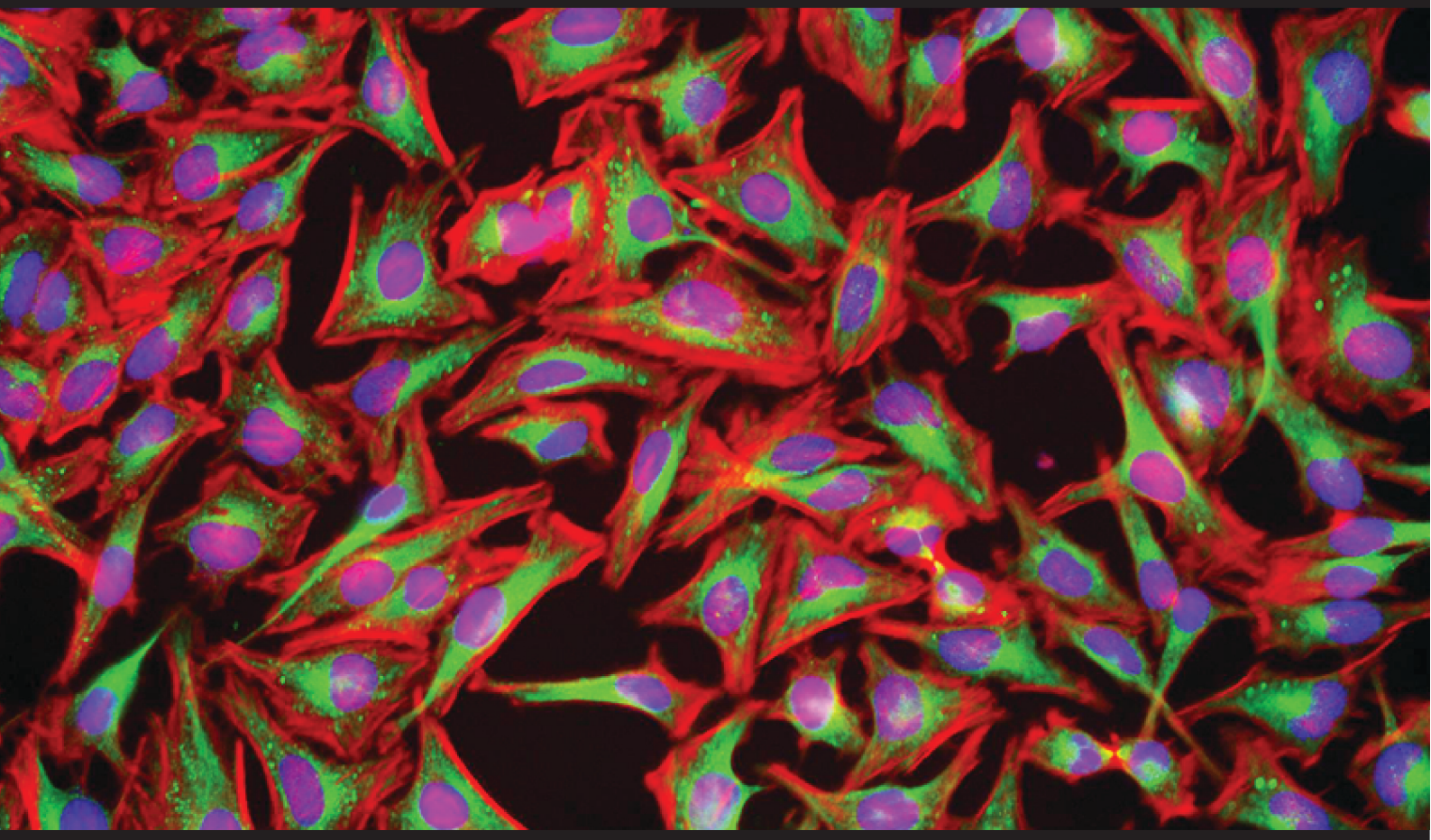
FHTTA 2018 Meeting

Program and Abstract Booklet

October 24-25th 2018
The John Curtin School of Medical Research
The Australian National University
Canberra, Australia



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Program – Day 1 (24th October)

Day 1: Wednesday 24th October

8:50-9:00 Conference Welcome (including Acknowledgement of Country)

SESSION 1: Introduction to High Content Screening and Imaging Analysis

9:00-9:45 Introduction to High Content Screening: from Imaging to Analysis
Kaylene Simpson (Peter Mac, VIC)

9:45-10:20 High-Content Phenotypic Screens: From Live Cells to Machine Learning
Keynote: Myles Fennell (Memorial Sloan Kettering Cancer Centre, USA)
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10:20-10:50 **Morning Tea (JCSMR Foyer)**

10:50-11:35 Machine Learning in Phenotypic Pre-Screening, Recognition, and Classification of Bacteria
Keynote: Bartek Rajwa (Purdue University, USA)
Sponsored by FHTTA

11:35-11:55 A High Throughput Approach to Identifying Compound Inhibitors of mRNA Processing and Export
Kirsty Carey (Peter Mac, VIC)

11:55-12:15 Identification of Novel FDA-Approved Inhibitors of Zika Virus Infection
Amanda Aloia (Flinders University, SA)

12:15-12:35 Identification of Therapeutic Targets for the Treatment of Diamond Blackfan Anaemia Using a High-Throughput Screening-Based Approach
Amee George (Australian National University, ACT)

12:35-13:35 **Lunch (JCSMR Foyer)**

Program – Day 1 (24th October)

SESSION 2: 3D Screening

13:35-14:10 Dynamic 3D Imaging of Patient-Derived Tumour Organoids for Drug Screening Applications
Keynote: Shannon Mumenthaler (University of Southern California, USA)
Sponsored by Perkin Elmer

14:10-14:45 High-Content Screening in Human Cardiac Organoids Identifies Key Proliferative Pathways Without Functional Side-Effects
Enzo Porrello (Murdoch Children's Research Institute, VIC)

14:45-15:15 **Afternoon Tea (JCSMR Foyer)**

15:15-15:35 3D Bioprinting of HTP Tumour Spheroids for Precision Medicine
Lakmali Atapattu (Children's Cancer Institute Australia, NSW)

15:35-15:55 Drug Discovery Phenomics
Tayner Rodriguez (Griffith Institute, QLD)

15:55-16:40 **Open discussion on the Future of HCS in Australia**
Sponsored by the Australian Phenomics Network
Moderated by Michael Dobbie – CEO

17:00-18:30 **Networking Cocktail Function (JCSMR Foyer)**
Sponsored by Tecan

Program – Day 2 (25th October)

Day 2: Thursday 25th October

8:55-9:00 Conference Messages

SESSION 3: CRISPR Screening

9:00-9:35 Synthetic CRISPRs: Novel Approaches to Study Gene Function
Iva Nikolic (Peter Mac, VIC)

9:35-10:10 Unravelling Biology and Identifying Targets with Functional Genomics Approaches Supported by LentiArray CRISPR Library Screens
Keynote: Jonathan Chesnut (ThermoFisher Scientific, USA)
Sponsored by ThermoFisher Scientific

10:10-10:40 **Morning Tea (JCSMR Foyer)**

10:40-11:15 Powering the Synthetic Biology Revolution
Keynote: Reed Hickey
Sponsored by Twist Bioscience

11:15-11:35 Defining Novel Modifiers of the Cellular Response to Unedited Endogenous dsRNA
Jacki Heraud-Farlow (SVI, VIC)

11:35-11:55 A whole genome CRISPR screen to identify mechanisms that determine resistance and sensitivity to the mutant p53 reactivator, APR-246
Nick Clemons (Peter Mac, VIC)

11:55-12:15 Unlocking HDR-mediated Nucleotide Editing by identifying high-efficiency target sites using machine learning and high throughput screening
Gaetan Burgio (Australian National University, ACT)

12:15-13:15 **Lunch (JCSMR Foyer)**

Program – Day 2 (25th October)

SESSION 4: Image Analysis Workshop

13:15-13:45 Microscopium: Interactive Exploration of Large Imaging Datasets for Unbiased Phenotype Discovery
Genevieve Buckley (Monash Micro Imaging, VIC) *Sponsored by FHTTA*

13:45-14:15 Mapping Phenotypic Plasticity in the Actin Cytoskeletal System via Unbiased Chemical Screening
John Lock (UNSW, NSW) Sponsored by *FHTTA*

14:15-14:35 Multilabelling – Highly Multiplexed, High-Content Confocal Imaging
Volker Hilsenstein (Monash Micro Imaging, VIC)

14:35-14:55 Using High-Content Time-Lapse Imaging to Investigate Effects of Oncogenic Mutations in Melanocyte-Keratinocytes-Fibroblast Interaction
Duka Skalamera (MMRI, University of Queensland, QLD)

14:55-15:25 **Afternoon Tea (JCSMR Foyer)**

SESSION 5: Emerging Technologies

15:25-16:00 The Promise of siRNA Therapeutics
Keynote: David Evans (Sirnaomics, USA)
Sponsored by Millennium Science

16:00-16:20 Intelligent Image-Activated Cell Sorting
Nao Nitta (Japan Science and Technology Agency, Japan)

16:20-16:55 Utilisation of High Throughput Cytometers to Expand Screening Capabilities
Keynote: Jennifer Smith (Harvard University, USA)
Sponsored by FHTTA

16:55-17:00 **Conference Wrap Up and Close**

Introduction to High Content Screening: from Imaging to Analysis

Kaylene Simpson
Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre

This introductory presentation will cover a basic understanding of quantitative high content imaging and analysis and offer a number of practical considerations to factor in when preparing to work in this field.

Keynote: High-Content Phenotypic Screens: From Live Cells to Machine Learning

Myles Fennell

Memorial Sloan Kettering Cancer Centre, USA

Oncogene-induced senescence is a potent barrier to tumorigenesis that limits the expansion of pre-malignant cells. A unique form of immune surveillance involves a secretory program referred to as the senescence-associated secretory phenotype (SASP). Among their many functions, SASP proteins mobilize immune cells and modify their activities, for example, by recruiting natural killer (NK) cells or altering macrophage polarization to kill and/or engulf senescent cells. We have developed a live-cell imaging assay for immune cell mediated targeting of premalignant senescent cells. Using multi-parametric image analysis, we quantified the chemotaxis and clumping of NK cells around the senescent cells, in addition to cell death, cell number and morphology of both populations. This assay was used to screen a panel of bioactive compounds. The potential impact of this work is to discover mechanisms whereby NK cells can be made to recognize and kill tumours and also activate recognition of tumours by NK cells.

TFEB is a transcription factor and master regulator of lysosomal biogenesis leading to protein degradation; important in pathogenesis of neurodegeneration and cancer. We used this model to screen small molecule and siRNA libraries and highlight various methods for hit identification and stratification using conventional and machine-learning techniques.

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Keynote: Machine Learning in Phenotypic Pre-Screening, Recognition, and Classification of Bacteria

Bartek Rajwa

Purdue University, USA

The majority of tools for enumeration, recognition, and classification of microorganisms are based on the physiological or genetic properties. However, there is also an enormous interest in devising label-free, reagent-less, rapid phenotypic methods that would operate utilizing the biophysical signatures of microbial samples without the need for labeling and reporting biochemistry. Such techniques could find use in biosurveillance, sterility testing, drug development, compound screening, water quality assurance and various other fields. Raman spectroscopy, LIBS, and MALDI-TOF are examples of such approaches. Elastic light scattering (ELS) – one of the most fundamental optical processes whereby electromagnetic waves are forced to deviate from a straight trajectory by non-uniformities in the medium that they traverse – can be employed to provide a very inexpensive and accurate implementation of the label-free phenotypic screening concept. The ELS screening system may operate independently or be employed within the context of established microbiological assays. The presented work demonstrates a rapid data processing pipeline for colony screening and selection, taking advantage of machine-learning (ML) and computer-vision tools for discovery and classification of ELS patterns formed by interaction between laser light and colony structures. Owing to reproducible morphological differences in internal colony organization, the method can robustly classify the samples on the genus, species, or even serotype level. The presentation will also discuss the fundamental limitations of the traditional supervised ML approaches, namely the reliance on a priori defined features and assumption of exhaustiveness of the training data. Alternative procedures employing representation learning paired with manifold learning and non-parametric clustering offer a unique and exciting new framework for a non-exhaustive ML that could be generalized from ELS to other fields dealing with the problem of emerging classes and a need for simultaneous classification and class discovery.

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A High Throughput Approach to Identifying Compound Inhibitors of mRNA Processing and Export

Kirsty Carey and Vi Wickramasinghe

Peter MacCallum Cancer Centre

Cancers with altered RNA processing are heavily reliant on gene expression to ensure production of oncogenic protein isoforms. This creates novel vulnerabilities in cancer cells that can be therapeutically exploited using compounds that affect the gene expression pathway, such as nuclear export of mRNA. Compounds inhibiting all other steps of the gene expression pathway are promising therapeutic candidates, and many have reached clinical trials. Thus, development of RNA export inhibitors offers a unique opportunity to develop innovative, targeted cancer therapeutics. We have developed and optimised a sensitive, high-throughput assay to screen 25,000 natural “drug-like” compounds examining their effects on cell death and RNA localisation within breast cancer cells. Coupled with advanced image analysis, it is possible to reproducibly detect and quantify phenotypes relating to mRNA processing and export including nuclear retention and sub-cellular compartmentalisation of mRNA. We have demonstrated the first ever compounds inhibiting mRNA export. 383 compounds were taken forward to determine dose dependency and to validate the phenotypes observed in the primary screen with a validation rate of 20%. Future characterisation of compounds identified in our screen and their mode of action will facilitate our understanding of mRNA processing and export and will hopefully lead to development of pharmacologically relevant compounds which can eventually advance to clinical trials as cancer therapeutics.

Identification of Novel FDA-Approved Inhibitors of Zika Virus Infection

Nicholas S Eyre, D Anfiteatro, E Kirby, G Bracho Granado, **Amanda L Aloia,** and Michael R Beard

University of Adelaide and Flinders University

Zika virus was first identified in 1947 and since then Zika virus infections have been recorded in Africa, Asia and South America. Zika virus came to prominence in 2015 after an outbreak in Brazil that was associated with microcephaly. It was declared a “public health emergency of international concern” by the World Health Organization in 2016. Infection during pregnancy is now a demonstrated cause of microcephaly. There is no vaccine and no approved pharmacological treatment for Zika virus.

We developed an image-based method for measuring Zika virus infection in cultured cells and used this to screen ~2900 known drugs for their ability to decrease Zika virus infection while showing limited cellular toxicity. We found 15 compounds that met our selection criteria. Several of these compounds fell into 4 distinct compound classes. We selected 2 of these compounds for further analysis and have since confirmed their antiviral effect against Zika virus as well as the related flaviviruses dengue virus and West Nile virus (Kunjin strain). These compounds target a cellular pathway that has not previously been associated with flavivirus replication. Our ongoing work is focused on characterising the mechanism of action of these compounds.

Identification of Therapeutic Targets for the Treatment of Diamond Blackfan Anaemia Using a High-Throughput Screening-Based Approach

Amee J. George[1,2], Lorena Nuñez Villacis[1], Mei S. Wong[1,3], Priscilla Soo[1], Maurits Evers[1], Nadine Hein[1], Sheren J. Al-Obaidi[1], Jeannine Diesch[2], Megan Pavy[1], Perlita Poh[1], Cathryn M. Gould[4], Piyush Madhamshettiwar[5], Lorey K. Smith[2], Jun Chen[6], Melissa Ilsley[6], Hamish S. Scott[7], Katherine M. Hannan[1], Thomas J. Gonda[8], Kaylene J. Simpson[1], Johan Flygare[7], Richard B. Pearson[1], and Ross D. Hannan[1-3]

[1]ACRF Department of Cancer Biology and Therapeutics, The John Curtin School of Medical Research, The Australian National University, Acton, ACT. [2]ANU Centre for Therapeutic Discovery, The John Curtin School of Medical Research, The Australian National University, Acton, ACT. [3]The Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC. [4]Garvan Institute of Medical Research, Darlinghurst, NSW. [5]Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre, East Melbourne, VIC. [6]Lund Stem Cell Centre, Biomedical Centre, Lund University, Lund, S-22184, Sweden. [7]Centre for Cancer Biology, Adelaide, SA. [8]School of Pharmacy, University of Queensland, Wooloongabba, QLD

One molecular mechanism to account for the impaired proliferation and cell death associated with bone marrow failure in Diamond Blackfan Anaemia (DBA) is the aberrant activation of the nucleolar surveillance pathway. In this model, mutations or insults that disrupt ribosome biogenesis result in the sequestration of the E3 ubiquitin ligase murine double minute 2 (MDM2) by free RPs (predominantly the 60S RPs, L5 and L11) in a complex with 5S rRNA, leading to the accumulation of p53 and subsequent induction of cell cycle arrest or apoptosis. In the case of DBA, the nucleolar surveillance response is aberrantly activated, and elevated p53 protein results in preferential apoptosis or cell cycle arrest of the erythroid progenitors required for red blood cell development and normal development. Moreover, it has also been proposed that the reduced levels of functional ribosomes in surviving erythroid cells exhibit altered translation of mRNAs that encode proteins critical for erythropoiesis. Our studies are built on the central hypothesis that understanding the molecular mechanism(s) by which DBA-causing ribosomal protein mutations activate p53, leading to the death of erythroid progenitors, will allow us to identify new therapeutic targets and drugs for the treatment of patients with Diamond-Blackfan Anaemia, as well as other ribosomopathies.

Continued…..

To address this, we have used a high content screening-based approach to perform genome-wide loss-of-function (RNAi) and gain of function (ORF overexpression) screens, and have also screened compound libraries of all current clinically approved therapeutics, to identify the critical genes and pathways implicated in the p53-mediated nucleolar surveillance response due to ribosomal protein S19 insufficiency (as observed in DBA). We will present our current data, which has uncovered a suite of novel genes/biological processes involved in this process, as well as a number of clinically approved therapeutics that can ameliorate nucleolar surveillance activation/p53 stabilisation. We are currently validating these targets/drugs using in vivo models. We predict that these studies will enable the development of novel treatments for patients with DBA.

Keynote: Dynamic 3D Imaging of Patient-Derived Tumour Organoids for Drug Screening Applications

Shannon Mumenthaler

University of Southern California, USA

The highly complex and evolving nature of cancer makes it challenging to study in the laboratory setting. To advance our biological understanding of cancer and improve treatment efficacy, we are utilizing quantitative high-content imaging to illuminate the dynamic interactions between cancer cells and their microenvironment within physiologically-relevant model systems. Patient-derived tumor organoids offer a rapid and scalable approach for patient-specific molecular and phenotypic characterization and drug screening. We highlight several 3D imaging-based workflows to phenotypically profile tumor organoids across scales from single cells to multicellular objects. When combined with machine learning and other image analysis techniques, we are able to rapidly and accurately classify cell types and cell behaviors. Using these approaches, we capture the dynamics and heterogeneity of drug response across patient-derived organoid models.

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High-Content Screening in Human Cardiac Organoids Identifies Key Proliferative Pathways Without Functional Side-Effects

Enzo Porrello

Murdoch Children's Research Institute, Department of Physiology, School of Biomedical Sciences, The University of Melbourne.

Human pluripotent stem cell-derived cardiomyocytes are emerging as a powerful platform for cardiovascular drug discovery and toxicology. However, standard 2D cultures are typically immature, which limits their capacity to predict human biology and disease mechanisms. To address this problem, we have recently developed a high-throughput bioengineered human cardiac organoid (hCO) platform, which provides functional contractile tissue with biological properties similar to native heart tissue including mature, cell cycle-arrested cardiomyocytes. Here, we take advantage of the screening capabilities of our mature hCO system to perform functional screening of 105 small molecules with pro-regenerative potential. Our findings reveal a surprising discordance between the number of pro-proliferative compounds identified in our mature hCO system compared with traditional 2D assays. In addition, functional analyses uncovered detrimental effects of many hit compounds on cardiac contractility and rhythm. By eliminating compounds that had detrimental effects on cardiac function, we identified two small molecules that were capable of inducing cardiomyocyte proliferation without any detrimental impacts on function. High-throughput proteomics on single cardiac organoids revealed the underlying mechanism driving proliferation, which involved synergistic activation of the mevalonate pathway and a cell cycle network. In vivo validation studies confirmed that the mevalonate pathway was shut down during postnatal heart maturation in mice and statin-mediated inhibition of the pathway inhibited proliferation and heart growth during the neonatal window. This study highlights the utility of human cardiac organoids for pro-regenerative drug development including identification of underlying biological mechanisms and minimization of adverse side-effects.

3D Bioprinting of HTP Tumour Spheroids for Precision Medicine

Lakmali Atapattu[1,2,3], Robert H. Utama[1,3], Aidan O'Mahony[5], Christopher Fife[5], Jongho Baek[4], Kieran O'Mahony[5], Julio Ribeiro[5], Katharina Gaus[4], J. Justin Gooding[1,3,*] and Maria Kavallaris[1,2,3,*]

[1]Australian Centre for NanoMedicine (ACN), UNSW Sydney. [2]Children Cancer Institute. [3]Centre of Excellence in Convergent Bio-Nano Science & Technology.
[4]EMBL Australia Node in Single Molecule Science, UNSW Sydney. [5]Inventia Life Science Pty. Ltd.

Cancer is the leading cause of disease-related death of Australians. Predicting patient response to cancer therapy is still a major challenge. Recently, precision medicine has gained momentum in cancer management. Most precision medicine approaches focus on genetic analysis with minimal phenotypic testing. The lack of robust and effective tumour models to study drug responses in high throughput (HTP) has limited phenotypic testing. 2D cell cultures are the most frequently used for phenotypic assays, however they often respond to therapy differently to the original tumour. On the other hand, widely used *in vivo* systems are a closer representation of human response although they are inconsistent and slow for clinical utility. 3D-tumouroids (spheroids developed from tumour, accessory cells and extra-cellular matrix) that closely mimic pathology and genetics of human tumours are important models to predict drug response. A key challenge for the routine application of tumouroids in precision medicine is the lack of HTP and uniform production of tumouroids in a biocompatible matrix. To address this, we have developed a HTP method of producing tumouroids embedded inside a tissue-like matrix using a custom-built drop-on-demand 3D bioprinter. We have successfully 3D bioprinted glioblastoma, neuroblastoma and lung cancer spheroids that maintain all the biological characteristics typically found in a spheroid. In particular, the bioprinted spheroids were shown to be viable, proliferating, and preserved the apoptotic and cancer-stemness characteristics. Using super-resolution lattice-light sheet microscopy, we showed the spheroids maintained their structural integrity. The potential application of the 3D bioprinted spheroids for high-throughput drug screening in 3D environments was demonstrated using doxorubicin as the model drug. 3D-bioprinting of patient-derived cancer cells for precision medicine applications is now underway. Freshly extracted patient derived xenograft cells form tumouroids upon bioprinting and exhibits dose dependent drug response. Thus, HTP 3D bioprinting has enormous potential to accelerate precision cancer medicine.

Drug Discovery Phenomics

Tayner Rodriguez and Vicky M. Avery

Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia

Cancer is the leading cause of disease-related death of Australians. Predicting patient response to cancer therapy is still a major challenge. Recently, precision medicine has gained momentum in cancer management. Most precision medicine approaches focus on genetic analysis with minimal phenotypic testing. The lack of robust and effective tumour models to study drug responses in high throughput (HTP) has limited phenotypic testing. 2D cell cultures are the most frequently used for phenotypic assays, however they often respond to therapy differently to the original tumour. On the other hand, widely used *in vivo* systems are a closer representation of human response although they are inconsistent and slow for clinical utility. 3D-tumouroids (spheroids developed from tumour, accessory cells and extra-cellular matrix) that closely mimic pathology and genetics of human tumours are important models to predict drug response. A key challenge for the routine application of tumouroids in precision medicine is the lack of HTP and uniform production of tumouroids in a biocompatible matrix. To address this, we have developed a HTP method of producing tumouroids embedded inside a tissue-like matrix using a custom-built drop-on-demand 3D bioprinter. We have successfully 3D bioprinted glioblastoma, neuroblastoma and lung cancer spheroids that maintain all the biological characteristics typically found in a spheroid. In particular, the bioprinted spheroids were shown to be viable, proliferating, and preserved the apoptotic and cancer-stemness characteristics. Using super-resolution lattice-light sheet microscopy, we showed the spheroids maintained their structural integrity. The potential application of the 3D bioprinted spheroids for high-throughput drug screening in 3D environments was demonstrated using doxorubicin as the model drug. 3D-bioprinting of patient-derived cancer cells for precision medicine applications is now underway. Freshly extracted patient derived xenograft cells form tumouroids upon bioprinting and exhibits dose dependent drug response. Thus, HTP 3D bioprinting has enormous potential to accelerate precision cancer medicine.

Synthetic CRISPRs: Novel Approaches to Study Gene Function

Iva Nikolic, Karla Cowley, Robert Vary, Kaylene Simpson
Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre

Since its recent discovery, CRISPR/Cas9 has triggered a revolution in biomedical research and is rapidly becoming an indispensible tool for many research applications. High-throughput CRISPR screens display remarkable results with low levels of off-targets and biological variation, making the hit identification easier and potentially improving the success of their clinical translation. These screens, however, typically run in a pooled format, where all gene knockout constructs are assembled in a single large collection, and are interrogated in the context of gross overall cell survival, which limits the scope of biological questions asked. Here we describe an arrayed CRISPR platform based on the synthetic CRISPR reagents, which enables individual gene knockout in a single well coupled with versatile downstream analyses using high-content microscopy. The synthetic CRISPRs induce highly efficient and rapid gene knockout, offering several advantages over vector-based systems: streamlined experimental setup, short assay length, strong phenotypic penetrance in the absence of selection, and short half-life of CRISPR constructs within the cell. In addition, drawing on our extensive experience with liquid handling robotics, we developed fully automated pipelines for the delivery of synthetic CRISPRs into the cells as well as all subsequent analyses, making it an ideal tool for both discovery and validation work.

Keynote: Unravelling Biology and Identifying Targets with Functional Genomics Approaches Supported by LentiArray CRISPR Library Screens

Jonathan Chesnut

Thermo Fisher Scientific, USA

Identifying and validating targets that underlie disease mechanisms and can be addressed to provide efficacious therapies remains a significant challenge in the drug discovery and development process. Mechanisms of RNAi have provided the use of siRNA and shRNA to knock-down RNA and suppress gene function. However, depending on the nature of the targets, cells, biology and end-point assays, these approaches may suffer variously from their transient nature, design complexity, incomplete knock-down or off-target effects. The use of CRISPR (clustered regularly interspaced short palindromic repeat)-associated Cas9 nuclease and guide RNA (gRNA) provides a strong alternative that can produce transient or long-lasting impact, straightforward design, knock-out of genes and increased specificity. Here we demonstrate a knock-out screening approach that utilizes the Invitrogen™ LentiArray™ CRISPR library to interrogate the impact of individual gene knock-outs in various systems, including the NFκB pathway as measured by a functional cell-based assay. We describe the library design concepts, assay development, as well as screening results and validation of specific identified hits. We expect these approaches to be scalable to the entire human genome and portable to multiple cell types and end-point assays including both high-throughput plate-based assays and high-content imaging-based assays.

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Keynote: Powering the Synthetic Biology Revolution
Reed Hickey

Twist Bioscience, USA

Twist Bioscience enables the design-build-test cycle and is disrupting the synthetic biology industry through its innovative DNA synthesis platform. By miniaturizing synthesis volumes by a factor of 1000 and up to 1 million in the case of the most expensive reagent. Twist Bioscience combined it's semiconductor-based platform with proprietary software, scalable commercial infrastructure and an e-commerce platform to create an integrated technology platform that enables us to achieve high levels of quality, precision, automation, and manufacturing throughput at a significantly lower cost than our competitors. Twist is leveraging its unique technology to manufacture a broad range of synthetic DNA-based products, including synthetic genes, tools for next generation sample preparation, and antibody libraries for drug discovery and development for a wide range of commercial enterprises.

Ginkgo Bioworks, the largest user of synthetic DNA globally

Ginkgo Bioworks, the organism company, is bringing biotechnology to the consumer goods markets, enabling fragrance, cosmetic, nutrition, food, agriculture and pharmaceuticals to make better products. They have agreed to purchase 1.3 billion base pairs of DNA from Twist over the next four years to support their growth.

Top Three Pharmaceutical Company

For the last two years Twist Bioscience has been working with one of the top three pharmaceutical companies in the world to improve their discovery library synthesis supporting the development of biologics. Moving to Twist Bioscience has shortened the discovery time line allowing the company to focus on downstream assay development. Vanderbilt University is one of the four institutions funded under the DARPA Pandemic Prevention Program (P3). The goal of the P3 program is to generate a response in 60 days to provide transient immunity to a population and halt the spread of infectious disease before it becomes a full-scale pandemic. Twist Bioscience was chosen as the primary supplier for generation of expression constructs generated from patient antibody diversity. Twist Bioscience is enabling accelerated discovery across a broad area of research applications from synthetic biology to biologics discovery in a wide range of industries

Sponsored by Twist Bioscience

TWIST BIOSCIENCE

Defining Novel Modifiers of the Cellular Response to Unedited Endogenous dsRNA

Jacki Heraud-Farlow[1,2], Scott Taylor[1], Alistair Chalk[1,2], Iva Nikolic[4], Kaylene Simpson[4,5], Carl Walkley[1,2,3]

[1]St.Vincent's Institute of Medical Research, Fitzroy,Victoria, Australia.; [2]Department of Medicine, St. Vincent's Hospital, University of Melbourne, Fitzroy,Victoria, Australia; [3]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Victoria Australia; [4]Victorian Centre for Functional Genomics, ACRF RPPA Platform, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; [5]The Sir Peter MacCallum Department of Oncology, the University of Melbourne, Parkville, Victoria, Australia.

Adenosine-to-inosine (A-to-I) editing of double-stranded RNA (dsRNA) by ADAR proteins is a highly prevalent form of RNA base modification in higher eukaryotes. The primary physiological function of ADAR1 is to edit long dsRNA structures in endogenous RNAs, resulting in a change in secondary structure. Non-edited endogenous dsRNA would otherwise be recognised as non-self/viral dsRNA by the innate immune system. In the absence of editing the cytosolic dsRNA sensor MDA5 oligomerises on endogenous dsRNAs and signals to the mitochondrial protein MAVS leading to the ongoing production of interferon-stimulated genes (ISGs) and a permanent antiviral state that is extremely detrimental. Causative mutations have been identified in both *Adar1* and *Ifih1* (MDA5) in individuals with the rare auto-inflammatory disease, Aicardi-Goutieres syndrome (AGS).

We have developed a cell culture model for Adar1-editing deficiency which recapitulates the in vivo biology in order to perform a genome-wide screen for genes which modify the phenotype. I will present data describing the screening approach and the initial results from the screen to identify novel genes that rescue/modify the cellular response to unedited endogenous dsRNA.

A Whole-Genome CRISPR Screen to Identify Mechanisms that Determine Resistance and Sensitivity to the Mutant p53 Reactivator, APR-246.

Kenji Fujihara, and **Nicholas Clemons**

Peter MacCallum Cancer Centre, Melbourne, Australia

The transcription factor p53 plays a central role in defence against cancer and its functional inactivation occurs frequently, predominately through missense mutations. Subsequently, mutant p53 protein often accumulates in cancer cells and can have gain-of-function oncogenic activities that drive tumourigenesis and chemotherapeutic resistance. Thus, a number of strategies have been developed to target mutant p53 directly, including several low molecular weight compounds have been identified that reactivate and restore wild-type function to mutant p53. The most clinically advanced of these is APR-246 (also known as PRIMA-1met), which is currently in early phase clinical trials, including in patients with oesophageal cancer at the Peter MacCallum Cancer Centre.

Whilst APR-246 undoubtedly can restore normal p53 transcriptional activity to some mutant p53 proteins, we recently established that methylene quinuclidinone (MQ, the active derivative of APR-246) also binds and depletes glutathione, a major cellular antioxidant, to drive tumour cell death. Furthermore, we showed that the amino acid transporter, SLC7A11, a key determinant of glutathione biosynthesis, governs sensitivity to APR-246, independent of mutant p53. Thus, it is clear that mutant p53 protein is not the only biomarker and determinant of APR-246 response.

Currently, in collaboration with the Victorian Centre for Functional Genomics, we are performing genome wide CRISPR knockout and activation screens to identify additional molecular mechanisms that govern resistance and sensitivity to APR-246. In preliminary results from the knockout screen we have identified multiple genes involved in folate and formate metabolic pathways to regulate cellular response to APR-246 *in vitro*. Consistent with these findings, altering cell culture medium folate and formate concentrations alter sensitivity to APR-246. Thus, our preliminary screen has identified new potential strategies to target mutant p53 cancers in combination with APR-246, and potential biomarkers that could help guide the use of APR-246 in the clinic.

Unlocking HDR-mediated Nucleotide Editing by identifying high-efficiency target sites using machine learning and high throughput screening

[1,2]Aidan O'Brien, [2]Laurence Wilson, [2]Denis Bauer and [1]**Gaetan Burgio**

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Novel precision genetic technologies such as CRISPR-Cas9 genome editing technology offer novel avenues to better understand the mechanisms of diseases and predict the likelihood for a single nucleotide variant to be pathogenic. Using CRISPR-Cas9 we are able to precisely modify the mouse or the human genome by creating knockout or a specific single nucleotide change to enable the study of the function of the gene of interest. The generation of these models lies on the ability of Cas9 to create a double strand break in the DNA and the repair to occur via the error prone Non-Homologous End Joining (NHEJ) or the precise Homology direct Repair (HDR) mechanisms. A large body of work has been recently dedicated to improve the technology to generate efficiently knockout or knock-in mouse models (point mutations, tags or floxed alleles).

Here, we investigate the variable efficiency-governing factors on a novel mouse dataset of point mutation replacement using machine learning. We found the distance to the protospacer adjacent motif sequence (PAM) and the sequence composition of the repair template (ssODN) to be a governing factor, where different regions of the ssODN have variable influence, which reflects the underlying biophysical mechanism. Our model improves HDR efficiency by 83% compared to traditionally chosen targets. We are being validated these finding using high-throughput screening in mouse embryonic stem cells using distance to the PAM, ssODN symmetry and length and sgRNA efficiency as parameters. Using our findings, we developped CUNE (Computational Universal Nucleotide Editor), which enables users to identify and design the optimal targeting strategy using traditional base editing or --for-the-first-time -- HDR-mediated nucleotide changes.

Microscopium: Interactive Exploration of Large Imaging Datasets for Unbiased Phenotype Discovery

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The large volume of data produced by high content screening (HCS) presents a considerable challenge for distilling relevant insights from a multitude of irrelevant details. For this reason most HCS analyses focus on just one or two predetermined phenotypes (such as cell count or viability, or the total amount of a target protein). Current software tools for high content analysis often rely on predefined workflows for highly specific tasks, whether open source (CellProfiler, HCS-Analyzer, Advanced Cell Classifier, OpenHiCAMM) or closed (MetaXpress, Opera/Operetta, InCell Analyzer). When machine learning is used, it generally requires expensive and time-consuming human supervision. While several groups have made advances in unsupervised machine learning for high content screens, these remain highly technical and inaccessible to most researchers. For widespread adoption it is critical that unsupervised techniques are paired with an intuitive user interface for the non-computer scientist. We present microscopium, a free, open-source tool for the exploration of high content screening data. Microscopium uses machine learning to group images according to similarity, and presents the clustered results interactively through a web app. We demonstrate microscopium's capacity for large data handling using the open drug dataset BBBC021 and as others have found, we show that image clustering by similarity can recover mode-of-action information. Further, we will demonstrate how this discovery can be made interactively using our tool.

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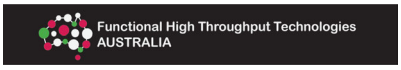

Mapping Phenotypic Plasticity in the Actin Cytoskeletal System via Unbiased Chemical Screening

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The multitude of proteins regulating F-actin suggest the potential for virtually unlimited phenotypic plasticity in actin organisation. At the same time, actin regulators also constitute a largely unexploited pool of prospective drug targets that could facilitate more precise and selective actin modulation, potentially side-stepping long-standing issues with cardiac and respiratory toxicity, for example. Screening of 114,400 structurally diverse compounds using high-content F-actin imaging provided avenues to systematically map the extent and limits of F-actin phenotypic plasticity, whilst simultaneously enabling detection of new compounds with potential as actin modulators. To explore plasticity, we applied unbiased phenotypic clustering to reveal just 27 recurrent actin phenotypes. This implies surprisingly low plasticity given the combinatorial potential of actin regulators and the scope of chemical perturbations applied. In terms of actin-drug discovery, more than 2600 unknown compounds impacted actin organisation and we employed 3 strategies based on phenotypic comparison to enhance prediction of underlying mechanisms: 1) comparison with known actin modulators embedded in the screen (>260 compounds identified); 2) comparison with known genetic perturbations (e.g. 3 novel talin-binding compounds identified), and; 3) retrospective integration of additional known compounds into the original screen data (revealing 2 ROCK inhibitors). Notably, this retrospective integration strategy may provide near-unlimited capacity to extend mechanistic analyses in this and other phenotypic screens. In summary, we use large scale screening to not only identify new compounds with potential in actin and broader cytoskeletal regulation, but also use this large dataset to begin delineation of the structure and limits of phenotypic plasticity in this critical cellular system.

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Multilabelling - Highly Multiplexed, High-Content Confocal Imaging

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We present Multilabelling, an implementation of highly multiplexed high-content imaging of cells on a commercial, off-the-shelf confocal microscope. The method is based on performing repeated, fully automated cycles of fluorescent labelling, imaging and photobleaching of the sample on the microscope stage. The fluorescent labelling is performed using an automated pipetting needle fitted to the microscope stand. We assembled a panel of commercially available dyes and primary labelled antibodies, selected to visualize a range of subcellular structures. We demonstrate imaging of up to 15 different labelled structures, creating an organelle fingerprint of each cell. In each experiment, labelling and imaging are performed in multiple wells, including wells where cells have been exposed to treatments such as siRNA knockdown or addition of drugs. The organelle fingerprint enables us to analyse the effects of such treatments on many different cellular structures simultaneously. In order to facilitate interactive analysis of the data we have developed a dedicated, layer-based viewer for the high-dimensional image data. Using a CellProfiler and Python-based image analysis pipeline we extract quantitative per-cell measurements of intensity and morphology in each channel. We visualise the extracted data with interactive linked plots, with the ability to link each data point back to the original image.

Using High-Content Time-Lapse Imaging to Investigate Effects of Oncogenic Mutations in Melanocyte-Keratinocytes-Fibroblast Interaction

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MMRI, TRI, University of Queensland

Melanocytic cell interactions are integral to skin homeostasis and affect the outcome of multiple diseases, including cutaneous pigmentation disorders and melanoma. These interactions are typified by complex interplay of multiple molecular components so that their exploration requires high-throughput approach. We have established an automated-microscopy platform for high-resolution analysis of melanocyte-keratinocyte-fibroblast interaction in microwell plates. The system relies on combinatorial interrogation of melanocyte genotypic variants with oncogenic mutations using lentiviral vectors. Transduced melanocytes in co-culture with either HACAT keratinocytes or primary fibroblasts were examined by automated microscopy in both end-point and time-lapse imaging assays. Fluorescent markers were used to distinguish between cell types and track co-culture induced changes in melanocyte morphology, such as the keratinocyte-stimulated increase in dendricity. Quantification of cell phenotypes using open-source software and machine-learning algorithms distinguished between oncogenic mutations that can disrupt normal melanocytic cell interactions from those that have no effect. Here we describe the effects of NRAS.Q61K, one of the most common melanoma-associated mutations which reduced keratinocyte-induced melanocyte dendricity and increased melanocyte migration resulting in keratinocyte avoidance. This mutant also altered interaction with primary dermal fibroblasts, suggesting that in addition to known role in activating mitogen signalling, NRAS.Q61K may also contribute to cancer progression by conferring avoidance of restrictive cell-cell interactions. Examination of other mutations as well as melanocyte genetic variants is in progress and will identify potential therapeutic targets as well as increase understanding of skin cell interactions.

Keynote: The Promise of siRNA Therapeutics

David Evans
Sirnaomics, USA

The identification of RNAi provided a breakthrough in our ability to silence genes within mammalian cells and hence offered the promise for therapeutics able to treat diseases driven by overexpression of such genes. With the recent approval of Patisiran from Alnylam, siRNAs have now completed the cycle from understanding the key design criteria for their function, using them to identify and validate targets driving disease and now their validation as viable therapeutics. Patisiran is undoubtedly the first of many such approvals from multiple companies focused on siRNA therapeutics - including several pending from Sirnaomics.

However, while there are many tools that allow digital design of siRNAs that can silence gene targets, many diseases such as cancer require inhibition of multiple genes in parallel and the genes that need to be targeted may change with time and stage of disease. We therefore need vehicles that can deliver more than one siRNA into the same cell concomitantly. Modification of these delivery vehicles with ligands to home to specific cell types in tissues may provide better therapeutic efficacy with a higher safety margin. This presentation will discuss our experiences in identification of multiple siRNAs with synergistic effects, development of delivery vehicles that can carry multiple siRNAs, and early steps we are taking in new screening methods that are being implemented to help rapidly identify and validate functional targeting ligands.

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Intelligent Image-Activated Cell Sorting

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I introduce our novel machine intelligence technology known as “intelligent Image-Activated Cell Sorting (intelligent IACS)” [Cell 175, 1 (2018)]. This technology builds on a unique integration of high-throughput cell microscopy, focusing, and sorting techniques on a unique software-hardware platform and hence performs fully automated operation for data acquisition, data processing, decision making, and actuation. Also, I present two unique applications in microbiology and hematology enabled by the technology and discuss how technology enables machine-based scientific discovery in diverse biological and medical sciences.

Utilisation of High Throughput Cytometers to Expand Screening Capabilities

Jennifer A. Smith
ICCB Longwood Screening Facility, Harvard Medical School, USA

The ICCB-Longwood Screening Facility at Harvard Medical School has been supporting high throughput screens and microplate automation projects for nearly 20 years. ICCB-Longwood has been developing cell-based phenotypic and high content assays since its inception. The complexity of screens continues to increase as the field of chemical genomics expands and technologies advance. A multitude of assays are supported with multimode plate readers and high content microscopes. However, it has only been in the last decade that there have been robust solutions for high content screens of non-adherent mammalian cells. We initially accomplished this with TTP LabTech's Acumen laser scanning cytometer and most recently using IntelliCyt's iQue Screener PLUS. The ability to conduct high throughput flow cytometry experiments has expanded the breadth of assays ICCB-Longwood is able to support, particularly those of immune cell maturation and differentiation. This talk focuses on how scientists at ICCB-Longwood have developed screens that rely on the iQue for assay quantitation and its accompanying ForeCyt software for analysis

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