, Baruch S Blumberg Institute, Pennsylvania, USA.

This study was conducted to define the role of Dachshund in prostate cancer, through assessing human prostate cancer samples and through genetic deletion in the mouse. Prostate cancer (PCa), the second leading cause of death in American men. A better molecular understanding of the disease is necessary in order to develop novel targeted therapies of metastatic PCa. Known genetic drivers to tumor initiation include PTEN and NKX3.1 deletions, rearrangements of the *TMPRSS2* gene to the oncogenic ETS transcription factor, *ERG,* and genetic predisposing factors include DNA-repair gene mutations. DACH1, initially cloned as an inhibitor of *Elipse* in *Drosophila*, was found to be reduced in abundance in several malignancies including breast and prostate cancer.

Results

Interrogation of the genomic sequence of prostate cancer from >490 patients from 5 population cohort showed homozygous deletion of DACH1 in 18% ($N=61$), 11% ($N=136$), 10% ($N=492$), 7% ($N=103$) and 3% ($N=150$). DACH1 gene deep deletions was more prevalent in the metastasis than in the primary tumors. AR activity levels (AR score derived from expression of AR target genes) showed a significant increase of AR score in the DACH1 deletion group as compared to Normal ($P=2\times10^{-5}$ by t-test) and ERG1 mutation groups ($P=0.003$ by *t*-test). The Transgenic Adenocarcinoma Mouse Prostate (TRAMP) transgenic, *Dach1*$^{fl/fl}$, and Probasin-Cre, *ROSA26*$^{mT/mG}$ transgenic mice were used to generate a prostate epithelial cell specific *Dach1* gene knockout mouse (Probasin-Cre-*Dach1*$^{fl/fl}$ *ROSA26*$^{mT/mG}$-TRAMP) lines. Prostate specific deletion of the murine *Dach1* gene enhanced progression of prostatic intraepithelial neoplasia (PIN), associated with increased prostate epithelial cell proliferation, epithelial mesenchymal transition (EMT), DNA damage and inflammation. DACH1 bound and restrained the AR and was recruited to ARE in a casodex-dependent manner.

Conclusions

DACH1 gene deletion may define a distinct subclass of prostate cancer that may benefit from PARP inhibitors, and platinum-based chemotherapy.

DOI: 10.1530/oncolabs.1.P041

P042

PARP inhibitor and CX-5461 combination therapy as a novel treatment strategy for castrate-resistant prostate cancer

Laura H Porter[1], Mitchell G Lawrence[1,2], Nicholas K Choo[1], Shahneen Sandhu[2], Hong Wang[1], Melbourne Urological Research Alliance (MURAL)[1], David Pook[1], Elaine Sanij[2], Richard B Pearson[2], Ross Hannan[2], Luc Furic[2], Renea A Taylor[1,2] & Gail P Risbridger[1,2]

[1]Monash University, Clayton, Australia; [2]Peter MacCallum Cancer Centre, Melbourne, Australia.

Abstract unavailable.

DOI: 10.1530/oncolabs.1.P042

P043

Activation of p53 in combination with endocrine and CDK targeted therapies in ER+ breast cancer

Neil Portman[1], Heloisa Milioli[1], Aliza Yong[2], Rhiannon Coulson[2], Sarah Alexandrou[1], Natasha Lam[2], Sue Haupt[3], Ygal Haupt[3], C Elizabeth Caldon[1] & Elgene Lim[1]
[1]Garvan Institute of Medical Research and St Vincent's Clinical School, Darlinghurst NSW, Australia; [2]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; [3]Peter MacCallum Cancer Centre, Melbourne, Australia.

Estrogen Receptor (ER) signalling, upregulation of the cyclin/CDK pathway, and suppression of p53 form a critical axis controlling proliferation of ER positive breast cancer. In this setting, mutation of p53 is relatively rare and suppression of p53 function can be achieved via regulators MDM2 and MDMX. Activation of p53 by inhibition of MDM2 is a promising therapeutic target in p53 wildtype tumours and several drugs are currently in clinical trials. We hypothesised that the MDM2 inhibitor NVP-CGM097 (Novartis, Phase I) will synergise with treatments that target ER signalling and cyclin/CDK activity by disrupting the complex feedback mechanisms that promote cell cycle entry and growth in ER positive breast cancer. We investigated the activity of CGM097 *in vitro* and *in vivo* in combination with selective ER degraders (SERDs). We show that CGM097 is an effective monotherapy *in vitro* and that response depends upon p53 status. CGM097 synergises with SERDs to inhibit proliferation, causing downregulation of cell cycle associated transcripts, cell cycle arrest, senescence and apoptosis. *In vivo*, CGM097 is as effective as endocrine therapy in an endocrine sensitive breast cancer PDX and resensitises an endocrine resistant PDX to endocrine therapy. CDK4/6 inhibitors are poised to become the new standard of care for advanced ER positive breast cancer. Using *in vitro* models of treatment naïve and CDK4/6-inhibitor resistant breast cancer, we show that CGM097 synergises with CDK4/6 inhibitors to strongly reduce proliferation and proliferation-associated transcripts in the treatment naïve setting; and causes cell cycle arrest and an accumulation of markers of senescence in models of Palbociclib resistance. In conclusion, MDM2 inhibition suppresses several proliferative pathways, including those deregulated in the acquisition of treatment resistance, and offers a rational therapeutic option for treating advanced and treatment resistant ER positive breast cancer.

DOI: 10.1530/oncolabs.1.P043

P044

Epithelial mesenchymal transition, stromal density, and chemo-resistance in breast cancer (BrCa)

Andrew Redfern[1], Veenoo Agarwal[1], Lisa Spalding[2], Tony Blick[3], Alexander Dobrovic[4] & Erik W Thompson[4]
[1]Fiona Stanley Hospital, Perth, Australia; [2]The University of Western Australia, Perth, Australia; [3]Queensland University of Technology, Brisbane, Australia; [4]Olivia Newton-John Cancer Research Institute, Melbourne, Australia.

Introduction
The process of Epithelial Mesenchymal Transition (EMT) involves the transition of cells from a differentiated epithelial phenotype to a less differentiated mesenchymal phenotype. Mammographic breast density (MBD) refers to the proportion of high opacity area on a mammogram. EMT may be triggered in cancer cells by a range of therapies including cytotoxic chemotherapy, with cell line and animal suggesting chemoresistance may result. High MBD in patients being treated for BrCa also associates with chemoresistance, correlating with lower pathological complete response rates (pCR) in a pilot study although impact on longer term outcomes was not reported[1]. Linking these two stimuli, EMT can also be induced by artificial high-density stroma, where it also leads to chemoresistance *in vitro*[2].
Aims
Here we set out to explore the link between poor outcome after NAC and EMT in a clinical patient cohort, and to ascertain the molecular drivers through which EMT is triggered in this setting. Further we looked to confirm the association of high MBD with poor chemoresponse, and to assess whether this chemoresistance is mediated through EMT with the same drivers.
Key Findings
In a pilot cohort of 50 NAC-treated locally advanced BrCas, development of EMT correlated with a significant increase in mortality (78 v 25%, $P=0.03$). In a subsequent 240-patient cohort MBD higher percent breast density divided by tertile correlated with trends to inferior clinical shrinkage (58 v 40%, $P=0.08$) and higher relapse rate (35 v 22%, $P=0.05$). EMT induction is being assessed and correlated with both breast density and outcome in this second cohort. On a subgroup of 50 patients within the second cohort a broad nanostring assay has looked at expression changes transcription factors known to drive EMT (EMT-TFs), to ascertain which factors control EMT in the context of either chemoresistance and/or high breast density. All EMT-TFs measured were numerically more strongly induced in relapsing patients, the change reaching significance for Snail-3 (OR=1.8, $P=0.04$) and borderline significance for TWIST-1 (OR=2.4, $P=0.08$). Validation of links between Snail-3 and Twist-1 protein expression with EMT in the full 240 patient cohort is underway.
Implications
Both high MBD and EMT correlate with chemoresistance with a mechanistic association between MBD and EMT being explored. Specific EMT-TFs are implicated, targeting of which could attenuate chemoresistance.
References
1. Elsamany *et al*. *Breast*. 2015 Oct;24(5):576–581
2. Raviraj *et al*. *Clin Exp Metastasis* 2012, 29(3):273–292.
DOI: 10.1530/oncolabs.1.P044

P045

Single-cell transcriptomics reveals marked heterogeneity for intrinsic molecular subtype and cellular function in estrogen receptor positive breast cancer

Daniel Roden[1,2], Sunny Wu[1,2], Kate Harvey[1], Chia-Ling Chan[1], Ghamdan Al-Eryani[1,2], Aurelie Cazet[1,2], Elgene Lim[1,2] & Alexander Swarbrick[1,2]
[1]The Kinghorn Cancer Center and Cancer Research Division, Garvan Institute of Medical Research, Sydney, Australia; [2]St Vincent's Clinical School, Faculty of Medicine, UNSW, Sydney, Australia.

Breast cancer is a heterogeneous disease that can be classified into a number of molecular subtypes that predict prognosis and influence clinical treatment. Cellular heterogeneity is also evident within breast cancers and plays a key role in their development, evolution and metastatic progression. How clinical heterogeneity relates to cellular heterogeneity is poorly understood. We have approached this question using single-cell RNA-Seq on 1000s of individual cells from well-established *in vitro* and *in vivo* models, as well as clinical samples of Estrogen Receptor positive (ER+) breast cancer. Supervised and unsupervised approaches have identified cellular populations with transcriptional signatures of diverse cancer associated phenotypes, including proliferation, hypoxia and treatment resistance. In particular, distinct sub-populations of cells with a heterogeneous mix of molecular subtypes and signatures suggesting innate resistance to endocrine therapies have been identified. Gene regulatory networks were then used to identify transcription factor regulons that are active in individual cells, leading us to identify potential transcriptional drivers (such as: KLF5 and E2F7) of the putative endocrine resistant cells. This approach has been extended into a number of clinical ER+ breast cancers, highlighting a complex ecosystem of tumour-associated cells and identified a heterogeneous mix of epithelial cells expressing transcriptional markers of both luminal and basal cells. This is a somewhat confounding finding in ER+ breast cancers and highlights the potential power of single-cell approaches to identify specific cellular populations that could contribute to malignancy or relapse following treatment. Overall, our results suggest a high degree of cellular heterogeneity within breast cancers that can be functionally dissected into sub-populations with transcriptional phenotypes of potential clinical relevance. In particular, the identification of cells associated with treatment resistance hints at ways in which single-cell

genomics could be used to predict and track variable treatment response and resistance during breast cancer treatment.

DOI: 10.1530/oncolabs.1.P045

P046

A miR-194-regulated transcriptional network is associated with progression to androgen receptor-independent prostate cancer

Rayzel C Fernandes[1,2], Kate Dredge[3], Andrew G Bert[3], John Toubia[3], Katherine A Pillman[3], Philip A Gregory[3], Theresa E Hickey[1], Wayne D Tilley[1,2], Gregory J Goodall[3] & Luke A Selth[1,2]
[1]Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, The University of Adelaide, Adelaide, SA 5005, Australia; [2]Freemasons Foundation Centre for Men's Health, Adelaide Medical School, The University of Adelaide, Adelaide, SA 5005, Australia; [3]Centre for Cancer Biology, University of South Australia, Adelaide, SA 5000, Australia.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression programs and have a critical role in both normal biology and disease. We previously identified microRNA-194 (miR-194) as an important driver of prostate cancer metastasis, although the molecular mechanisms by which it mediates these effects are not well understood. This study aimed to identify target genes and pathways that are responsible for miR-194's pro-metastatic activity. By integrating transcriptomics with a cutting-edge molecular technique that delineates miRNA:mRNA interaction sites, HITS-CLIP (high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation), we characterised the complete set of miR-194 target genes (its 'targetome') in prostate cancer cells. MiR-194 targets approximately 160 genes in prostate cancer - predominantly through canonical binding to 3'UTR regions - many of which are involved in key metastatic pathways. Interestingly, miR-194 activity was inversely correlated with androgen receptor (AR) activity in clinical metastatic cohorts, an observation explained mechanistically by AR-mediated repression of miR-194 expression. In concordance with these findings, miR-194 activity is significantly elevated in neuroendocrine prostate cancer (NEPC) and double-negative prostate cancer (DNPC), both of which are aggressive AR-independent subtypes. Interestingly, miR-194 enhanced trans-differentiation of epithelial LNCaP cells to neuroendocrine-like cells, a function mediated at least in part by miR-194 targeting of FoxA1, a critical regulator of AR signalling. Importantly, targeting miR-194 in aggressive models of NEPC effectively inhibited cell growth. Overall, our study provides new insights into miR-194 function in prostate cancer progression, cancer cell plasticity and the emergence of aggressive AR-independent disease subtypes.

DOI: 10.1530/oncolabs.1.P046

P047

IL6/STAT3 co-opts ER regulatory elements to drive metastasis in breast cancer

Rasmus Siersbæk[1], Valentina Scabia[2,*], Igor Chernukhin[1,*], Eva Papachristou[1], Rebecca Broome[1], Andrew R Green[3], Stacey Joosten[4], Sanjeev Kumar[1,5], Ruben Alvarez[1], Sankari Nagarajan[1], Silvia Glont[1], Soleilmane Omarjee[1], Sarah Aitken[1,5], Kamal Kishore[1], Emad Rakha[3], Clive D'Santos[1], Wilbert Zwart[4], Alasdair Russell[1], Cathrin Brisken[2] & Jason S Carroll[1]
[1]Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge CB2 0RE, UK; [2]ISREC – Swiss Institute for Experimental Cancer Research, School of Life Sciences, Ecole polytechnique fédérale de Lausanne (EPFL), SV2.832 Station 19, 1015 Lausanne, Switzerland; [3]Nottingham Breast Cancer Research Centre, Division of Cancer and Stem Cells, Nottingham University and Nottingham University Hospitals NHS Trust, Nottingham City Hospital, Nottingham NG5 1PB, UK; [4]Division of Oncogenomics, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands; [5]Addenbrookes Hospital, Cambridge CB2 0QQ, UK.
*Equal contribution.

Interleukin 6 (IL6) signaling has been associated with an aggressive and metastatic phenotype in multiple solid tumors including breast cancer, but its mechanism of action in mediating tumor progression and treatment response is not clear. By exploiting a clinically relevant intraductal xenograft model of estrogen receptor positive (ER+) breast cancer, we demonstrate that IL6 increases both primary tumor growth and distant metastases. By integrating pre-clinical models and clinical specimens, we show that signal transducer and activator of transcription 3 (STAT3) mediates IL6-induced activation of a metastatic gene program from enhancer-elements shared with ER and its pioneer factor FOXA1. Although IL6 activated STAT3 and ER/FOXA1 share cis-regulatory regions, STAT3 drives transcription independent of ER and FOXA1 function, and the IL6/STAT3 gene program is not influenced by ER-targeted therapies, decoupling these two important pathways. This demonstrates that ER/FOXA1 and IL6/STAT3 are two parallel, but independent actionable pathways controlling breast cancer progression.

DOI: 10.1530/oncolabs.1.P047

P048

Exploring the clinical significance of interactions between oestrogen and progesterone receptors in breast and endometrioid adenocarcinomas by proximity ligation assay

Cameron E Snell[1,2], Deborah Smith[1,2], Madeline Gough[1,2], Cheng Liu[2], Kathryn Middleton[3,5], Christopher Pyke[4,5], Catherine Shannon[3,5], Natasha Woodward[3,5], Theresa E Hickey[6], Jane E Armes[2] & Wayne D Tilley[6]
[1]Cancer Pathology Research Group, Mater Research Institute – The University of Queensland, Translational Research Institute, Queensland, Australia; [2]Department of Anatomical Pathology, Mater Pathology, Mater Hospital, Brisbane, Australia; [3]Department of Medical Oncology, Mater Hospital, Brisbane, Australia; [4]Department of Breast and Endocrine Surgery, Mater Hospital, Brisbane, Australia; [5]Mater Research Institute – The University of Queensland, Translational Research Institute, Brisbane, Australia; [6]Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, Australia.

Recent pre-clinical studies indicate that activated progesterone receptor (PR) (particularly the PR-B isoform) binds to oestrogen receptor-α (ER) and reprograms transcription toward better breast cancer outcomes. We investigated whether ER and PR interactions were present in breast and endometrial tumours and associated with clinical parameters including response to endocrine treatments. We developed a proximity ligation assay to detect ER and PR interactions in formalin-fixed paraffin-embedded tissues. The assay was validated in a cell line and patient-derived breast cancer explants. The assay was applied to a cohort of 229 patients with ER-positive and HER2-negative breast cancer with axillary nodal disease and another cohort of 100 patients with early-stage endometrioid adenocarcinoma treated with a levonorgestrel-releasing intrauterine device (Mirena). In breast cancer, a higher frequency of ER:PR-B interaction correlated with increasing patient age, lower tumour grade and mitotic index. A low frequency of ER:PR-B interaction was associated with higher risk of relapse. In multivariate analysis, ER:PR-B interaction frequency was an independent predictive factor for relapse, whereas PR expression was not. In subset analysis, low frequency of ER:PR-B interaction was predictive of relapse on adjuvant aromatase inhibitor (HR 4.831, $P=0.001$), but not on tamoxifen (HR 1.043, $P=0.939$). Results of the endometrioid adenocarcinoma cohort are to be detailed during the meeting. This study demonstrates that ER:PR-B interactions have utility in predicting patient response to adjuvant AI therapy in breast cancer. ER and PR interactions are potentially associated with response to progestogen in endometrial cancer.

DOI: 10.1530/oncolabs.1.P048

P049

DNA demethylation agents as a therapeutic approach in endocrine-resistant breast cancer

Clare Stirzaker[1,2], Kee Ming Chia[3], Neil Portman[3], Heloisa Helena Milioli[3], Samuel Clifton[1], Joanna Achinger-Kawecka[1], Shalima Nair[1], Elgene Lim[2,3] & Susan J Clark[1,2]
[1]Epigenetics Research Laboratory, Genomics and Epigenetics Division, Garvan Institute of Medical Research, Sydney, Australia; [2]St Vincent's Clinical School, UNSW Sydney, Sydney, Australia; [3]The Kinghorn Cancer Center, Darlinghurst, Sydney, Australia.

Seventy percent of breast cancers are classified as estrogen-receptor positive (ER+) and ER is the key proliferative driver in these tumours. Clinically, ER+ patients receive ER-targeted (endocrine) therapies to inhibit ER activity and whilst these agents reduce the risk of recurrence, up to 43% patients develop drug

resistance within 15 years. Hence, identification of mechanisms underlying these resistant mechanisms could extend the use of endocrine-therapies. Profound alterations to the genome-wide DNA methylation landscape occur in the early stages of cancer and continue to alter throughout the acquisition of drug resistance. We have previously identified DNA hypermethylation as an important contributor to endocrine-resistance resulting in reduced ER binding and decreased gene expression of key regulators of ER-activity (Stone *et al*., Nat Comms 2015). Here, we aim to determine whether DNA demethylation agents may be efficacious in reversing endocrine-resistance and restoring sensitivity to endocrine therapy. As a pilot study we have evaluated the efficacy of decitabine, a DNA methyltransferase inhibitor, in combination with endocrine-therapies on the growth of endocrine-resistant patient-derived xenograft (PDX) models (Gar15-13 and HCI-005). Our results demonstrate that decitabine treatment alone reduced the proliferation of these PDX models, with decitabine further augmenting the effect of endocrine-therapies. At end point harvested tumours were assessed for genome-wide methylation alterations using the Illumina MethylationEPIC microarray. We show that decitabine treatment induces DNA demethylation, enriched primarily at promoter and enhancer elements. Furthermore, gene expression profiling indicates an elevation in ER signaling and this increase in ER activity by decitabine may underlie the added efficacy when endocrine-therapy and decitabine are combined. Overall our results provide promise for the potential efficacy of demethylation agents in a preclinical model. Further work is currently being done in different endocrine-resistant patient-derived xenograft models to determine if this may be a suitable therapeutic approach for endocrine resistant ER+ breast cancer.

DOI: 10.1530/oncolabs.1.P049

P050

Preclinical development of CDDD3-14, a potent and selective inhibitor of CDK4/6 for the treatment of breast cancer

Laychiluh Bantie, Solomon Tadesse, Jimma Likisa, Mingfeng Yu, Benjamin Noll, Gary Heinemann, Robert Milne, Hugo Albrecht & Shudong Wang
Drug Discovery and Development, University of South Australia Cancer Research Institute, Adelaide, Australia.

Deregulation of the CDK4/6-cyclin D-Rb-E2F pathway is common in subtypes (e.g. ER+/HER2−) of breast cancer, and activation/amplification of cyclin D1 (CCND1) and CDK4/6, or deletion/mutation of CDKN2A gene that encodes p16INK4a are the major mechanisms. Aberration in the upstream pathways such as PI3K/Akt/mTOR can also lead to the deregulation of the CDK4/6 axis, which drives carcinogenesis and development of resistance to therapies. Therefore, inhibition of CDK4/6 is a rational approach for effectively treating breast cancer. We have identified a highly potent inhibitor of CDK4/6, CDDD3-14, that showed excellent selectivity for CDK4/6 over a panel of >360 human kinases. It held the growth and proliferation of Rb-proficient cancer cell lines, including those cancers of breast, colon, prostate, pancreatic, lung and melanoma, and was more potent than palbociclib, a FDA-approved CDK4/6 inhibitor. CDDD3-14 arrested breast cancer cells in G1 phase of the cell cycle, prevented their colony formation and induced senescence. It blocked the phosphorylation of Rb protein and inhibited the E2F-transcription programs leading to reduced level of cyclins E2, A2, B1, TS and TOPOIIα. Moreover, CDDD3-14 possessed high oral bioavailability, and demonstrated marked in vivo anti-tumor efficacy in a MCF-7 breast cancer xenograft model ($T/C = 18\%$, $P < 0.0001$) without causing any histopathological changes to animal organs including blood, bone marrow, intestine, liver, heart and kidneys of animal. In conclusion, we have identified CDDD3-14 as a highly potent and selective inhibitor of CDK4/6 that is highly efficacious in preclinical breast tumor models. Our data suggest that CDDD3-14 is a highly promising drug candidate for the treatment of cancers.

DOI: 10.1530/oncolabs.1.P050

P051

Targeting HP1-alpha for prevention and treatment of neuroendocrine prostate cancer

Xinpei Ci, Dong Lin, Colin Collins, Nada Lallous, Michael Hsing, Art Cherkasov & Yuzhuo Wang
Department of Urologic Sciences, Vancouver Prostate Centre, UBC, Canada.

NEPC is a lethal subtype of PCa frequently arising from adenocarcinoma via NE transdifferentiation following ADT. In AACR-PCF West Dream Team series of sequential biopsies of over 300 CRPC biopsies, NEPC was discovered in 17% of cases, making neuroendocrine transdifferentiation one of the most common mechanisms underlying ADT resistance. However, a mechanistic understanding of both NEPC development and its aggressiveness remain elusive. Research in this field has been hampered by a lack of relevant preclinical cancer models. We have developed a panel of unique, clinically-relevant PCa PDX models, including the first-in-field PDX model of complete transdifferentiation of prostatic adenocarcinoma (LTL331) to NEPC (LTL331R). Using transcriptomic analyses in these models, we have identified a heterochromatin gene signature in NEPC. Longitudinal analysis of the LTL331/331R model revealed that among those heterochromatin-related genes, HP1α expression is increased early, rises steadily during NEPC development, and remains elevated in fully developed NEPC. Its elevated expression is further confirmed in clinical NEPC samples. HP1α knockdown dramatically inhibits NEPC cell proliferation, completely ablates colony formation, and induces apoptotic cell death, ultimately leading to tumor growth arrest. Its ectopic expression significantly promotes NE transdifferentiation in adenocarcinoma cells. Mechanistically, HP1α reduces expression of AR and REST, two crucial transcription factors silenced in NEPC, by enriching the repressive histone mark H3K9me3 on their respective gene promoters. These observations indicate a novel mechanism underlying NEPC development mediated by abnormally expressed heterochromatin genes, with HP1α as an early functional mediator and a novel therapeutic target in NEPC. Subsequently, we have developed small molecule inhibitors (SMIs) of HP1α using an in silico drug discovery pipeline. This SMI series is actively under preclinical development. Significance: Heterochromatin proteins play a fundamental role in NEPC, illuminating new therapeutic targets for this aggressive disease, accordingly new SMIs are on the way to come.

DOI: 10.1530/oncolabs.1.P051

P052

Novel and highly selective CDK9 inhibitors suppress proliferation of triple negative breast cancer (TNBC) cells *in vitro*

Jean M Winter[1], Ebtihal H Mustafa[1], Shudong Wang[2], Luke A Selth[1], Theresa E Hickey[1] & Wayne D Tilley[1]
[1]Dame Roma Mitchell Cancer Research Laboratories, Adelaide Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, SA 5000, Australia; [2]School of Pharmacy and Medical Sciences, The University of South Australia, Adelaide, South Australia, SA 5000, Australia.

This study evaluates the efficacy of two newly developed selective CDK9 inhibitors (CDK9i) across a panel of TNBC cell lines. MDA-MB-453, MFM-223, MDA-MB-468 and MDA-MB-231 TNBC cells were treated with increasing concentrations of two novel and highly selective CDK9 inhibitors and the effect on proliferation, apoptosis and expression of CDK9 targets determined. MDA-MB-453 and -468 cells showed significant growth inhibition with as little as 150 nM of CDK9i, evident 3 days after commencement of treatment. Both MDA-MB-231 and MFM-223 cells were less sensitive to the CDK9 inhibitors, with MDA-231 cells requiring at least 300 nM to suppress growth. MFM-223 cells did not demonstrate any growth inhibition after 7 days of culture with CDK9i concentrations up to 1.2 μM. Protein expression of CDK9 targets, including RNA Polymerase II (RNAPII), phosphorylated-RNAPII, the proto-oncogene C-MYC, and apoptotic marker cleaved caspase-3, were examined by Western blot after optimal CDK9i exposure across each cell line. CDK9i suppressed phosphorylated-RNAPII, but not total RNAPII, indicative of targeted CDK9 inhibition. The master transcription factor C-MYC, which is highly expressed in TNBC, was downregulated, and cleaved-caspase-3 was upregulated with CDK9i treatment. These data demonstrate cell specific efficacy of novel CDK9 inhibitors in cell line models of TNBC via transcriptional suppression of proto-oncogenes and upregulation of apoptotic pathways. Future studies will identify molecular markers of response to CDK9 inhibition and evaluate these novel inhibitors in TNBC patient derived xenografts.

DOI: 10.1530/oncolabs.1.P052

Author Index