CRISPR DOWN UNDER

CRISPR DOWN UNDER 2026

Abstract Booklet

24 – 25 March 2026

Melbourne, Australia

WELCOME

CRISPR Down Under returns for its third edition, bringing together researchers, clinicians, industry partners, and emerging scientists from across Australia and around the world to explore the rapidly evolving landscape of CRISPR and genome engineering technologies. Since its inception, the meeting has aimed to foster collaboration across disciplines and provide a forum for the CRISPR community to exchange ideas, showcase discoveries, and build new partnerships.

This year's program highlights advances spanning CRISPR biology, genome and epigenome engineering, functional genomics, translational gene therapies, and emerging ethical considerations surrounding genome editing technologies. The meeting features keynote presentations from leading international experts, invited talks from researchers across the region, contributed oral presentations, flash talks, and poster sessions showcasing outstanding work from early-career scientists. We are also excited to host a panel discussion, "***To Edit or Not to Edit*** – Translating Gene Editing to Real World Applications," bringing together perspectives from academia, industry, and bioethics.

Our thanks to all sponsors, presenters, and delegates for their tremendous support and enthusiasm for CRISPR Down Under 2026. Without their contributions, this meeting would not be possible.

We hope you leave with new collaborations, fresh ideas, and perhaps a few carefully edited perspectives.

Warm Regards,
The 2026 Organising Committee

MEETING INFO

Dates:
24-25 March 2026

Location:
Melbourne Connect

Hashtag:
#CDU2026

X: @CRISPR_Aus

Acknowledgement of Country

We acknowledge the Traditional Owners of the lands on which this meeting takes place, the Wurundjeri Woi-wurrung people of the Kulin Nation, and pay our respects to their Elders past and present. We extend that respect to all Aboriginal and Torres Strait Islander peoples attending this meeting.

COMMITTEE & CONTACT INFORMATION

2026 Organising Committee

Convenor

Gurjeet Kaur Gill Jagjeet Singh

Committee Members

Emily Lelliott
Patrick Constantinescu
Christina Koenig
Eddie La Marca
Jovita D Silva
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PROGRAM AT A GLANCE

Day 1 - Tuesday 24 March

08:00 Registration

09:00 - 10:40
CRISPR Molecular Mechanism

10:40 - 11:10
Morning Tea

11:10 - 12:10
CRISPR Tools For Genome Interrogation

12:10 - 13:40
Lunch • 1-on-1 with IDT

13:40 - 15:10
Functional Genomics

15:10- 15:30
Afternoon Tea

15:30 - 16:30
Genome and Epigenome Engineering

16:30 - 18:30
Poster Session I
Networking Reception

Day 2 - Tuesday 25 March

08:00 Registration

09:00 - 10:35
CRISPR Evolution and Diversity

10:35 - 11:05
Morning Tea

11:05 - 12:25
Immunoengineering and Cancer Therapeutics

12:30 - 14:00
Lunch • (1-on-1 with IDT) •
Poster Session II

14:00 - 15:05
Responsible Innovation and Ethics
Panel Discussion
To Edit or Not to Edit - Translating Gene Editing to Real World Applications

15:05 - 15:35
Afternoon Tea

15:35 - 16:45
Translating CRISPR: Delivery and Diagnostics

16:45 - 17:00
Closing Ceremony

KEYNOTE SPEAKERS

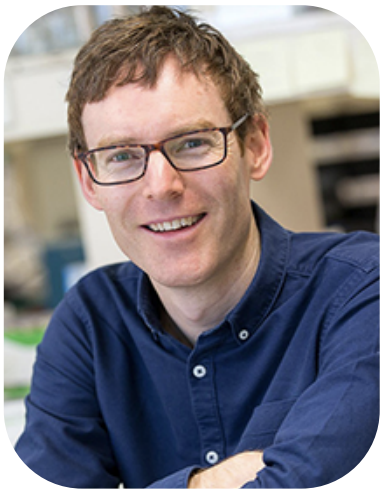
Dr. Ekaterina Semenova

Rutgers University, USA

What does Cas13 Really Cleave?
tRNA cleavage as a Core Mechanism of CRISPR-Cas13 Immunity

Ekaterina Semenova, PhD, is Laboratory Director and Assistant Research Professor at the Waksman Institute of Microbiology, Rutgers University (USA). She earned her B.Sc. in Molecular Biology from Novosibirsk State University (Russia) and began her scientific career studying microbial diversity in Lake Baikal.

Following completion of her PhD, Dr. Semenova joined the laboratory of Konstantin Severinov at Rutgers University, where she established a research program focused on bacteriophage–host interactions and CRISPR-Cas adaptive immunity. Her work has contributed to several key advances in the CRISPR field, including discoveries related to seed sequence recognition, primed spacer acquisition, CRISPR adaptation intermediates, and Cas13-dependent tRNA cleavage as an antiphage defense mechanism. Her research continues to advance understanding of RNA-targeting CRISPR systems and their applications.



Dr. Peter Fineran

University of Otago, New Zealand

CRISPR-Cas systems in phage-bacterial interactions:
biology and exploitation

Peter Fineran is Professor at the University of Otago, New Zealand, where he leads the Phage–Host Interactions (Phi) Laboratory. He obtained a BSc (Hons) in Biochemistry from the University of Canterbury, New Zealand, and completed his PhD and post-doctoral training at the University of Cambridge, UK.

His research focuses on the interactions between bacteriophages, other mobile genetic elements, and their bacterial hosts, particularly the mechanisms and evolution of CRISPR-Cas and other phage defence systems. His team has also studied strategies used by phages to evade bacterial immune systems and explored the development of bacteriophages in therapeutic applications. Professor Fineran has published over 140 research articles, filed multiple patents, and received numerous awards, including the Fleming Prize from the Microbiology Society (UK). He is a Fellow of the Royal Society of New Zealand.

PANEL DISCUSSION

To Edit or Not to Edit:
Translating Gene Editing to Real-World Applications

Gene editing technologies are rapidly transitioning from laboratory discovery to real-world applications. This panel brings together perspectives from academia, industry, bioethics and consumer advocacy to explore the challenges and opportunities in translating these technologies into practice.

Peter Fineran
Professor
University of Otago
New Zealand

Ekaterina Semenova
Associate Professor
Rutgers University
USA

Christopher Gyngell
Bioethicist
University of Melbourne

Con Panousis
Vice President
Drug Discovery
CSL

MODERATOR

Gavin Knott
Group Leader
Monash Biomedicine Discovery Institute
Monash University

Nathalie Cook
Accredited Practising Dietician
and Consumer Advocate

DISCUSSION THEMES

- Responsible translation of gene editing technologies
- Clinical and regulatory considerations
- Industry perspectives on therapeutic development
- Ethical and societal implications of genome editing
- The role of patient and consumer voices in biotechnology

Wednesday 25 March **14:25 - 15:05** **The Forum, Melbourne Connect**

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Olivia Newton-John Cancer Research Institute

Session 1: CRISPR Molecular Mechanisms

Brooke Hayes

Monash University, Australia

Invited Speaker

In Defence of Cas13

About The Speaker

Brooke Hayes is a protein biochemist and structural biologist at Monash University. Her research focuses on bacterial defence and persistence in polymicrobial environments, with the aim of understanding how diverse molecular factors influence bacterial survival.

Following an MSc at the University of Otago, Brooke completed her PhD at the Monash University Biomedicine Discovery Institute in 2023, where she investigated the structure and function of protein toxins associated with the type VI secretion system.

She is currently a postdoctoral research fellow studying the CRISPR-Cas13 nuclease family, with a focus on substrate specificity, cleavage mechanisms, and the role of accessory proteins in modulating activity. In recognition of her early-career research, Brooke was awarded the 2025 Lorne Proteins Anders Early Career Researcher Award.

Session 1: CRISPR Molecular Mechanisms

Honglin (Kevin) Chen

Peter MacCallum Cancer Centre, The University of Melbourne

Oral

The Landscape of On-target and Collateral Activity of Various CRISPR-Cas13 Enzymes in Human Cells and Zebrafish

Abstract

Honglin Chen [1], Wenxin Hu [1], Mohamed Fareh [1]

[1]*PeterMacCallum Cancer Centre, Melbourne, VIC, Australia*

Targeted manipulation of cellular transcriptomes and proteomes with sequence-specific RNA targeting tools is crucial for comprehending biological and pathological processes, including viruses and cancer. CRISPR-Cas13 systems represent a recently discovered class of RNA-guided nucleases capable of selectively degrading single-stranded RNA. Unlike classical RNAi or Cas9-based approaches, Cas13 enzymes use long spacer sequences (22–30 nt), enabling highly specific target recognition. However, some Cas13 orthologues were reported to exhibit collateral RNase activity mediating indiscriminate degradation of nearby non-target RNA molecules, raising concerns for specificity.

In this study, we investigated the silencing efficiency and specificity of different Cas13 orthologues in human cells and zebrafish embryos. Using quantitative fluorescence assays, flow cytometry, cell viability analysis, western blotting, transcriptomics, and mass spectrometry-based proteomics, we systematically assessed on-target activity, off-target effects, and collateral RNA degradation.

We evaluated three Cas13 variants and found that LwaCas13a and PspCas13b exhibit high specificity with no detectable collateral activity in either human cells or zebrafish. In contrast, RfxCas13d displayed pronounced collateral cleavage with unexpected characteristics. Specifically, targeting highly abundant transcripts led to excessive activation of RfxCas13d, triggering widespread degradation of non-target RNAs and resulting in cellular toxicity. In zebrafish embryos, this collateral activity caused developmental defects when highly expressed RNAs were targeted, whereas embryos with moderate target RNA levels developed normally. Importantly, we observed that collateral damage was influenced by the subcellular localisation of the target RNA, leading to tissue- and organ-specific phenotypes.

Together, our results reveal a mechanistic link between transcript abundance and RfxCas13d collateral activity. Once a critical threshold of target abundance is reached, RfxCas13d unleashes irreversible collateral RNA degradation, leading to cell toxicity and developmental defects. This property highlights potential therapeutic applications, such as selectively inducing cytotoxicity in cancer cells with high target RNA expression while sparing normal tissues.

Session 1: CRISPR Molecular Mechanisms

Khoa Nguyen
The University of Melbourne

Oral

Harnessing Machine Learning to Design High-Efficacy CRISPR-Cas13b Guide RNAs

Abstract

Khoa Nguyen[1], Kerry A. Mullan[1], Syed Faraz Ahmed[1,2], Wenxin Hu[3,4], Priyank Rawat[3], Ahmed Abdul Quadeer[1,2], Rebecca J. Bengtsson[1], Danielle Anderson[2,5], Morgan Freney[5], Moe El Mohamad[6], Sharon Lewin[6,7,8], Wei Zhao[6], Mohamed Fareh[3,4], Matthew McKay[1,2,5]

[1]*Department of Electrical and Electronic Engineering, University of Melbourne, Australia*
[2]*Department of Microbiology and Immunology, University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Australia*
[3]*Peter MacCallum Cancer Centre, Melbourne, Australia*
[4]*Sir Peter MacCallum Department of Oncology, University of Melbourne, Australia.*
[5]*Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital, Australia*
[6]*Department of Infectious Diseases, University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Australia*
[7]*Victorian Infectious Diseases Service, Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia*
[8]*Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia*

Originally evolved in bacteria as an immune mechanism, CRISPR-Cas13b is a programmable ribonuclease that uses 30-nucleotide guide RNAs (gRNAs) to silence intracellular RNAs. The ability of CRISPR-Cas13b to silence diverse RNAs demonstrates high potential for robust therapeutics in antiviral and cancer treatment, as well as molecular diagnostic applications. However, limited understanding of the sequence features that influence the gRNA silencing efficacy hampers the rational design of gRNAs.

To address this challenge, we leveraged an in-house dataset capturing the silencing efficacies of 265 gRNAs targeting multiple transcripts, measured using an experimental reporter assay. We trained twelve machine learning (ML) models—including linear, tree-based, kernel-based, and neural network architectures—to learn the relationship between gRNA sequences and their silencing efficacy. Models underwent the evaluation using 10-fold cross-validation with hyperparameter optimization. The top-performing models—Lasso regression, Convolutional Neural Network (CNN), and Long Short-Term Memory (LSTM)—achieved median mean absolute errors (MAEs) of 0.153, 0.142, and 0.123, respectively.

Predictions of the top-performing models can diverge on certain gRNAs; Reliance on any single model might result in large errors for a subset of these highly discordant gRNAs. To mitigate this variability, we developed an ML model from an ensemble of top-performing models and achieved reduced prediction errors on these gRNAs. We further validated this ensemble model by applying its predictions to select effective gRNAs targeting the SARS-CoV-2 Nucleocapsid gene. When evaluated on the reporter system, a top-ranked gRNA (NCP163) had a prediction efficacy of 91% and achieved 97% silencing efficacy in vitro. Live-virus evaluations are under way to assess the efficacy of NCP163 in suppressing SARS-CoV-2 replication. These results demonstrate that ML models can accurately predict Cas13b gRNA silencing efficacy, with the potential to enable rapid prioritization of effective gRNAs and streamline therapeutic development.

Session 2: CRISPR Tools for Genome Interrogation

Lisanne Spenkelink

University of Wollongong, Australia

Invited Speaker

dCas9 and nCas9 as tools to study DNA replication

About The Speaker

Lisanne Spenkelink is a physicist and biophysicist whose research focuses on developing single-molecule approaches to study DNA replication and other complex biomolecular mechanisms. She obtained her BSc and MSc in Physics in the Netherlands before joining the laboratory of Antoine van Oijen at the University of Groningen for her PhD, where she investigated bacterial DNA replication at the single-molecule level.

She later moved with the van Oijen lab to the University of Wollongong and completed a joint PhD between the University of Groningen and the University of Wollongong in 2018. Lisanne subsequently worked as an Associate Research Fellow at the University of Wollongong, developing new single-molecule techniques to study the more complex eukaryotic DNA replication system.

In 2022, she was awarded an NHMRC Investigator Grant. Her current research focuses on advancing single-molecule visualisation methods to uncover the mechanisms that underpin dynamic biomolecular processes.

Session 2: CRISPR Tools for Genome Interrogation

Ali Motazedian

Peter MacCallum Cancer Centre, The University of Melbourne

Oral

Characterising the functional landscape of the human PRC2 complex by base editing at single cell resolution

Abstract

Ali Motazedian[1,2,3], Maria Faleeva[1,2,3], Henrietta Holze[1,2,3,4], Kapil Mcinerney[1,2,3], Andrea Gillespie[1,2,3], Oliver Sinclair[1,2,3], Shellaina J.V. Gordon[1,2,3], Andrew Das[1,2,3,5,6], Ching-Seng Ang[7], Tom Caradoc-Davies[8], Ruby H. P. Law[9], Pascal Falter-Braun[4,10], Alan F. Rubin[5,6], Mark A. Dawson[1,2,3,11]

[1]*Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia*
[2]*Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC 3010, Australia*
[3]*Collaborative Centre for Genomic Medicine, Peter MacCallum Cancer Centre and The University of Melbourne, Parkville, VIC 3000, Australia*
[4]*Institute of Network Biology (INET), Molecular Targets and Therapeutics Center (MTTC), Helmholtz Center Munich, German Research Center for Environmental Health, Munich-Neuherberg, Germany*
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[9]*Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800, Australia*
[10]*Microbe-Host Interactions, Faculty of Biology, Ludwig-Maximilians-Universität (LMU) München, Planegg-Martinsried, Germany*
[11]*Department of Haematology, Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Melbourne, VIC 3000, Australia*

Hundreds of germline and somatic mutations in PRC2 members have been annotated in human diseases; however, the functional consequence for most remains unknown. Using an innovative multiplexed strategy involving five separate CRISPR base editors, two cytosine base editors (CBE), adenine base editor (ABE) and two dual base editors (DBEs), coupled to a high-throughput single cell screen, we systematically identified the precise loss of function variants in the transcripts of EZH2, EED and SUZ12. Enriching and capturing the full-length transcripts in single cells using Oxford Nanopore Technologies (ONT) sequencing allowed comprehensive coverage of our gene targets, enabling robust detection of nucleotide variants. We identified many human mutations in both structured and disordered regions of the core complex members. With a multidisciplinary approach incorporating structural modelling, proteomics and chromatin-based assays we show that PRC2 activity is abolished through a variety of mechanisms including impaired chromatin binding, reduced substrate (SAM) access, compromised allosteric activation, weakened protein-protein interactions or formation of a catalytically inert complex with EZHIP. Remarkably, we also show that a single site, EZH2 A677, can be leveraged to precisely tune PRC2 for specific methylation states unveiling potential new strategies for therapeutic intervention.

Session 2: CRISPR Tools for Genome Interrogation

Mollie Schubert

Sr. Innvation Product Manager, Gene Writing and Editing, Integrated DNA Technologies (IDT)

Industry Presentation

End-to-end genotoxicity assessment services for rapid development of CRISPR-based therapies

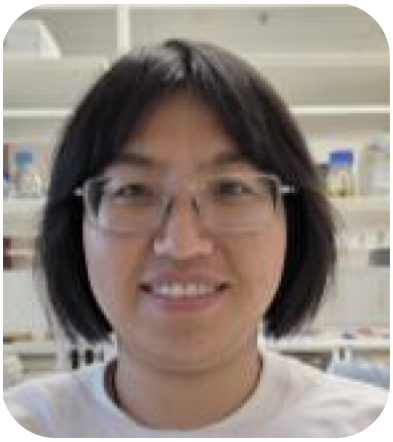
About The Speaker

Mollie Schubert joined IDT in January 2013 where she has been an Innovation Product Manager for the Gene Writing and Editing business unit since May 2022. In this role she manages a team of two associates and is responsible for annual strategic planning portfolio strategy and execution of new product development projects. Previously, Mollie spent 9 years in the Molecular Genetics and CRISPR R&D group at IDT as a research scientist where she focused on studying CRISPR gene editing which included high-throughput screening of CRISPR-Cas9 guides, optimizing the composition and delivery of synthetic guide RNA reagents complexed to recombinant CRISPR nucleases, and developing methods for efficient gene editing with a focus on improvements to homology directed repair. Mollie holds a Master of Science and Bachelor of Science in Biochemistry from Iowa State University and is currently pursuing an MBA from the University of Iowa Tippie College of Business.

Session 3: Functional Genomics

Lu Wang

QIMR Berghofer Medical Research Institute, Queensland, Australia



Invited Speaker

Identification of Breast Cancer-Associated lncRNAs Using 3D and In Vivo CRISPR-Cas13d Screen

About The Speaker

Dr. Lu Wang is an early-career breast cancer genomic scientist in QIMR. She focuses on understanding how the noncoding genome modulates breast cancer development, with the ultimate goal of identifying novel therapeutic targets. She completed her Ph.D. at QIMR with Queensland University of Technology in 2023. She was awarded ACRF Prize for Cancer Research Excellence 2025. Dr. Wang has expertise in non-coding RNA biology, developing 2D/3D CRISPR–Cas13 systems and CROP-seq platforms to uncover regulatory networks driving breast cancer progression.

Abstract

Long noncoding RNAs (lncRNAs) have outnumbered protein-coding genes, yet the majority have unknown function. We previously discovered over 800 lncRNAs at breast cancer risk regions. However, developing a low-cost and accurate 3D model to identify functional genes in high-throughput CRISPR screens is challenging. Here, we devised a scalable breast cancer spheroid model and performed a pooled CRISPR-Cas13d RNA knockdown screen (~20,000 guides) in 3D and in vivo. We identified 50–100 lncRNAs involved in cell proliferation, with about 30% overlap between 3D and in vivo hits. Our findings highlight lncRNAs as mediators of breast cancer risk and introduce a robust spheroid CRISPR platform for mapping phenotypes associated with lncRNAs.

Session 3: Functional Genomics

Ebtihal Mustafa

Peter MacCallum Cancer Centre, The University of Melbourne

Oral

CRISPR-Cas9 Functional Screens and Single-Cell Transcriptomics Define Molecular Drivers and Therapeutic Vulnerabilities in Esophageal Adenocarcinoma

Abstract

Ebtihal Mustafa [1,2],Julia Milne [1,2], Kameng Wu [1], Katherine Papastratos [1], Niko Thio [1,2], Wayne Phillips [1,2,3], Nicholas Clemons [1,2]

[1] *Division of Cancer Research, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.*
[2] *Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia.*
[3] *Department of Surgery (St Vincent's Hospital), The University of Melbourne, Parkville, Victoria, Australia.*

Esophageal adenocarcinoma (EAC) is an aggressive malignancy with poor prognosis due to limited therapeutic options. Despite extensive genomic profiling, functional characterisation of EAC driver genes has remained limited, slowing the development of targeted therapies. Here, we integrated a human Barrett's esophagus-derived tumourigenesis model with pooled CRISPR-Cas9 loss-of-function screening and single-cell RNA sequencing (scRNA-seq) to identify tumour suppressors driving early EAC and delineate their transcriptional consequences. High-grade dysplastic Barrett's cell lines expressing Cas9 were transduced with a pooled library targeting 56 candidate tumour suppressor genes. In vivo screens revealed that loss of multiple genes, including PTEN, STK11, MAP2K7, MAP3K1, and EPHA3, reproducibly promoted tumour formation in NSG mice. Perturb-seq analysis of edited cells revealed four major transcriptional programmes associated with driver genes: (i) cell cycle regulation, (ii) metabolic reprogramming, (iii) RNA processing and protein homeostasis, and (iv) cell-cell communication and motility. Integration with TCGA EAC datasets showed mutually exclusive patterns of mutations across clusters, reflecting functional redundancy.

To study the effect on therapy response, CRISPR screens under carboplatin or paclitaxel treatment identified six genes, NIPBL, PBRM1, AXIN1, RNF43, TGFBR2, and FAM196B, whose loss confers dual resistance, along with additional genes mediating drug-specific resistance. Transcriptional profiling of these chemoresistance-associated perturbations revealed coordinated dysregulation of chromatin dynamics, mitotic spindle assembly, DNA repair, cytoskeletal organisation, and stress response pathways. Collectively, our study provides a comprehensive functional map of EAC molecular drivers, links transcriptional programmes to tumour initiation and chemoresistance, and highlights convergent phenotypic pathways that may be targeted therapeutically. These findings establish a scalable framework for prioritising candidate genes and developing intervention strategies in this genetically heterogeneous malignancy.

Session 3: Functional Genomics

Yexuan Deng

Olivia Newton-John Cancer Research Institute (ONJCRI), Australia

Oral

A novel, high-density CRISPR activation platform for mapping cancer dependencies and resistance pathways ex vivo and in vivo

Abstract

Yexuan Deng[1234], Sarah T. Diepstraten[134], Margaret A. Potts[1234], Amy Heidersbach[5],Christina König[1234], Kristel M. Dorighi[5,] Lin Tai[13], Andrew J. Kueh[1234], Lauren Whelan[13], Catherine Chang[3], Felix Brown[34], Gemma L. Kelly[34], Jean-Philippe Fortin[6], Benjamin Haley[57], John E. La Marca[1234], Marco J. Herold[1234]

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[2] *School of Cancer Medicine, La Trobe University, Bundoora, Melbourne, Australia*
[3] *The Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Australia*
[4] *Department of Medical Biology, University of Melbourne, Parkville, Melbourne, Australia*
[5] *Department of Molecular Biology, Genentech, Inc., South San Francisco, California, USA*
[6] *Computational Sciences, Genentech, Inc., South San Francisco, California, USA*
[7] *Current address: Université de Montréal, Centre de recherche de l'Hôpital Maisonneuve-Rosemont*

CRISPR activation (CRISPRa) enables precise, locus-specific upregulation of gene expression, offering potential for both ex vivo and in vivo applications. However, the lack of scalable, high-coverage tools has limited its use in comprehensive genetic screens, particularly in murine models. Here, we introduce Partita, a next-generation, whole-genome CRISPRa sgRNA platform designed for unparalleled efficiency and depth in gene activation studies.

Partita employs a high-density targeting strategy, deploying ten sgRNAs per transcription start site, structured into five gene family–specific sub-libraries to maximize transcriptional induction. To demonstrate its capabilities, we performed a series of large-scale screens: an in vitro enrichment/depletion screen in immortalised bone marrow–derived macrophages (iBMDMs); whole-genome CRISPRa screens in a double-hit lymphoma model to uncover genes driving resistance to pro-apoptotic drugs (venetoclax, nutlin-3a, etoposide); and an in vivo whole-genome screen identifying accelerators of Myc-driven lymphomagenesis.

Each experiment revealed both expected and novel regulators of cellular phenotypes, with a high validation rate in secondary assays. By enabling robust, high-throughput gain-of-function screening, Partita unlocks new avenues for functional genomics and expands the toolkit for discovering key drivers of biological processes across diverse research fields.

Session 3: Functional Genomics

Liam Neil

Olivia Newton-John Cancer Research Institute (ONJCRI), Australia

Flash | Poster P1-01

Using genome-wide CRISPR-Cas9 knockout screens to sensitise breast cancer to natural killer (NK) cell-mediated killing

Abstract

Liam Neil[1,2], Akash Srivaths[1,2], Tirta Djajawi[1,2], Aleen Alhawani[3,] Stephin Vervoort[3], Belinda Yeo[4], Conor Kearney[1,2], Bhupinder Pal[1,2]

[1]*Olivia Newton John Cancer Research Institute, Melbourne, Victoria, Australia*
[2]*Latrobe University School of Cancer Medicine, Melbourne, Victoria, Australia*
[3]*Walter and Eliza Hall Institute, Melbourne, Victoria, Australia*
[4]*Austin Health, Melbourne, Victoria, Australia*

Breast cancer is the most common cancer among women worldwide and diagnosis rates are steadily increasing. Current therapeutic strategies largely rely on toxic chemotherapies and hormone-based approaches to limit the growth and spread of breast tumours. Despite the progression of immunotherapy as a treatment in many different cancers, it is still lagging for breast cancer patients with only approximately 10% being eligible for the treatment and less than half of these eligible recipients seeing effec ve treatment results. Immunotherapy in breast cancer is heavily reliant on antigen presentation and CD8+ T cell-mediated killing, however breast tumours are known to have low levels of antigen presentation. Therefore, harnessing natural killer (NK) cells which kill tumour cells without requiring specific antigen presentation is a promising yet underexplored avenue to develop new and improved immunotherapy for breast cancer patients.

Using a genome-wide in vitro CRISPR Cas9-knockout screen in human triple-negative breast cancer cells, we have identified several novel targets in breast cancer that can enhance NK cell-mediated killing. When removing our primary target, PTPN11, there is a significant increase in NK cell-mediated killing in vitro. This has also been shown to significantly reduce the primary tumour growth in in vivo syngeneic breast tumour models. The NK cells isolated from these tumours upon cessation of the experiment display increased activation and proliferation, suggesting the loss of PTPN11 from breast cancer increases the anti-tumour activity of the NK cells infiltrating the tumour. Furthermore, we are targeting PTPN11 in metastatic in vivo syngeneic tumour models to determine its role in preventingbreast cancer dissemination and testing its potential synergy with current immunotherapies."

Session 3: Functional Genomics

Antonin Serrano

Collaborative Centre for Genomic Cancer Medicine, University of Melbourne

Flash | Poster P1-02

Investigating chemotherapy resistance using whole genome CRISPR-cas9 screening in pancreatic cancer organoids

Abstract

Antonin Serrano[1,2], Nuoyi Cao[1,2], Twishi Gulati[3], Belinda Lee[4], Tracy L Putoczki[4], Sean Grimmond[1,2], Kaylene J. Simpson[3], Frederic Hollande[1,2]

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[4] *The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.*

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with a five-year survival below 15% in Australia. Most patients are diagnosed with advanced diseases, where cytotoxic chemotherapy is the only modality to prolong survival. FOLFIRINOX is currently the most efficacious and commonly used regimen in PDAC, but most patients rapidly develop resistance and relapse within a short period of time. Chemoresistance is a major bottleneck towards improving the survival outcome of PDAC patients, but the underpinning mechanisms are poorly understood.

Aiming to identify genes crucial for the survival of PDAC cells under FOLFIRINOX, we performed a whole genome CRISPR-Cas9 loss of function screen under chemotherapeutic pressure using patient derived organoids (PDOs). In the primary screen, 19114 genes were invalidated individually in PDOs derived from a primary PDAC tumour. Genes whose invalidation sensitised PDAC cells to FOLFIRINOX were identified and the top 1000 candidates were selected for a secondary screen. We performed the secondary screen in 4 PDOs. >30 robust gene hits were identified, including candidates that are novel in the context of chemoresistance, as well as a few genes that have been previously described in the literature. Currently, we are exploring the mechanism of action of the top candidates and their druggability or the possibility of drug repurposing. The ultimate outcomes of this project will not only contribute to the knowledge of chemoresistance but also foster translation towards novel combination treatments for PDAC patients.

Session 3: Functional Genomics

Leo He

MaxCyte, Inc., Cell and Gene Therapy Services, Waltham, MA,

Industry Presentation | Poster P1-11

Investigating chemotherapy resistance using whole genome CRISPR-cas9 screening in pancreatic cancer organoids

Abstract

Leo He, Ashley Lester, Elijah E. Dabney, Andrew Hollinger, Douglas R. Smith, Thomas E. Mullen

MaxCyte, Inc., Cell and Gene Therapy Services, Waltham, MA,

The specificity of CRISPR-Cas9 gene editing is essential for therapeutic safety and efficacy. Off-target activity can lead to unintended genomic alterations with potential functional or clinical consequences. This study presents a variant-aware, gene-editing risk assessment platform integrating computational and biochemical methods to support rational guide RNA (gRNA) selection and off-target site nomination for therapeutic genome editing. We evaluated eight candidate PCSK9 guides using Guide Profiler™, an in silico tool that screens against the human reference genome and 3,502 haplotype-phased genomes to assess off-target burden and biological risk. Three guides were prioritized and further screened using Guide Select™, a multiplexed biochemical assay that identifies cleavage events across genetically diverse backgrounds. Among the candidates, PCSK9-1 demonstrated the lowest off-target activity. For comprehensive characterization, we employed ONE-seq™, a high-sensitivity, variant-aware assay that integrates in vitro editing with deep sequencing to nominate and biochemically validate genome-wide off-target sites. ONE-seq analysis confirmed minimal cleavage at high-risk loci for PCSK9-1, supporting its candidacy for therapeutic development. Together, this approach demonstrates how combining computational ranking, variant-aware screening, and deep off-target nomination enables more informed gRNA selection and risk assessment. By accounting for population-scale genetic diversity, this strategy enhances the confidence, safety, and regulatory readiness of CRISPR-based therapies.

Session 4: Genome and Epigenome Engineering

Fatimah Jalud

Olivia Newton-John Cancer Research Institute (ONJCRI), Australia

Oral

Hidden Drivers in Non-Coding Regions: UTR Alterations Promote Immune Checkpoint Dysregulation and Oncogenic Activity

Abstract

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We developed a CRISPR/Cas9-based system to generate defined chromosomal translocations that encode for fusion genes commonly implicated in leukaemia. Using this approach, we showed that CRISPR-engineered kinase fusions can drive cytokine-independent growth and can be blocked by tyrosine kinase inhibitors, providing a rapid model for the interrogation of novel putative oncogenic lesions. We then extended this strategy to model non-coding alterations, including disruptions of untranslated regions (UTRs).

UTRs regulate mRNA stability, localisation, and translation through interactions with microRNAs and RNA-binding proteins, acting as post-transcriptional checkpoints that regulate oncogene activity. Despite their regulatory importance, UTR alterations are frequently excluded from diagnostic pipelines, and their role in immune checkpoint and gene dysregulation remains poorly understood in haematologic malignancies. In a patient with primary mediastinal B-cell lymphoma, we identified a novel CD274::PDCD1LG2 fusion generated by an in-frame deletion spanning the CD274 3' UTR, extending into the PDCD1LG2 5' UTR.
This fusion removes conserved regulatory elements that normally suppress PD-L1 and produces a chimeric protein with abnormal PD-L2 trafficking. Using CRISPR, we recreated both the endogenous fusion and targeted CD274 3' UTR deletions in a lymphoma cell line. Functional assays showed the fusion was sufficient to drive PD-L1 upregulation, though at slightly lower levels than UTR deletion alone, demonstrating that CD274::PDCD1LG2 can independently promote immune-checkpoint dysregulation via loss of UTR control.

Building on this principle, we hypothesise that UTR alterations may similarly dysregulate oncogenes in leukaemia. RNA-seq data from clinical cohorts of ETV6::RUNX1 B-ALL patients revealed a recurrent RAG1::IFTAP fusion. As RAG1 harbours conserved 3' UTR regulatory elements, we propose that UTR disruption may contribute to its misregulation. Notably, excess RAG1 activity drives genomic instability and is a recognised cooperating lesion in multiple haematologic malignancies.

These findings highlight UTR disruption as a broader, underappreciated mechanism of immune evasion and oncogene activation in blood cancers.

Session 4: Genome and Epigenome Engineering

Sarah Williams

Peter MacCallum Cancer Centre, The University of Melbourne, Australia

Flash | Poster P1-03

Investigating how CRISPR-defined chromatin regulators maintain epithelial integrity to prevent neoplastic tumours

Abstract

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Epithelial tissues maintain barrier function and regulated proliferation through apical-basal polarity. When polarity is disrupted, tissues activate elimination mechanisms to protect integrity. Failure of these processes can initiate tumorigenesis. However, the molecular pathways that safeguard epithelial structure and suppress abnormal growth remain poorly defined. CRISPR-based approaches now enable precise dissection of these protective mechanisms. I hypothesised that transcriptional repressors linked to the TNF and Hippo signalling pathways, together with chromatin modifiers, act to preserve epithelial integrity by preventing neoplastic overgrowth. Targeted CRISPR perturbations can reveal how these epigenetic regulators prevent neoplastic transformation. This study aimed to identify chromatin regulatory proteins that suppress epithelial tumourigenesis and to define their mechanisms of action within established signalling pathways using CRISPR-based functional genomics in Drosophila melanogaster. A genetic screen using RNA interference and the Gal4–UAS system was performed to deplete candidate transcriptional repressors associated with TNF, Hippo, and chromatin-modifying complexes. Candidate hits were validated with CRISPR/Cas9-engineered mutants and transcriptional reporter assays to assess growth regulation and gene repression.

The screen identified three chromatin regulators, G9a, HDAC1, and Su(var)3-3, as candidate tumour suppressors. The top candidate, the histone methyltransferase G9a, was validated using CRISPR/Cas9-engineered mutants and transcriptional reporter assays to assess epithelial growth regulation and gene repression. CRISPR/ Cas9-mediated loss of G9a induced striking epithelial overgrowth, revealing a direct requirement for this enzyme in epithelial growth suppression. Reporter assays showed that G9a cooperates with the co-repressor CtBP to silence pro-growth genes, linking chromatin repression to polarity and Hippo pathway control. Integrating CRISPR genome engineering with epithelial biology uncovered a chromatin-based tumour-suppressor mechanism mediated by the CtBP–G9a complex. Ongoing CRISPR editing of HDAC1 and Su(var)3-3 will define their distinct epigenetic contributions and clarify whether multiple chromatin-modifier pathways converge to maintain epithelial homeostasis and prevent tumour initiation.

Session 4: Genome and Epigenome Engineering

Kevin Sek

Peter MacCallum Cancer Centre, The University of Melbourne, Australia

Flash | Poster P1-04

Targeting the epignome for precision CAR-T therapy

Abstract

Dr Kevin Sek[1], Woon Xuan Hong[1], Oliver Yu[1], Kah Min Yap[1], Dane Vassiliadis[1], Phil Darcy[1]

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The anti-tumour activity of endogenous or adoptively transferred tumour-specific T cells is highly dependent on their differentiation state. CAR-T cells that are less differentiated have better therapeutic effects due to their ability for self-renewal and long-term persistence. While 'armoured' CAR-T cells have shown some promise for treating solid cancers, current engineering approaches utilizing lentiviral vectors result in constitutive expression, and 'inducible' vectors lack specificity or potency. For example, our lab has demonstrated in a recent publication in Nature, while WT-FOXO1 expression in CAR-T cells promotes a stem-like phenotype and enhancing persistence and therapeutic efficacy, the expression of constitutively active FOXO1-ADA mutant, locks cells in a persistent memory state leading to T cell dysfunction. To address this challenge, we have developed a CRISPR homology directed repair (CRISPR-HDR) strategy enabling armouring gene expression only within the tumour site upon CAR-T activation.

Published in Nature and Nature Communications, we demonstrate delivery of potent cytokines IL-12 and expression of pro-effector A1 adenosine receptor, to drive effector CAR-T differentiation and enhanced tumour control in preclinical solid cancer mouse models. To build on this platform, we have utilized mult-omic integration of Nanopore DNA-seq, RNA-seq, ATAC and single-cell sequencing (DNA+RNA), to identify novel epigenetic networks associated with CAR-T cell activation and memory states. Critically, we identified novel cis-regulatory-gene circuits based on DNA-methylation marks (5hmc and 5mc), which we have utilized to rewire CAR-T cells for context-aware and dynamic payload delivery. We demonstrate delivery of payloads to not just CAR-T cells which dynamically upregulate expression upon T cell activation but also to resting CAR-T cells that downregulate payload expression only upon T cell activation, highlighting the potential of this platform for precision reprogramming of CAR-T cell therapy for solid cancers.

Session 4: Genome and Epigenome Engineering

Hanieh Noeparast

Western Sydney University, Australia

Flash | Poster P1-05

Rapid and sensitive detection of Diaporthe citri causing melanose in Australian finger lime (Citrus australasica) using an RPA-CRISPR/Cas12a molecular diagnostic assay

Abstract

Hanieh Noeparast[1], Michelle Mak[1], Sunil Panchal[1], Paul Holford[1], Frank Bedon[2], Matias Silva Campos[2]

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All citrus species in coastal orchards are susceptible to a fungal disease known as melanose, caused by the fungus Diaporthe citri F.A. Wolf. Melanose infection results in preharvest and postharvest loss of citrus crops by harming the tree and leaves and reducing fruit marketability. Rapid and accurate identification of D. citri makes early intervention possible and supports sustainable orchard management and effective control of its dispersion. However, conventional diagnostic approaches such as morphological identification or PCR-based methods are time-consuming and lab-dependent and may lack specificity when other closely related species are present. In this study, we develop and validate a rapid Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas12a-based diagnostic assay for the detection of this fungal pathogen in finger lime (Citrus australasica), which is a citrus species endemic to Australia. After amplification of the target region using Recombinase Polymerase Amplification (RPA), Cas12a-mediated collateral cleavage activity produces visible fluorescence signals within 30 minutes. Upon binding of the guide RNA (gRNA)–Cas12a complex to the target D. citri DNA, the activated Cas12a enzyme cleaves a fluorescent single-stranded DNA reporter and generates a detectable fluorescence signal.

This diagnostic method shows high specificity, with no cross-reactivity with host plant genomic DNA and no signal generated in the presence of other closely related Diaporthe species. The RPA amplification and CRISPR-Cas12a detection workflow is completed within 30 minutes and can be used as a novel and promising diagnostic tool for rapid, sensitive, and sustainable detection of this fungal pathogen. This study provides a reproducible approach for developing CRISPR-based diagnostic platforms for other agriculturally important fungal pathogens to improve disease management, enhance biosecurity, and support sustainable crop protection strategies.

Session 5: CRISPR Evolution and Diversity

Gaetan Burgio

Australian National University, Australian Capital Territory, Australia

Invited Speaker

Voyage into the mechanisms of CRISPR-Cas12a cleavage activities

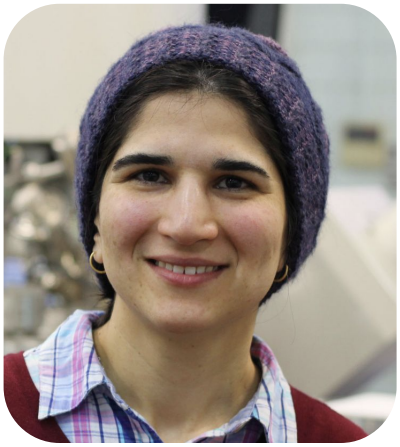
About The Speaker

A/Prof Gaetan Burgio, MD, PhD is leading a research laboratory at the John Curtin School of Medical Research, the Australian National University, Australia. Gaetan completed a medical degree at Paris and then completed a PhD in mammalian genetics at the Pasteur Institute (Paris) and the National Museum of Natural History (Paris). He migrated to Australia in 2008 and joined Prof Simon Foote's laboratory as a postdoctoral fellow. In 2015 Gaetan established his independent research and his laboratory at the Australian National University at Canberra, Australia. Gaetan's research aims to gain a fundamental understanding in the interaction between a pathogen and its host. He has a strong interest on prokaryotic immune defences with a specific emphasis on single strand positive RNA viruses and the development of RNA and CRISPR technologies for gene therapy of molecular detection. His research program uses a combination of computational biology, microbiology, biochemistry and RNA biology to dissect these mechanisms and develop technologies.

Session 5: CRISPR Evolution and Diversity

Marjan-Hadian Jazi

Monash University, Australia



Invited Speaker

Discovery and Design of Novel CRISPR-Cas13 Effectors

About The Speaker

Marjan is a research fellow in the Monash Biomedicine Discovery Institute with a background in computer science and expertise in machine learning techniques. She leverages her programming skills to advance data analysis and artificial intelligence, contributing to the development of cutting-edge solutions in structural biology. Additionally, she is focused on creating novel methods for designing proteins using advanced AI methodologies.

Abstract

Type VI CRISPR-Cas systems encode Cas13, a diverse family of RNA-targeting nucleases central to bacterial immunity and widely adapted for RNA manipulation, diagnostics, and antiviral applications. Despite their versatility, the full structural and mechanistic diversity of Cas13 enzymes remains poorly understood. Leveraging machine learning, we systematically investigated a dataset of around 6,000 Cas13 homologs identified from metagenomic datasets. Structural clustering, phylogenetics, and voxel-based spatial analysis allowed us to uncover previously uncharacterized structural variants.

We used AlphaFold2 [1] to predict the structures of the proteins in our dataset. These predictions provided insights into the family. This is based on the principle that similar folds suggest similar functions, even with low sequence identity. We then computed a pairwise TM-score [2] matrix for the Cas13 homologs, similar to [3]. Unsupervised clustering of this matrix yielded eight distinct structural groups within the family. To explore each group's structural landscape, we used a voxel-based encoding [4] of each 3D protein structure in a grid, with voxel occupancy based on atomic presence and weight. Our results reveal uncovered group-specific variations around the core of the enzyme, suggesting potential differences in RNA recognition, catalytic efficiency, and substrate specificity , contributing to a deeper understanding of Cas13 functional diversity and informing future applications. Our observation of a structural similarity conserved within enzyme core across the identified groups aligns with recent evidence suggesting the structure of early Cas13 proteins [5]. The group-specific variations surrounding this core likely represent evolutionary adaptations driving the functional diversification within this enzyme family.

Session 6: Immunoengineering and Cancer Therapeutics

Michelle Fraser

Senior Director of Cell and Gene Therapy Business Globally, Revitty

Industry Presentation

Advancing Genome Editing Precision: Comparative Insights into CRISPR-Cas9, Base Editing, and AI-Engineered Enzymes

About The Speaker

Michelle Fraser, PhD is Head of Cell and Gene Therapy at Revvity, where she leads strategic initiatives supporting the development and translation of advanced cell and gene therapies. Her work focuses on integrating technologies, services and analytical platforms to accelerate the discovery, development and manufacturing of next-generation therapeutics. Michelle previously led Revvity's base editing and next-generation sequencing product portfolios and holds a PhD in mycology as well as a Graduate Diploma in Science and Technology Commercialisation from the University of Adelaide

Abstract

As our mechanistic understanding of CRISPR-based genome editing deepens, the distinctions among editing platforms are becoming increasingly well-defined. In parallel, approaches for evaluating editing efficiency, fidelity, and off-target effects are evolving from early development through clinical application. Recent advances have uncovered unexpected cellular responses that influence the selection and optimization of editing systems. Artificial intelligence–engineered nucleases and deaminases, incorporating features beyond those of natural variants, are exhibiting enhanced performance in preclinical studies.

At Revvity, we are extending beyond life sciences tools provision to develop proprietary gene-editing and delivery platforms. Our comparative analyses of CRISPR-Cas9 and base editors have revealed marked differences in p53 activation and DNA damage responses—findings with significant implications for clinical translation. Furthermore, by decoupling the DNA-binding and -modifying components of base editing systems, we identified deaminase recruitment kinetics—not intrinsic enzymatic activity—as a key determinant of on-target precision and off-target editing. These insights establish a promising strategy to improve editing specificity and advance the safe clinical deployment of base editing technologies.

Session 6: Immunoengineering and Cancer Therapeutics

Daniel Layton

Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia

Invited Speaker

From Screens to Solutions: CRISPR Knockouts for Next Generation Infectious Disease Countermeasures

About The Speaker

Dr Daniel Layton is a Senior Research Scientist in CSIRO Health and Biosecurity, working at the interface of host-pathogen biology and translational countermeasure development. His research applies genome-wide CRISPR screening approaches (including GeCKO-style libraries) and engineered knockout cell lines to identify host factors that shape infection outcomes and immune signalling. Daniel combines these functional genomics tools with infectious disease models and immunology to map pathways that drive viral replication, pathology, and protective responses, with a track record spanning influenza and other emerging threats.

Session 4: Genome and Epigenome Engineering

Wenxin Hu

Peter MacCallum Cancer Centre, The University of Melbourne

Oral

Systematic silencing of oncogenic fusion transcripts with ultra-precise design of CRISPR-Cas13b

Abstract

Wenxin Hu[1,2], Honglin Chen[1,2], Joshua ML Casan[1,2], Carolyn Shembrey[1,2], Lauren M Brown[3,4,5], Timothy P. Hughes[6], Deborah L. White[6], Ilia Voskoboinik[1,2], Joseph A Trapani[1,2], Paul G Ekert[1,2,3,4,5], Teresa Sadras[1,2] and Mohamed Fareh[1,2,7,*]

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**Correspondence: Mohamed.fareh@petermac.org*

Oncogenic gene fusions are critical drivers of cancer development, yet most of these aberrant genes remain challenging to target with current therapeutic approaches. Thus, there is a need for a programmable platform for the systematic targeting of oncogenic fusion genes in a personalized manner.
CRISPR-PspCas13b (PspCas13b) is a programmable RNA nuclease that has the potential to offer potent, selective, and personalized silencing of various fusion transcripts. In this study, we show that the recognition and cleavage of the breakpoint sequence by PspCas13b enables potent and selective silencing of various fusion transcripts without off-targeting wildtype variants that share extensive sequence homology. We unveil a surprising RNA cleavage, trimming and ligation mechanism near PspCas13b binding site, which generates out-of-frame translation-incompetent fusion transcript isoforms in various human cells.

PspCas13b RNA cleavage enables potent silencing of BCR::ABL1 in patient-derived cancer cells, a critical driver of chronic myeloid leukemia (CML). Notably, targeting the breakpoint sequence also enables efficient degradation of BCR::ABL1 mutant transcripts (e.g., T315I) that often drive resistance to clinically approved Tyrosine Kinase Inhibitor (TKI) drugs and cancer relapse. Silencing T315I BCR::ABL1 RNA in drug-resistant CML cells triggers extensive transcriptomic and proteomic remodelling, causing terminal cell differentiation, cell cycle arrest, and apoptosis, whereas TKI drugs used in the clinic were ineffective, showing no significant impact on the transcriptome or proteome of cancer cells carrying the T315I mutation. Collectively, this study provides a new conceptual framework for systematic, specific, and personalized targeting of undruggable or drug-resistant oncogenic transcripts with ultra-precise design of PspCas13b.

Session 6: Immunoengineering and Cancer Therapeutics

Yu-Kuan Huang

Peter MacCallum Cancer Centre, The University of Melbourne, Australia

Oral

Antigen-heterogeneous solid tumour targeting with tumour-localised T cell engager–expressing T cells

Abstract

Yu-Kuan Huang[1,2], Thang Hoang[1,2], Dat Minh Nguyen[1,2], Phil Darcy[1,2], Cheng-I Wang[3], Paul Beavis[1,2]

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[3]*Singapore Immunology Network, A*STAR, Singapore.*

Adoptive cell therapy (ACT) and bispecific T-cell engagers (TCEs) are powerful immunotherapeutic strategies that redirect T cells to eliminate tumour cells. Chimeric antigen receptor (CAR) T cells are genetically engineered to mediate sustained, antigen-specific cytotoxicity, whereas TCEs are synthetic antibodies that transiently link T cells to tumour antigens via CD3 binding, enabling rapid activation without the need for genetic modification. Despite their promise, both approaches are limited in solid tumours by heterogeneous antigen expression and off-tumour or dose-limiting toxicities. Our lab previously developed a CRISPR/HDR knock-in (KI) strategy that drives payload expression in T cells under the control of the tumour-localised, T cell activation-inducible NR4A2 promoter. Here, we investigate tumour-specific armouring of T cells with TCEs to improve efficacy and safety against antigen-heterogeneous solid tumours.

Murine CAR T cells and OT-I T cells were engineered to secrete HER2-targeting TCEs either (i) constitutively via retroviral transduction or (ii) tumour-localised via CRISPR/HDR KI into the NR4A2 locus. TCE-specificity and T-cell activation were assessed via flow cytometry. Therapeutic efficacy, and toxicity were evaluated in vivo using immunocompetent HER2-transgenic mouse models bearing subcutaneous or mammary fat pad tumours expressing different tumour antigens.

Both constitutive and tumour- localised TCE-expressing T cells showed enhanced anti-tumour efficacy across solid tumour models, accompanied by increased T-cell activation and expansion. However, 30% of mice treated with constitutively TCE-secreting T cells developed severe weight loss (>20%), consistent with systemic toxicity. In contrast, this adverse effect was abrogated when TCE-expression was regulated via NR4A2. Tumour-localised TCE control splenic T-cell localisation, circulating serum TCE levels, and expression of exhaustion-associated markers PD-1 and CD101 on intratumoural cytotoxic T cells.

Tumour-localised control of TCE delivery in ACT enhances therapeutic efficacy while improving safety. This strategy provides a promising solution for targeting antigen-heterogeneous solid tumours while minimising systemic toxicity.

Session 6: Immunoengineering and Cancer Therapeutics

Felix O' Hagan

Peter MacCallum Cancer Centre, The University of Melbourne, Australia

Flash | Poster P2-01

CRISPR-based modulaton of lymph node homing improves CAR T-cell functon in solid tumours

Abstract

Felix O'Hagan[1,2], Yu-Kuan Huang[1,2], Ian Parish[1,2], Paul Beavis[1,2]

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Chimeric Antigen Receptor (CAR) T-cells are highly susceptible to exhaustion upon activation, which limits their efficacy against solid tumours. Our lab recently demonstrated that overexpression of the transcription factor FOXO1 in CAR T-cells significantly improves tumour control through enhanced polyfunctionality and persistence (Chan et al., 2024 Nature). Notably, these cells exhibit increased residency within the tumour-draining lymph node (tdLN). While it is well established that lymph nodes are critical sites for T-cell priming through interactions with antigen-presenting cells, it is unknown if they modulate CAR T-cell responses. Our laboratory has recently developed CRISPR/Cas9-based methodologies that enable knockout or regulated expression of genes of interest (Chen, Yap et al., 2025 Nature), which can be leveraged to test our hypothesis that homing CAR T-cells to the tdLN is beneficial for solid tumour control.

Expression of the lymph node homing receptor CCR7 was modified using retroviral overexpression or CRISPR/Cas9-mediated knockout in murine anti-Her2 CAR T-cells. CAR T-cells were adoptively transferred into a syngeneic breast cancer model, tumour burden was assessed, and tissues were analysed by flow cytometry to determine the underlying mechanisms at play.

Knockout of CCR7 abrogated the enhanced anti-tumour efficacy of FOXO1-expressing CAR T cells, implicating lymph node homing as a key aspect of their enhanced efficacy. Consistent with this observation, CCR7 overexpression in CAR T-cells exhibited significantly improved tdLN residence and intratumoural polyfunctionality. CCR7 overexpression was also associated with the emergence of a CD62L-effector memory population in the tdLN of Her2-tumour-bearing mice, suggesting these cells originate from the tumour. In future work our CRISPR methodologies will be employed to interrogate whether constitutive or induced expression of CCR7 leads to improved therapeutic activity.

Collectively these findings suggest enforced lymph node homing can enhance CAR T-cell therapies against solid tumours and may represent a targetable axis to improve the next generation of CAR.

Session 6: Immunoengineering and Cancer Therapeutics

Yi Tian Ting
Monash University

Flash | Poster P2-02

Why Equal TCR Engineering Is Not Equal: CRISPR-Engineered TCR-Tregs Outperform Lentiviral Approaches

Abstract

Yi Tian Ting[1]

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Autoimmune rheumatic diseases such as systemic lupus erythematosus and Sjögren's syndrome represent a major unmet clinical need. Current treatments still rely heavily on broad-spectrum steroids and biologics, with durable, drug-free remission remaining rare and relapse common after treatment withdrawal.

Antigen-specific regulatory T cell (Treg) therapy offers a promising next-generation approach. Using single-cell RNA sequencing (10x Genomics), we identified a bona fide antigen-specific T cell receptor (TCR) from naturally occurring Tregs isolated from a healthy donor. This TCR was introduced into primary Tregs from healthy donors and patients using lentiviral vectors, achieving >80% transduction efficiency at an MOI of 2. Lentivirally engineered TCR-Tregs demonstrated a 2–3-fold improvement in antigen-specific suppression of pro-inflammatory T cells compared with non-engineered polyclonal Tregs.

To overcome limitations associated with viral TCR delivery, we applied a single-step CRISPR knock-out/knock-in strategy. By deleting the endogenous TCR and precisely inserting the therapeutic TCR into the TRAC locus, we generated uniform antigen-specific TCR-Tregs with controlled receptor expression. CRISPR-engineered TCR-Tregs showed approximately 20-fold greater immunosuppressive activity than lentivirally engineered TCR-Tregs, driven by ~40-fold higher surface expression of the therapeutic TCR.

These findings reveal that therapeutic TCRs are not functionally equivalent across engineering platforms or across donor T cell repertoires, particularly for autoreactive or low-affinity TCRs where mispairing and expression competition limit efficacy. Overall, this work demonstrates that CRISPR-based TCR replacement enables superior potency, consistency, and functional performance, supporting its development as a scalable regulatory T cell therapy for autoimmune rheumatic diseases

Session 6: Immunoengineering and Cancer Therapeutics

XiaoJing (XJ) Ong

Peter MacCallum Cancer Centre, The University of Melbourne a

Flash | Poster P2-03

Overcoming CAR-T manufacturing barriers for T-cell lymphoma using CRISPR-HDR

Abstract

XJ. Ong[1,2], T. Nguyen[1], K. Simpson[1,2], V. Qin[1,2], X. Wang[1,2], E. Roussel[1,2], E. ORourke[1], R. Castle[1], J. Casan[2,3], C. Weyden[2,3], P. Beavis[1,2], HM. Prince[2,3], J. Zhu[1,2], C. D'Souza[1,2], P. J. Neeson[1,2]

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The recent success of CAR-T cell therapies has revolutionized treatment for relapsed/refractory (r/r) B-cell lymphomas. However, patients with r/r T-cell lymphomas (TCL) continue to face limited treatment options and poor clinical outcomes, with no approved CAR-T therapies available. While CAR-T cell therapy is a promising therapeutic option for TCL, its development is hindered by the difficulty in differentiating malignant from healthy T cells due to antigen sharing and limited healthy T cells for autologous CAR-T production. Hence, to circumvent these obstacles, we sought to develop allogeneic CAR-T cells (AlloCAR) using healthy donor T cells.

Utilising CRISPR-Cas9 Homology-directed Repair (HDR), we generated KIR3DL2-directed AlloCAR-T cells by double knock-in of CAR into T-cell receptor Alpha Constant (TRAC) and HLA-E into the 2-microglobulin (2M) gene locus. This strategy not only eliminates endogenous T-cell receptor expression to prevent graft-versus-host disease but also prevents graft rejection by knocking out MHC-1 molecules. The overexpression of HLA-E further inhibits host NK-cell-mediated cytotoxicity against AlloCARs.

AlloCARs were successfully generated with >50% double-knock-in and >80% double-knock-out efficiency, achieving a 60-fold expansion over 10 days (n = 3). AlloCARs demonstrated comparable anti-tumor efficacy to lentiviral-transduced CAR-T cells (lentiCAR) against TCL cell lines. Notably, AlloCARs exhibited a more stem-cell memory-like phenotype (CD45RA+ CD62L+) and higher proliferative capacity against TCL cells in a 5-day CTV Proliferation Assay compared to lentiCAR, consistent with a more physiological expression of CAR molecules under the TRAC promoter. AlloCARs also exhibited robust anti-tumour potency against ex vivo TCL patient tumour samples.

In summary, we developed a CRISPR-engineered off-the-shelf CAR-T therapy with potent activity against TCL cell lines and ex vivo patient samples. This provides a rational strategy to overcome manufacturing barriers of CAR-T therapy for TCL and supports AlloCAR-T cells as a promising therapeutic strategy.

Session 6: Immunoengineering and Cancer Therapeutics

Jacinta Macdonald

Institute for Biomedicine and Glycomics, Griffith University, Queensland, Australia

Flash | Poster P2-04

CRISPR-mediated gene editing to validate targets of malaria parasite drug leads

Abstract

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[4]*Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee DD15EH, UK*

In 2024, there were around 282-million cases and 610,000 deaths due to the parasitic disease malaria. Malaria prevention and control remains a challenge due to the lack of effective vaccines and antimalarial drug resistance. To address this, the identification of new drugs with novel targets is a priority. In vitro evolution of resistance and whole genome analysis (IVEWGA) followed by target validation using reverse genetics and other tools, is a technique commonly employed to identify putative targets and, or resistance mechanisms of novel drug leads. We have previously demonstrated that the indoloquinolizidine alkaloid, alstonine, and novel 1,3,4-oxadiazoles inhibit the in vitro growth of Plasmodium falciparum malaria parasites by different mechanisms than current malaria drugs.

To further understand the action of these compounds, we used IVEWGA to identify putative genes that may be linked to compound action. A single nucleotide polymorphism (SNP) and a copy number variation in the gene encoding the putative inner-mitochondrial membrane protein, MPV17, was detected in alstonine-resistant parasites, and two SNPs in the palmitoyltransferase DHHC7 was detected in 1,3,4-oxadiazole-resistant parasites. Introduction of these SNPs into wildtype P. falciparum using CRISPR/Cas9 gene editing resulted in resistance to the selection compound, indicating an involvement of these genes in compound action. Orthogonal approaches such as solvent proteome profiling will be used in future work to confirm target versus resistance mechanisms. In addition, the CRISPR-edited parasite lines generated in this work are new tools for target-based screens to expand our antiplasmodial toolkit.

Session 7: Responsible Innovation and Ethics

Christopher Gyngell

The University of Melbourne

Invited Speaker

The Ethics of Polygenic Genome Editing

About The Speaker

Christopher Gyngell is an Associate Professor in the Department of Paediatrics at the University of Melbourne and Team Leader of the Biomedical Ethics Research Group at the Murdoch Children's Research Institute. His research focuses on the ethical, legal, and social implications of emerging genetic technologies, with a particular focus on genome editing and genomic data. He has published widely on the ethics of gene editing in journals including Nature, Bioethics, and the Journal of Medical Ethics.

Abstract

Advances in genome editing technologies such as CRISPR-Cas9 have made it possible to precisely alter human DNA. While early research has focused on single-gene disorders, recent developments raise the prospect of editing multiple genetic variants simultaneously to reduce the risk of common polygenic diseases such as cancer, heart disease, and mental health disorders. Drawing on modelling of polygenic risk scores and genome-wide association studies, this talk explores the potential of genome editing to reduce the disease burden of polygenic diseases. It also examines the key ethical concerns this use of the technology raises, including safety, inequality, effects on future generations, and the line between therapy and enhancement.

Session 8: Translational CRISPR: Delivery and Diagnostics

Fei Deng

School of Biomedical Engineering, University of New South Wales, Australia

Oral

Advancements in CRISPR-based Diagnostics

Abstract

Fei Deng[1],

[1]*School of Biomedical Engineering, University of New South Wales, Australia*

Control of CRISPR/Cas12a trans-cleavage is crucial for biosensor development. Here, we show that small circular DNA nanostructures which partially match guide RNA sequences only minimally activate Cas12a ribonucleoproteins. However, linearizing these structures restores activation. Building on this finding, an Autocatalytic Cas12a Circular DNA Amplification Reaction (AutoCAR) system is established which allows a single nucleic acid target to activate multiple ribonucleoproteins, and greatly increases the achievable reporter cleavage rates per target. A rate-equation-based model explains the observed near-exponential rate trends. Autocatalysis is also sustained with DNA nanostructures modified with fluorophore-quencher pairs achieving 1 aM level (<1 copy/uL) DNA detection (106 times improvement), without additional amplification, within 15 mins, at room temperature. The detection range is tuneable, spanning 3 to 11 orders of magnitude. We demonstrate 1 aM level detection of SNP mutations in circulating tumour DNA from blood plasma, genomic DNA (H. Pylori) and RNA (SARS-CoV-2) without reverse transcription as well as colorimetric lateral flow tests of cancer mutations with ~100 aM sensitivity.

Dr Deng is currently an NHMRC EL1 Fellow and NSW Cancer Institute ECR Fellow. Dr Deng's research interests include point-of-care biosensing devices, CRISPR biosensing device and in vivo biosensing device. To date, Dr Deng has published over 50 journal articles, including Nature Communications, Nucleic Acids Research, AFM, Trends in Analytical Chemistry, Small, Water Research, BBI etc. In addition, he has secured over $12M research grants, including AEA, CRCP, NHMRC Idea Grant, ARC Discovery Project etc. His biosensor research has been internationally recognized, leading to a 2025 NSW Premier's Awards (Finalist), an ECR Award from Royal Society of NSW, and an ECR Award from ECAN.

Session 8: Translational CRISPR: Delivery and Diagnostics

Wei Deng

The University of Technology Sydney (UTS), Australia

Invited Speaker

Lipid nanoparticle-delivered CRISPR-mediated VEGFA gene editing for in vitro retinal disease treatment

About The Speaker

A/Prof Wei Deng is Head of the Nanomedicine group in the School of Biomedical Engineering at UTS. Her multidisciplinary research integrates nanobiotechnology, oncology, biomedical science and pharmaceutical manufacturing to develop innovative technologies for the treatment of cancer and genetic disorders. Wei received her PhD in nanobiotechnology from Macquarie University. She began her academic career in nanocarrier delivery research as a DECRA Fellow at Macquarie University, later establishing her own laboratory at UNSW before joining UTS, where she leads the Nanomedicine Group. Wei has published numerous high-impact papers across materials science, pharmaceutics, biochemistry and biomedical engineering, and holds multiple patents in the field of gene/drug delivery, several of which have been licensed to industry. Her flagship innovation focuses on developing safe, affordable and effective gene delivery platforms to accelerate the clinical translation of gene therapies, including CRISPR.

Session 8: Translational CRISPR: Delivery and Diagnostics

Alexandra Farcas

Technical University of Cluj-Napaca, Romania

Oral

Computational enhancement of non-viral CRISPR/Cas9-gold nanoparticle delivery platform design

Abstract

Alexandra Farcas[1,2]

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CRISPR/Cas9-based gene therapy offers several advantages over conventional gene therapy approaches by enabling precise targeted genome editing. A primary challenge in deploying this technology lies in developing gene delivery vectors capable of selectively targeting specific genetic mutations. In the context of treating inherited disorders such as muscular dystrophy, in vivo delivery of CRISPR/Cas9 components has been achieved using oligonucleotide-functionalized gold nanoparticles (GNPs). Given that current CRISPR/Cas9-GNP clinical trials are in preliminary stages, it is crucial to design delivery vectors with minimal risk of insertional mutagenesis, informed by molecular insights into the underlying biological systems. We employed a two-step computational optimization process to enhance the design of CRISPR/Cas9-Gold-based delivery vehicles. First, we optimized the DNA loading capacity on GNPs of various sizes within nanoparticle-oligonucleotide conjugates. Subsequently, we conducted molecular dynamics simulations of the Cas9/sgRNA complex to assess its structural stability and interactions within the delivery system. This systematic approach is critical for improving the efficacy of nanoparticle-mediated CRISPR/Cas9 delivery.

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Session 8: Translational CRISPR: Delivery and Diagnostics

Michael Keating

Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia

Oral

Packaged CRISPR lipid nanoparticles as a research tool and treatment modality for cardiovascular disease

Abstract

Michael F. Keating[1, 4], Christine Yang[1], Yingying Liu[1], Kevin Liu[1], Douglas P. Loesch[2], Scott C. Ritchie[3], Michael Inouye[3], Brian G. Drew[1,4,5]

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[4]*Baker Department of Cardiometabolic Disease, University of Melbourne, Melbourne, Victoria, Australia*
[5]*Central Clinical School, Department of Medicine, Monash University, Melbourne, Victoria, Australia*

Coronary artery disease (CAD) is a significant health-burden worldwide and there exists a need to explore new strategies to treat disease onset and progression.

To achieve this, we employed a multi-ancestry genetic meta-analysis of plasma proteins that were associated with risk for developing CAD, from participants in the UKB and All of Us cohorts. From this we identified known causal mediators (APOE, PCSK9) and novel candidate plasma proteins that significantly associated with CAD risk. One such novel candidate was ITIH4, a liver enriched secreted factor which was associated with increased CAD risk. To further our mechanistic understanding of ITIH4, we harnessed CRISPR editing technology delivered with lipid nanoparticles to the liver of mice. Specifically, Cas9 transgenic mice were injected with encapsulated gRNAs targeting GFP (control), the low-density lipoprotein receptor (LDLR) alone, or dual targeting of both LDLR and ITIH4 in combination. Mice were fed a western diet to drive hypercholesterolemia and atherosclerotic phenotypes and followed for 20 weeks.

At the study endpoint liver and plasma were collected for analysis. We confirmed that CRISPR editing resulted in a 60% ($p<0.0001$) decrease in hepatic LDLR protein expression compared to control. Mice receiving dual targeting had a 55% ($p<0.001$) and 85% ($p<0.0001$) decrease in hepatic LDLR and ITIH4 expression respectively. Circulating levels of ITIH4 were also substantially decreased in dual targeted mice. Reduced hepatic LDLR expression led to an increase in plasma cholesterol levels in edited mice (7.7 vs 9.82 vs 8.20 mmol/L, control vs LDLR alone vs combination; $p<0.01$), with ITIH4 depletion demonstrating a significant reduction in various disease-associated plasma lipids, including cholesterol.

Our results suggest that targeted knockdown of ITIH4 using CRISPR technology may protect against hypercholesterolemia that drives CAD in mammals. Secondly, our model offers a highly feasible and cost-effective approach to validate potential disease-causing genes in the liver.

POSTER SESSION I

Tuesday 24 March

16:30 - 18:30

P1-01* **Liam Neil**
Using genome-wide CRISPR-Cas9 knockout screens to sensitise breast cancer to natural killer (NK) cell-mediated killing

P1-02* **Antonin Serrano**
Investigating chemotherapy resistance using whole genome CRISPR-Cas9 screening

P1-03* **Sarah Williams**
Investigating how CRISPR-defined chromatin regulators maintain epithelial integrity to prevent neoplastic tumours

P1-04* **Kevin Sek**
Targeting the epigenome for precision CAR-T cell therapy

P1-05* **Hanieh Noeparast**
Rapid and sensitive detection of Diaporthe citri causing melanose in Australian finger lime (*Citrus australasica*) using an RPA-CRISPR/Cas12a assay

P1-06 **Michael Gitonobel**
CRISPR-Cas9 base-editing as a versatile tool to unravel causality of genetic variants in HEK293T cells

P1-07 **Wendy Jia**
Challenges in developing a novel screen to identify 3D genome architecture regulators

P1-08 **Barnaby Kelly**
Identification of the mechanisms underlying poor response to standard of care therapy in childhood and adolescent osteosarcoma through whole genome CRISPR screens

P1-09 **Lynda Truong**
FACS-based whole genome CRISPR screen complemented by a bespoke GFP reporter system to identify upstream regulators of SNAI1

P1-10 **Shourya Giri**
Experimental analysis of the genomic determinants of tissue-specific splicing

P1-11 **Leo He** *(MaxCyte)*
A variant aware framework for reducing off-target risks in therapeutic guide RNA selection

P1-12 **Tal Shamia** *(Synthego)*
AccuBase™: A GMP-grade protein-format cytosine base editor (CBE) enabling safer, high-performance gene editing

* Flash talk abstracts are included under their respective session abstracts and are identified by poster number

POSTER SESSION II

Tuesday 24 March

16:30 - 18:30

P2-01* **Felix O'Hagan**
CRISPR-based modulation of lymph node homing improves CAR T-cell function in solid tumours

P2-02* **Yi Tian Ting**
Why equal TCR engineering is not equal: CRISPR-engineered TCR-Tregs outperform lentiviral approaches

P2-03* **XiaoJing Ong**
Overcoming CAR-T manufacturing barriers for T-cell lymphoma using CRISPR-HDR

P2-04* **Jacinta Macdonald**
CRISPR-mediated gene editing to validate targets of malaria parasite drug leads

P2-05 **Andrea Di Pietro**
Rewiring gene regulatory networks to reverse melanoma cell dedifferentiation and overcome ICI resistance by CRISPR perturbation

P2-06 **Zak Janetzki**
Editing of HBV DNA and RNA in vitro using a combination of CRISPR/Cas9 cytosine-to-thymine base editors and CRISPR/Cas13b

P2-07 **Laura McCoullough**
The significance of viral genomic variability in developing Cas13b-based therapy for chronic hepatitis B infection

P2-08 **Kristie Dickson**
Mutant p53 and ovarian cancer: examining therapeutic opportunities using CRISPR-engineered p53 wild-type, knockout and gain-of-function ovarian cancer panels

P2-09 **Gaoyuan Wang**
Combining CRISPR screen, scRNAseq, and spatial techniques to study venetoclax resistance mechanism in aggressive lymphomas

* Flash talk abstracts are included under their respective session abstracts and are identified by poster number

Poster Session I

Michael Gitonobel

Monash University, Melbourne, Australia

Poster P1-06

CRISPR-Cas9 base-editing as a versatile tool to unravel causality of genetic variants that drive splicing changes in HEK293T cells

Abstract

Michael Gitonobel[1, 2, *], Kai Wei Tan[1, 2, *], Aaryan Chhabra[1], Aiswarya Balakrishnan[1, 3], Tamara Tongoi[2], Joseph Rosenbluh[2], Sridevi Sureshkumar[1], and Sureshkumar Balasubramanian[1, 2,$ *]

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The advent of large-scale genome-wide association studies (GWAS) allowed us to determine genetic variants associated with certain phenotypes or molecular traits. This development holds an immense value as variants associated with genetic diseases, even cancer, can now be identified. However, most studies typically do not have the possibility to experimentally demonstrate the causality of these variants; instead, they often proved the associations by observing different genetic variants carried by subpopulations with differing traits. Many such traits are caused by aberrant splicing, which formed defective mRNA transcripts and dysfunctional proteins. Nevertheless, there has not been a systematic effort to perturb the genetic variants associated with phenotypes to ascertain causal relationships with an experimental approach.

In this study, we used a CRISPR-Cas9 base editor-based assay to test for the causality of genetic variants that are associated with changes in splice-site usage. By performing hundreds of thousands of GWAS with the usage variation of individual splice sites in the human heart and testis tissues, we uncovered the association between thousands of SNPs and variation in splice site usage. We systematically selected variants for this study through a pipeline of stringent parameters. sgRNAs targeting these variants were cloned into CRISPR-Cas9 base editor-expressing plasmids, which were transfected into HEK293T cells. We subsequently performed RNA extractions and RT-PCR to compare splicing pattern in transfected and non-transfected cells, as well as DNA extractions and Sanger sequencing to verify successful base editing. Of the 9 SNPs successfully edited, we were able to prove that 7 of them were indeed causal in driving splicing changes. This study becomes one of the very first successful implementations of CRISPR-Cas9 base-editing to demonstrate the causality of genetic variants associated with molecular phenotypes and demonstrated the power of the CRISPR-Cas9 base-editing approach to demonstrate causality in eQTL/sQTL studies.

Keywords: CRISPR-Cas9 base-editing, GWAS, splice site usage, mRNA splicing, causative variant.

Poster Session I

Wendy Jia

Walter and Eliza Hall Institute of Medical Research (WEHI), The University of Melbourne

Poster P1-07

Challenges in developing a novel screen to identify 3D genome architecture regulators

Abstract

Wendy Jia[1,2], Hannah D Coughlan[1,2], Tom S Weber[1,2], Oliver Ozaydin[1,2], Jakob Schuster[1,2], Mathew Chu[1,2], Miles B Horton[1,2], WingFuk Chan[1,2], Daniel V Brown[1,2], Esther Bandala Sanchez[1,2], Michael J.G. Milevskiy[1,2], Sarahi Mendoza Rivera[1,2], Jane E Visvader[1,2], Matthew E Ritchie[1,2], Rory Bowden[1,2], Gordon K Smyth[1,3], Stephin J Vervoort[1,2], Shalin H Naik[1,2], Rhys S Allan[1,2,#], Timothy M Johanson[1,2,#]

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 # These authors contributed equally

The eukaryotic genome is a polymer structure organised in three dimensions (3D) to generate a hierarchical organisation that is crucial for cell function and identity. Despite efforts to characterise the molecular machinery driving this genome organisation, relatively few proteins have identified as universal genome organisers. To address this, we developed a CRISPR-based screen called 3CaPER (Chromatin Conformation Capture with Prime Editing and RNA knockdown) to uncover novel regulators of 3D genome organisation. This innovative screen couples Cas13d mediated knock-down (KD) of a panel of candidate genes with prime editing-driven cellular barcoding that can be read out using barcode enriched long read chromosome conformation capture (Capture Pore-C) to directly resolve the impact of target gene KD on genome organisation.

As a proof-of-concept study, we cloned a sub-library containing 28 gene candidates, including known 3D genome regulators and apoptotic pathway genes to account for cell death in the screen read out. Analysis of the sub-library screen revealed technical limitations leading to a weak signal to noise ratio, and thus the inability to connect gene KD with loss of genome architecture. Our experience highlights both the challenges and opportunities for high-throughput approaches to discover novel regulators of genome organisation.

Keywords: 3D genome, Prime editing, CRISPR screen, Nanopore sequencing, Capture Hi-C

Poster Session I

Barnaby Kelly

Hudson Institute of Medical Research, Monash University, Melbourne, Australia

Poster P1-08

Identification of the mechanisms underlying poor response to standard of care therapy in childhood and adolescent osteosarcoma through whole genome CRISPR cut screens.

Abstract

Barnaby Kelly[1,2], Elise Young[1,2,3], Jennie Do[1,2], Vincent Xue[1,2], Ron Firestein[1,2] Alejandro Sweet-Cordero[4], Leane Sayles[4], Murray Manning[5], Sefi Rosenbluh[5], Vijesh Vaghjiani[1,2] & Jason Cain[1,2]

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[4]*University of California, San Francisco (UCSF), California, USA.*
[5]*Monash Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia*

Osteosarcoma (OS) is the most common primary bone tumour affecting teenagers and young adults. Neoadjuvant chemotherapy increased 5-year survival rates from 10% to ~70% for patients with localised disease. However, ~70% of patients will develop metastatic disease during their treatment, with 5-year survival rates of 20% for recurrent and metastatic disease, often related to the generation of treatment resistance to agents such as Cisplatin. This study used CRISPR Cas9 and Cas12 screening technologies to identify mediators of cisplatin resistance and identify targets involved in DNA repair that drive chemotherapy drug resistance.

Following whole genome pooled CRISPR KO screens, we designed a custom Cas9-KO cisplatin resistance library (8699 constructs, containing 5 gRNAs per gene) and at an MOI of
0.3 and a minimum representation of 1000x validated resistance mechanisms across 4 osteosarcoma cell lines, U2OS, OS384, OS186 and HOS. Comparison of gRNA abundance between cisplatin treatment and vehicle control revealed genes that are sensitised to (decreased representation) or enhanced resistance to (increased representation) cisplatin.

The results revealed that loss of genes associated with DNA repair, DNA replication and anti-apoptotic signalling enhanced sensitivity to cisplatin. For example, targets involved in the Fanconi anaemia pathway and homologous recombination, such as FAAP24, FANCA and ATR were found to increase sensitivity to cisplatin. Furthermore, harmonisation of functional screening data with whole genome transcriptomic data from a novel cisplatin-resistant OS model highlighted functional gene dependencies and pathways to be targeted in both in vitro and in vivo drug studies.

Overall, this data provides insights into cisplatin resistance mechanisms in OS, reveals biomarkers of predictive cisplatin response, and identifies new therapeutic strategies to improve tumour response to cisplatin. Our ongoing work is validating these mechanisms using both repurposed and clinical trial therapeutics.

Poster Session I

Lynda Truong

Hudson Institute of Medical Research, Monash University, Melbourne, Australia

Poster P1-09

FACS-based whole genome CRISPR screen complemented by a bespoke GFP reporter system can successfully identify upstream regulators of SNAI1 in Acute Myeloid Leukaemia.

Abstract

Lynda Truong[1], Steve Lin[1], Claire Sun[1], Sukhpreet Singh[1], Jake Shortt[2], Catherine Carmichael[1]

[1] *Centre for Cancer Research, Hudson Institute of Medical Research, Clayton, VIC, Australia.*
[2] *Department of Medicine, School of Clinical Sciences, Monash Health, Clayton, VIC, Australia*

Acute Myeloid Leukaemia (AML) is one of the most genetically complex and aggressive cancers worldwide. ~1000 Australians are diagnosed with AML annually, with 5-year survival rates resting at 26%. Although most of the underlying genetic mutations have been identified, the inherent genetic heterogeneity across patients remains a limiting factor for current targeted therapies.

Our previous works have shown that SNAI1 protein expression is significantly upregulated in ~50% of AML patients and is correlated with worse overall prognoses. SNAI1 is not normally expressed during haematopoiesis, suggesting that it is upregulated in AML for a specific pathogenic reason. Thus, knocking it down or reducing its expression is predicted to have therapeutic benefit. So far, there has been no effective method to target SNAI1 chemically. To determine what is responsible for high SNAI1 expression in AML, we propose our reporter system in tandem with whole-genome CRISPR screening can determine which regulators are responsible for high SNAI1 expression in AML. We can then screen for druggable targets to identify pathogenic weaknesses in SNAI1 high AMLs.

We have developed a bespoke reporting tool for endogenous SNAI1 expression in AML to elucidate the mechanisms by which SNAI1 is highly expressed in AML and what upstream regulators may be responsible.

We have been able to identify 1820 positively enriched gene hits and 777 negatively enriched gene hits for repressing and promoting SNAI1 protein expression respectively. Analysis of SNAI1 promoting gene hits identified key regulators known to physically interact with SNAI1, transcription factors involved in chromatin remodelling, and oncogenic networks known to promote pathogenicity in AML but also in SNAI1-driven solid malignancies.

With these findings we will be able to compare the regulatory profile upstream of SNAI1 in AML to other well documented cancers. We hypothesise that the regulatory pathways promoting expression of SNAI1 are similar to what we find in current literature for SNAI1-driven solid malignancies.

Poster Session I

Shourya Giri

Monash University, Melbourne, Australia

Poster P1-10

Experimental analysis of the genomic determinants of tissue-specific splicing

Abstract

Shourya Giri, Aaryan Chhabra, Sridevi Sureshkumar and Sureshkumar Balasubramanian*

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Differential splicing is fundamental to transcriptome diversity in multicellular organisms, enabling fine-tuned gene expression and the generation of proteins with specialised functions. Tissue-specific splicing, a major form of context-dependent alternative splicing, involves preferential usage of distinct splice sites across tissues, often resulting in functional diversification of gene products. Classical models of splice-site selection emphasise canonical cis-regulatory elements (5' splice site, 3' splice site, branch point sequence and polypyrimidine tract), auxiliary enhancer and silencer motifs, RNA secondary structure, and recruitment of RNA-binding proteins (RBPs). However, in many tissue-specific contexts, these local genomic features are conserved across tissues, yet splice-site choice differs markedly. This discrepancy highlights a key limitation of existing models in fully explaining tissue-specific splicing regulation.

Although several RBPs exhibit tissue-specific expression, a systematic analysis of the underlying genomic determinants governing tissue-specific splice-site choice is lacking. Recently, we identified putative genomic determinants of tissue-specific splicing from a large-scale genome-wide association study (GWAS) involving over one million individuals (Chhabra and Balasubramanian, personal communication). Here, we test whether these determinants causally regulate tissue-specific splicing using CRISPR-based genome editing approaches.

I will present our latest findings from this ongoing project. Our work advances the mechanistic understanding of splice-site regulation and establishes a framework for rational, context-dependent modulation of splicing, with direct relevance to therapeutic engineering of gene expression in disease.

Poster Session I

Tal Shamia

Synthego

Poster P1-12

AccuBase™: A GMP-grade protein-format Cytosine Base Editor (CBE) enabling safer, high-performance gene editing

Abstract

Guanglei Li[1], Tianhong Xu[1], **Tal Shamia**[2], Hana Kim[2], and Casey Jowdy[2]

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Base editing has enabled precise single-base changes without inducing double-strand breaks (DSBs). AccuBase, Synthego's GMP-grade cytosine base editor (CBE), is an engineered, protein-based CBE that delivers the enhanced gene editing precision, safety, and quality necessary for CRISPR-based therapeutics. By converting C•G base pairs to T•A, AccuBase can introduce stop codons in coding sequences to achieve efficient gene knockouts, disrupt splice donor/acceptor sites for exon skipping, and silence genes with unprecedented control. This capability mirrors that of CRISPR nucleases for gene knockout while eliminating the risks of indels, chromosomal rearrangements, and persistent DNA disruption, making it particularly suited for therapeutic applications. Recently, the AccuBase CBE RNP has demonstrated its therapeutic potential in edited Natural Killer (NK) cells, achieving IND approval and advancing to clinical trials aimed at treating solid tumors.

Unlike mRNA-based CBEs that require translation, AccuBase is delivered as a ready-to-use ribonucleoprotein (RNP) complex, enabling immediate action and a short half-life, limiting editing to a brief, controlled window. The transient exposure dramatically minimizes genomic side effects and avoids triggering DNA-damage response (e.g., p53 activation). AccuBase demonstrates a superior safety profile, with no detectable off-target single-nucleotide variants; even multiplex editing across multiple loci yields virtually no detectable chromosomal translocations or large-scale indels. Furthermore, the engineered AccuBase achieves high knockout efficiencies in primary T cells, without compromising cell viability or editing precision. This controlled approach enables researchers to perform precise, multiplexed edits in a single step, accelerating research timelines while maintaining stringent safety requirements, features important for therapeutic developers.

Incorporating AccuBase alongside our best-in-class GMP-grade gRNAs, streamlines therapeutic development by providing a comprehensive, GMP-compliant editing solution. This bundled offering, complete with full quality documentation, allows developers to seamlessly incorporate AccuBase into clinical workflows. AccuBase is a next-generation CBE solution, providing researchers with a safer and more effective alternative to mRNA-based editors. By addressing key industry challenges, including editing efficiency, multiplexing capability, off-target risk mitigation, and regulatory readiness, AccuBase represents a critical advancement in the field of CRISPR-based gene editing, enabling the development of life-changing, therapeutic-grade solutions.

Poster Session II

Andrea Di Pietro

Peter MacCallum Cancer Centre, The University of Melbourne

Poster P2-05

Rewiring gene regulatory networks to reverse melanoma cell dedifferentiation and overcome ICI resistance by CRISPR perturbation

Abstract

Andrea Di Pietro[1,2], Lewis Au[1,2], Ruiqing Zhu[3], Patrick Crock[3], Niko Thio[3], Sean Macdonald[2], Fanny Bouquet[4], Mitch Levesque[5], Sonia Mailer[2,6], Kristy Barnes-Cullen[2,6], Karen Winch[2,6], Lavinia Spain[7], Aparna Rao[1,7], Shahneen Sandhu[1,7], David Gyorki[8], Grant McArthur[1,7,9], Paul J. Neeson [1,2]

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[8]*Division of Cancer Surgery, Peter MacCallum Cancer Centre, Melbourne, Australia*
[9]*Cancer Biology and Therapeutics Program, Peter MacCallum Cancer Centre, Melbourne, Australia*

Resistance to immune checkpoint inhibitor (ICI) therapy in melanoma is frequently associated with tumour-intrinsic transcriptional plasticity and chronic inflammatory signalling. In particular, sustained interferon-gamma (IFNγ) exposure can drive adaptive immune resistance, melanoma de-differentiation, and immune evasion. However, the gene regulatory networks (GRNs) supporting resistant melanoma cell states, and their potential as targets, remain poorly defined.
Lymph node melanoma metastases from untreated and ICI-treated patients (n = 42) were profiled using multi-omic single-cell sequencing (RNA- and ATAC-seq) and spatial transcriptomics (VisiumHD). Integrated analyses were performed to reconstruct melanoma cell–intrinsic GRNs associated with ICI resistance. Network inference and in silico perturbation analyses were used to identify candidate regulatory nodes. These targets are being prioritised for functional interrogation using CRISPR-based loss-of-function and transcriptional perturbation approaches in patient-derived melanoma cell lines.

ICI-resistant melanoma cells exhibited a conserved transcriptional programme characterised by chronic IFNγ and type I interferon responses, activation of IL6–JAK–STAT3 signalling, and a de-differentiated neural crest–like state. Spatial transcriptomics confirmed enrichment of IFNγ-response and de-differentiation signatures within resistant tumour regions, associated with immune exclusion of effector CD8+ T cells. GRNs in ICI-resistant melanoma cells showed distinct regulatory changes compared to untreated cases. We observed suppression of differentiation-associated transcription factors, such as KLF4, and reinforcement of inflammatory and stress-response pathways. In silico CRISPR perturbation of key GRN nodes predicted partial reversion of resistant transcriptional states and restoration of melanoma differentiation programmes.

Melanoma resistance to ICI therapy is driven by sustained IFNγ signalling that promotes changes in tumour cell–intrinsic GRN and transcriptional plasticity. Our integrative multi-omic framework identified actionable regulatory targets that can be directly edited using CRISPR-based functional genomics. These findings provide a rational foundation for genome editing strategies aimed at reprogramming resistant melanoma cells to restore tumour immunogenicity and therapeutic sensitivity.

Poster Session II

Zak Janetzki

Department of Infectious Diseases, The University of Melbourne

Poster P2-06

Editing of HBV DNA and RNA in vitro using a combination of CRISPR/Cas9 Cytosine to Thymine Base Editors and CRISPR/Cas13b

Abstract

Zak Janetzki[1], Laura McCoullough[1,2], Wendy Liaw[1], Chee Leng Lee[3], Hee Jung Kang[3], Thomas Payne[3], Stewart Fabb[3], Colin Pouton[3], Mohamed Farah[4,5], Joe Trapani[4,5], Margaret Littlejohn[1,2], Peter Revill[1,2]

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Current hepatitis B virus (HBV) treatments do not target the HBV covalently closed circular DNA (cccDNA) minichromosome reservoir, HBV integrated DNA nor HBV RNA. There is a desperate need to develop novel therapeutics that target HBV nucleic acids; to improve HBV cure rates.

CRISPR/Cas9 cytosine to thymine base editors (CBEs) are a promising approach as they utilise the CRISPR/Cas9 guiding system to introduce specific C:G to T:A edits into target DNA. Edits can be predicted and there is less chance of genome instability when targeting integrated HBV DNA. CRISPR/Cas13b has been previously utilised to target HBV RNA and successfully reduced HBV protein expression and replication in vitro and in vivo. However, its impact on HBV is transient and will likely require permanent edits introduced by CBEs to achieve sustained knockdown. The aim of this project is to determine the effect of combining CBEs with CRISPR/Cas13b on HBV replication and protein expression in vitro.

CBE sgRNA was designed to introduce a premature stop codon in a single HBV open reading frame to reduce HBV protein expression and replication. The Cas13b crRNA tested was previously shown to reduce HBV protein expression and replication in vitro. HBV-infected HepG2.NTCP cells were transfected with CBE and Cas13b mRNA and HBV replication markers and proteins were measured and compared to a non-targeting control sgRNA and crRNA.

At initial time points, a combination of CBE with Cas13b achieved greater knockdown of HBV proteins, DNA and RNA compared to CBE alone. This combination also had similar levels of knockdown compared to Cas13b alone indicating minimal interference by the CBE HBV-targeting sgRNA. Additionally, longitudinal studies are currently being performed to analyse the level of viral rebound of Cas9 CBE with Cas13b and HBV targeting guide RNAs. Further analysis of the impact on HBV cccDNA and sequencing will be performed to determine the extent of CBE activity.

This study is the first of our knowledge that has tested Cas9 with Cas13 simultaneously. These results will determine the utility of a Cas9 CBE and Cas13b combination to target HBV DNA and RNA, as an important first step towards developing this approach as a novel HBV therapeutic

Poster Session II

Laura McCoullough

Department of Infectious Diseases, The University of Melbourne

Poster P2-07

The significance of viral genomic variability in developing Cas13b-based therapy for chronic hepatitis B infection

Abstract

Mai Anh Thu Le[1,2], **Laura McCoullough**[1,2], Zak Janetzki[1,2], Wendy Liaw[1,2], Mohamed Fareh[3,4], Joseph Trapani[3,4], Peter Revill[1,2]*, Margaret LiDlejohn[1,2]*

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**These authors contributed equally to this work.*

Chronic hepatitis B infection, caused by the hepatitis B virus, is a global health burden that affects more than 254 million people globally. hepatitis B virus is a DNA virus that replicates through RNA intermediates. Current treatments suppress, but do not eliminate, viral replication. Therefore, novel therapies for chronic hepatitis B infection are urgently needed.

Bacterial CRISPR-Cas13b endonuclease can target viral RNA in mammalian cells through highly specific CRISPR RNAs (crRNAs). We have previously shown that CRISPR-Cas13b can target the HBV RNAs to strongly reduce HBV replication and protein expression in vitro. However, HBV genomic variability, with multiple genotypes worldwide, can generate mismatches between crRNAs and their target sites, potentially impairing Cas13b-crRNA recognition and antiviral activity. This project investigated the impact of HBV genomic variability on Cas13b activity in vitro.

HepG2 cells were co-transfected with plasmids expressing different HBV genotypes, PspCas13b and different HBV-targeting crRNAs. The impact of PspCas13b on the replication of different HBV genotypes was determined. crRNAs that were not fully complementary to the target sequence were modified to restore complementarity and tested against different HBV genotypes.

Three crRNAs were effective against all HBV genotypes tested, whilst two crRNAs were less effective against HBV genotypes that had sequence mismatches. Modifying the crRNAs to restore complementarity improved their efficacy. Three crRNA mismatches partially reduced PspCas13b activity, which varied across genotypes and target sites. Five or more mismatches completely abrogated PspCas13b activity. The nucleotide positions of the mismatches did not influence PspCas13b efficacy, suggesting that additional genotype-specific factors were involved.

This study further explored the impact of crRNA-target mismatches on PspCas13b efficacy in the context of different HBV genotypes. Whilst crRNA-target mismatches influenced PspCas13b efficacy, the impact was context-dependent and was improved by modifying the crRNA to restore complementarity to the target sequence.

Poster Session II

Kristie Dickson

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Poster P2-08

MUTANT P53 AND OVARIAN CANCER: EXAMINING THERAPEUTIC OPPORTUNITIES USING CRISPR ENGINEERED P53 WILD-TYPE, KNOCKOUT AND GAIN-OF-FUNCTION MUTANT OVARIAN CANCER PANELS

Abstract

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High-Grade Serous Ovarian Cancer (HGSOC) is characterised by mutation in TP53 in almost 100% of cases. These mutations result in either accumulation of mutant p53 (missense mutations) and/or loss of wild-type (WT) p53 (nonsense mutations), leading to loss of normal p53 tumour suppressor functions. We have used CRISPR engineering to generate isogenic ovarian cancer cell line panels differing at the p53 locus (WT, null and gain-of-function (GOF) mutations). To ensure isogenic panels have an identical genetic background, clonal cell lines were first derived from single parent cells with WT p53 or endogenous GOF mutations. Base pair editing CRISPR was used to engineer hotspot GOF missense mutations including Y220C (A>G) and R248Q (C>T) in WT p53 clonal lines. WT and GOF p53 was knocked out using CRISPR-Cas9, with sgRNA targeting regions in the DNA binding domain of p53. Resulting p53 modifications were confirmed by in-cell western, western blotting and Sanger sequencing, and characterised for expected functional consequences of mutant p53 including failure to activate its target genes (CDKN1A, PUMA, MDM2, and BAX), increased clonogenic potential, resistance to standard-of-care chemotherapy, and sensitivity to the p53 reactivating drug APR-246.

p53 targeting compounds were identified using two independent approaches – screening of theTP53 isogenic panel against a 160 drug compound library, and in silico analysis of publicly available data sets from the Broad and Wellcome Sanger institutes. As a proof of principle, the MDM2 inhibitor Nutlin-3A was found to target only WT p53. The glycogen synthase kinase-3 (GSK3) inhibitor, AR-A 014418 was identified as a potential mutant p53 targeting drug showing greatest effect against GOF p53 missense mutations. Several compounds were also found to specifically target the Y220C mutation, known to have a binding pocket. Isogenic panels of identical genetic background upon which a gene of interest is modified are powerful models for drug discovery.

Poster Session II

Gaoyuan Wang

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Poster P2-09

Combining CRISPR screen, scRNAseq, and spatial techniques to study venetoclax resistance mechanism in aggressive lymphomas

Abstract

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Venetoclax is a BCL-2-specific BH3-mimetic drug that is FDA approved for the treatment of Chronic Lymphocytic Leukemia (CLL) and Acute Myeloid Leukemia (AML). While initial treatment success was very high, recent reports suggest relapse of patients being treated with venetoclax. Hence, identifying resistance factors to venetoclax therapy is an area of great clinical relevance. While a plethora of resistance mechanisms have been described, we are particularly interested in signals provided by the tumour micro-environment that mediate drug resistance of haematopoietic cancers. It is known that the upregulation of BCL-2 family proteins, for example, results from microenvironmental signals that can help cancer cells evade apoptosis. We designed a CRISPR droplet sequencing (CROP-seq) based transcriptional activation library to upregulate genes that mimic signals from the microenvironment (~30 genes; 5sgRNAs/gene). The CROP-seq method allows detection of each cell's sgRNA along with its single cell transcriptomic landscape, providing gene expression signatures for individual gene perturbations.

In our in vitro study, we showed that CROPseq activation screening system works effectively to identify clinically known resistance factors for venetoclax treatment. We are now taking this research to the next level, combining CROPseq activation screens with another advanced technique, spatial transcriptomics, to enable us to investigate the impact of the tumour microenvironment on the response of cancer cells to venetoclax treatment in vivo. On top of the resistance mechanisms that we can identify from scRNAseq datasets, spatial transcriptomics allows us to examine how cells of the tumour microenvironment may affect drug sensitivity in vivo, better recapitulating what is happening in patients. In pilot experiment, we successfully identified our CROPseq-enabled blood cancer cells and also annotated surrounding host cells. The ultimate goal of this project is to obtain more insight into the pathways mediating venetoclax resistance and use this information to target synergistic pathways increasing the treatment response to venetoclax in blood cancer patients.

Keywords:

Lymphoma, venetoclax resistance, CRISPR activation screen, single-cell RNAseq, spatial transcriptomics