CRISPR Down Under 2025 – All Abstracts

Day One: Thursday 27th of February 2025

Day Two: Friday 28th of February 2025

Session One: Crispr Biology

Invited Speaker: Elizabeth Kellogg
Talk Title: TBA

Invited Speaker: Mohamed Fareh
Talk Title: The molecular basis of target recognition and cleavage by CRISPR-Cas13

Invited Speaker: Gavin Knott
Talk Title: TBA

Session Two: Functional Genomics *(Sponsored by Twist Bioscience & Decode Science)*

Invited Speaker: Marco Herold
Talk Title: Next Generation CRISPR tools to model and interrogate cancer

Speaker: Wei Jin

Abstract Title: Advancing the genetic engineering toolbox by combining AsCas12a knock-in mice with ultra-compact screening

Authors: Wei Jin1,2,3,4,#, Yexuan Deng1,2,3,4,5,#, John E. La Marca1,2,3,4,#, Emily J. Lelliott1,2,3,4, Sarah T. Diepstraten3,4, Christina König1,2,3, Lin Tai1, Valentina Snetkova6, Kristel M. Dorighi6, Luke Hoberecht7, Millicent G. Hedditch3, Lauren Whelan3, Geraldine Healey1, Dan Fayle1, Kieran Lau1, Margaret A. Potts1,2,3,4, Moore Z. Chen8, Angus P. R. Johnston8, Yang Liao1,2, Wei Shi1,2, Andrew J. Kueh1,2,3,4, Benjamin Haley6,9,*, Jean-Philippe Fortin7,*, Marco J. Herold1,2,3,4,*,^

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Full Abstract: CRISPR-Cas9 technology has revolutionized genetic research, particularly in the discovery of novel tumour drivers and resistance factors through CRISPR screens. Cas12a (Cpf1) distinguishes itself from Cas9 by short crRNAs and a unique PAM site . Additionally, intrinsic RNase activity simplify multiplexed gene targeting via the processing of individual crRNAs from a pre-crRNA-encoding RNA. Here, we present a mouse model that constitutively expresses enhanced Acidaminococcus sp.Cas12a (enAsCas12a) linked to an mCherry fluorescent reporter. We validated robust expression of Cas12a in our mouse model across multiple tissues, with a particular focus on haematopoietic organs, a key facet of our research. We demonstrate efficient single and multiplexed gene-editing in vitro, using primary and transformed cells from enAsCas12a mice. We further demonstrate successful in vivo gene-editing, using normal and cancer-prone enAsCas12a stem cells to reconstitute the haematopoietic system of wild-type mice. We next generated compact, genome-wide Cas12a knockout libraries targeting each gene with four crRNAs encoded across one (Scherzo) or two (Menuetto) vectors, and demonstrated the utility of these libraries across multiple screens: in vitro enrichment screening in lymphoma cells, in vitro drop-out screening in immortalised MDFs, and

in vivo enrichment screening in the haematopoietic lineage of mice to identify lymphoma-driving events. The consistency and robustness of the data extracted from each of our screens underscore the high effectiveness and broad potential of these new tools. Finally, we demonstrate CRISPR multiplexing via simultaneous gene knockout (via Cas12a) and activation (via dCas-SAM) using primary T cells and mouse dermal fibroblasts (MDFs). This highlights the compatibility of our enAsCas12a mouse model with other CRISPR technologies. Collectively, our enAsCas12a mouse model and accompanying crRNA expression libraries enhance genome engineering capabilities and complement current CRISPR technologies.

Speaker: Marina Leiwe

Abstract Title: Using a spatial CRISPR screen to identify regulators of the immune microenvironment in non-small cell lung cancer

Authors: Marina Leiwe1,2, Claire Marceaux1,2, Daniel Batey1, Velimir Gayevskiy1, Tracy Leong3, Marie-Liesse Asselin-Labat1,2

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

Perturb-map adds spatial resolution to pooled CRISPR screens, hence allowing the identification of extracellular gene functions within the native tissue environment.

As this approach is limited in the number of genes that can be studied in parallel, we curated a candidate gene library based on two RNA-seq datasets of lung adenocarcinoma patient biopsies: 1) comparing tumours sensitive or resistant to anti-PD-L1 therapy and 2) comparing tumours with high or low immune infiltration. Correlating these two transcriptomic datasets identified genes associated with both, response to immune checkpoint blockade and immune infiltration, suggesting these gene products may be involved in immunotherapy resistance via modulation of the tumour immune microenvironment.

We have completed a pilot screen depleting 12 candidate genes in murine lung carcinoma cells before injecting pooled knock-out cells into immunocompetent mice to grow orthotopic tumours. We identified two genes, which when depleted, decreased the number of tumour lesions as well as the percentage of total tumour burden compared to control lesions. Our ongoing work uses multiplex imaging techniques to investigate how the individual gene depletions influence composition of the immune microenvironment.

Genes identified in our study may suggest novel drug targets aiming to recruit immune cells to the tumour site and may improve patient outcome in combination with conventional immunotherapies like immune checkpoint blockade.

Speaker: Dane Vassiliadis

Abstract Title: Recursive CRISPR screening identifies a functional link between P300 and SF3B3 in transcriptional elongation control

Authors: Dane Vassiliadis 1,2, Jesse J. Balic1,2, Andrea Gillespie1,2, Olivia Braniff1,2, William Rothnie1,2, Oliver Sinclair1, Kelsy Prest1,2, Andrew Das1,2, Ching-Seng Ang3, and Mark A. Dawson1,2,4,^.Dane Vassiliadis1,2,*,^, Jesse J. Balic 1,2, Andrea Gillespie 1,2, Olivia Braniff 1,2, William Rothnie 1,2, Oliver Sinclair 1, Kelsy Prest 1,2, Andrew Das 1,2, Ching-Seng Ang 3, and Mark A. Dawson 1,2,4.

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Full Abstract: The expression of eukaryotic protein-coding genes is the product of transcriptional (e.g. initiation, elongation and termination) and co-transcriptional processes (e.g. splicing, modification and export). How diverse input signals are relayed to, and interpreted by transcription factors and associated co-factors to govern RNA Pol II activity remains unclear. Here we developed a gene expression reporter system traceable in real-time by flow cytometry and combined this with CRISPR/Cas9 screens to identify epistatic relationships underpinning transcriptional co-activator inhibition in human cells. We find that loss of the U2 spliceosome factor SF3B3 broadly desensitises cells to transcriptional co-activator inhibition, with short, exon-dense genes displaying the most prominent desensitisation. We show that splicing remains functional despite SF3B3 loss and that the desensitisation effect is mediated by the physical presence, but not the catalytic activity of the acetyltransferase and transcriptional co-activator P300. Mechanistically, SF3B3 depletion drives the redistribution of chromatinised P300 to newly licensed cis regulatory elements enriched for JUN/AP1; and perturbs the pause release and elongation dynamics of RNA Pol II. Moreover, the association of key transcriptional complexes, elongation factors and cyclin dependent kinases with RNA Pol II is reduced following SF3B3 depletion. Finally, using a CRISPR base editor screening approach, we performed tiled mutagenesis of P300 and discovered key residues in the histone acetyltransferase domain that either promote or prevent desensitisation to transcriptional coactivator inhibition. Together these data suggest a splicing-independent functional link between SF3B3, P300 and RNA Pol II required for the coordination and licensing of transcriptional elongation.

Day 1 Flash Talks

Speaker: Thomas Chadwick

Abstract Title: Utilising CRISPR/Cas9 screening to uncover novel immunotherapies for targeting bone metastases

Authors: Thomas B. Chadwick1,2, Joan So1,2, Laura Vojtech1,2, Amina Ismail1,2, Lap Hing Chi1,2, Kaylene J. Simpson1,3,4, Marco J. Herold5,6, Belinda S. Parker1,2

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Full Abstract: Despite improved detection and treatment of early-stage breast cancer, metastatic disease remains a clinical challenge as conventional and immune-based therapies offer little long-term survival benefit. About 75% of breast cancer metastases occur in bone, which can be treatment resistant and associated with severe morbidity and high mortality rates. The biological changes promoting outgrowth of disseminated cancer cells in bone are not fully understood, but one mechanism is microenvironment-induced immune suppression that renders bone metastases unresponsive to anti-tumour immunity. Our laboratory has previously identified a critical pathway associated with bone metastatic outgrowth and reduced cellular immunogenicity: suppression of tumour-intrinsic type I interferon (IFN) signalling, including hundreds of interferon-regulated genes crucial for innate and adaptive immune response and anti-tumour immunity. Thus, targeted approaches to restore IFN signalling in metastatic cells could have important therapeutic implications. This project used genome-wide CRISPR/Cas9 screening in bone metastatic lines in vitro to identify novel targets to restore IFN signalling. We transduced bone metastatic cells isolated from the syngeneic mouse models of breast cancer with an interferon-stimulated response element (ISRE)-GFP/Luc2 reporter system, which revealed significant reductions in ISRE activity both at baseline and with TLR-agonist stimulation compared to primary tumour lines. After Cas9 transduction, we performed a CRISPR/Cas9 knockout screen, and isolated GFP positive cells (indicating enhanced ISRE activity) via flow cytometry. This screen identified >100 genes that, when suppressed, restore type I IFN signalling, including confirmed targets relevant to the cGAS/STING signalling pathway. These key hits are being validated for their ability to alter IFN signalling and cellular immunogenicity in vitro and will be further validated for their effectiveness alongside traditional therapies in vivo. Identified through ISRE reporter and CRISPR/Cas9 screening, these gene targets offer novel therapeutic strategies for combatting late-stage breast cancer, enhancing IFN signalling in bone metastasis and increasing cancer vulnerability to immune-based therapeutics.

Speaker: Debolina Majumdar

Abstract Title: Genome-wide CRISPR knockout screen to identify host factors in avian influenza virus infection

Authors: Debolina Majumdar1, Balaji Manicassamy2, Emily Hann1, Daniel Layton1, Arjun Challagulla1

Affiliations: 1 CSIRO Health and Biosecurity, Australian Centre for Disease Preparedness, Geelong, VIC 3220, Australia. 2 Department of Microbiology and Immunology, University of Iowa, Iowa City, Iowa, USA

Full Abstract: Avian influenza (AI) poses a significant challenge to the global poultry industry, with current biosecurity measures and vaccination programs proving insufficient. Recent advances in gene editing technologies offer transformative potential by enabling complete resistance to infections within a single generation. However, successful implementation of gene editing relies on the identification of key host targets and molecular pathways that confer resistance to the diseases. Genome-wide CRISPR knockout (GeCKO) is a powerful tool that enables unprecedented opportunity to dissect the specific biological roles of genes in a massive-parallel approach under the influence of virus infection. Although numerous studies have identified host factors critical for influenza virus infection in human cells, comparable research in chicken cells remains unexplored, highlighting a significant gap in our understanding of host-pathogen interactions in avian species. In this study, we are conducting a GeCKO screen in chicken fibroblast DF1 cells to identify host factors required for AI infection. This will involve the development of a chicken GeCKO cell library, in which cells are targeted by gRNAs on a genome-wide scale. Subsequently, we will conduct an H7N7 AI infection survival screen on GeCKO library cells, performing multiple rounds of AI infection with virus-induced cell death serving as the readout. Following next-generation sequencing (NGS) and bioinformatics analyses, we will validate the highly enriched genes from the survival screen through a series of functional analyses. The outcomes of this GeCKO screening may provide new insights into the AI-host interface, enhance our understanding of host-directed strategies, and facilitate the discovery of novel gene-editing targets to mitigate AI in chickens.

Speaker: Yuchen Bai

Abstract Title: Promoting cell growth to prevent the spread of head and neck cancer

Authors: Yuchen Bai, Charbel Darido

Affiliations: Peter MacCallum Cancer Center

Full Abstract:

Head and neck cancer (HNC) presents significant challenges in treatment due to its high metastatic propensity, leading to poor survival rates. 50% of patients develop metastasis within 9-month diagnosis, nearly all within 5-year treatment, reducing overall survival below 10%. Current therapies focus on rapidly dividing cancer cells but face limitations as metastasis-prone cells often adopt slower growth patterns, evading treatment. This resistance fuels cancer recurrence and complicates effective intervention, highlighting an urgent need for innovative therapies. A paradigm shift is essential to overcome these challenges and effectively target metastatic progression in HNC.

To comprehensively explore metastatic HNC, single-cell RNA sequencing data from patients' primary and nodally invasive tumors was analyzed. Trajectory analysis, based on cycling and EMT scores, elucidated molecular changes along metastatic progression. SCIENIC reconstructed gene regulatory networks during the dynamic transcriptional shifts, identifying key regulators responsible for the switch between proliferation and metastasis. A combinatorial genetic screen was applied in vivo to confirm the the genetic architecture that guides cellular decisions in metastatic HNC.

We confirmed the mutually exclusive expression of proliferation and metastasis cascades in the primary tumors and lymph nodes of HNC. Cells fell continuously along a gradient of proliferation to metastasis progression, revealing distinct waves of gene regulation. We incorporate a combinatorial perturbation sequencing into our trajectory analysis to confirm the dependency of the proliferative shut-down on progression along the EMT continuum. Combined genetic alterations can efficiently reverse the metastasis into proliferation, implying the close interaction between proliferative and metastatic cascades co-regulating cellular state of metastatic HNC.

Our study combines single-cell trajectory analysis with high-throughput genetic screening, which constitutes a powerful approach for identifying upstream signals of pathways that regulate cellular phenotypes. Indeed, these experiments uncover a combination of transcription factors that drive cells towards metastasis, explaining how cells switch between the discrete cellular states.

Speaker: Liam Neil

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

Authors: Neil L1,2,, Liyanage C1,2, Brown R1,2, Srivaths A1,2, Vervoort S3, Yeo B4, Conor Kearney1,2,, Pal B1,2

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Full Abstract: Distant metastasis is a major cause of breast cancer related mortality. Approximately one-third of patients diagnosed with early Triple-negative breast cancer (TNBC) subtype will develop distant metastases, who often face highly toxic and largely ineffective treatments. The median overall survival for metastatic TNBC patients is 11-13 months. Natural killer (NK) cells are recognised as the first line of defence against metastasis in solid tumours, including breast cancer. Harnessing NK cells to target metastatic TNBC presents a promising yet underexplored treatment avenue. We aim to develop NK cell-based immunotherapies targeting metastatic breast cancer and improve patient outcomes.

Our preliminary data on different breast cancer cell lines indicates that NK-cell mediated killing of breast cancer cells is independent of MHC-class I expression, opposing what is typically expected in the current literature. Therefore, we completed genome-wide CRISPR screens in a highly metastatic TNBC cell line. In this screen, we identified gene candidates whose activation or suppression could potentially sensitise cancer cells to NK cell-mediated killing. Here, we have prioritised two genes for inhibition: one gene known to promote cancer cell growth and immune evasion, and a target gene that is essential for mitosis, of which inhibition could induce immunogenic cell death via NK and T cells. We have validated these targets in vitro confirming increased NK cell-mediated killing of cancer cells and future studies will involve in vivo experiments to confirm their role in reducing metastatic burden.

Speaker: Daisy Wilson Kocher

Abstract Title: CRISPR in pest species Tribolium Castaneum

Authors: Daisy WILSON KOCHER, Dr. Charles Robin

Affiliations: The University of Melbourne

Full Abstract: The red flour beetle, Tribolium castaneum, is an emerging model organism. This species is also a major pest of stored grains that impacts farmer's profits and food security globally. Agriculturalists have been using the fumigant phosphine to control T. castaneum infestations for decades. Unfortunately, resistance to phosphine has been recorded in many locations across sites world-wide. Compounding the problem, there is no alternative pesticide on the market that is as food-safe as phosphine. This project aims to use CRISPR-Cas9 technology to elucidate the identity / function of genes involved in phosphine resistance.

Gene knock-ins and gene knock-outs have both been performed in T. castaneum embryos using CRISPR-Cas9. In this study a family of Cytochrome-P450s (the CYP346B gene family) are targeted for gene knock-out because there is evidence that each of these genes are overexpressed in phosphine resistant T. castaneum These genes are adjacent to each other on chromosome 5 and arose from gene duplication, meaning that they can be targeted in tandem with CRISPR. Our design allows us to tease out the impacts of each gene on phosphine resistance phenotype. We have also performed a gene knock-out on Cytochrome-b5-fatty-acid-desaturase. This gene has been associated with phosphine resistance through variant linkage analysis. It is unclear whether a beetle homozygous for a loss-of-function alleles is viable, and our experiment will determine the answer. We also aim knock-in a wild-type copy of this gene to a phosphine resistant strain of T. castaneum in order to perform complementation tests. CRISPR is a tool that has and will continue to provide valuable insights into T. castaneum biology.

Speaker: Kah Min Yap

Abstract Title: Identifying Optimal Tumour-specific Promoters for CRISPR/Cas9 Engineering of Armoured CAR T Cells with Enhanced Safety and Efficacy

Authors: Kah Min Yap1,2, Amanda X. Y. Chen1,2, Imran G. House1,2, Phillip K. Darcy1,2 and Paul A. Beavis1,2

Affiliations: 1. Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, 2. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia

Full Abstract: Background: The tremendous success of chimeric antigen receptor-T (CAR-T) cell therapy in haematological malignancies has not been recapitulated in solid tumours, owing to tumour-induced immunosuppression, tumour heterogeneity and inefficient tumour trafficking. One promising solution includes "armouring" CAR-T cells with therapeutic transgenes. Indeed, we demonstrated that CAR-T cells engineered to express dendritic cell growth factor Flt3L could effectively engage host anti-tumour immunity crucial for overcoming antigen-negative relapse. However, synthetic promoters have demonstrated insufficiencies in driving tumour-restricted cytokine expression, which had caused systemic toxicities and trial termination. The advent of CRISPR/Cas9 gene-editing tool has enabled the precise engineering of CAR-T cells for safety and efficacy enhancements. We previously showed that CRISPR/Cas9-mediated knock-out (KO) of immunosuppressive gene A2AR enhanced CAR-T cell function. Now, we aim to exploit a CRISPR/Cas9-mediated knock-in (KI) strategy to leverage endogenous gene regulatory elements to restrict transgene expression to tumour for enhanced safety and efficacy.

Methods: Genome-wide RNA sequencing was performed on CAR-T cells isolated from tumours and spleens of mice. 27 genes upregulated in intratumoural relative to splenic CAR-T cells were identified as potential KI sites. As KI disrupts target gene expression, the impact of each gene KO on CAR-T cell function/phenotype was first assessed. 7 genes without adverse impact following KO had GFP knocked in.

Results: NR4A2 and RGS16 emerged as tumour-specific promoters upon KI. While NR4A2 was highly tumour-restricted and could deliver highly toxic cytokines (e.g., IL-12) without inducing toxicities in mice, RGS16 had high intratumoural expression and could mediate the efficacy of less potent cytokines (e.g., IL-2).

Conclusions: Endogenous tumour-specific promoters enabled the generation of IL-12- and IL-2-expressing CAR-T cells with enhanced safety and efficacy in syngeneic and xenogeneic mouse models that was concomitant with improved CAR-T cell polyfunctionality and activation of host anti-tumour immunity. Notably, this CRISPR-KI strategy was applicable using patient-derived CAR-T cells, demonstrating its clinical translatability.

Poster Session One

Speaker: Alice Salib

Abstract Title: Expanding the molecular biology toolkit for paediatric cancer: generation of a comprehensive library of CRISPR/Cas cell lines

Authors: Alice Salib1, Teresa Sadras2,3, Fatimah Jalud2,3, Tasnia Ibnat2,3, Paul G Ekert1,2,4,5,6

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Full Abstract: Personalised medicine initiatives like the Zero Childhood Cancer Program have highlighted the genomic heterogeneity of paediatric cancers, and the value of molecular characterisation to deliver more effective treatments. As novel variants are identified, there is a growing need to characterise their functions and therapeutic targetability. To address this, we are developing a comprehensive library of CRISPR/Cas-engineered isogenic paediatric cancer cell lines, enabling manipulation of gene expression through knockout (CRISPR/Cas9), knockdown (CRISPR/Cas13), or overexpression (CRISPRa) strategies. A nuclease-dead Cas13d variant (dCas13d) serves as a control. Leveraging these tools, we aim to functionally characterise candidate driver genes and novel variants in paediatric malignancies.

To date, we have generated 22 CRISPR/Cas cell lines, encompassing a wide range of paediatric cancers including neuroblastoma, medulloblastoma, rhabdomyosarcoma, osteosarcoma, and Ewing's sarcoma. Across the CRISPR/Cas13d lines generated, we observe variable doxycycline (dox)-induced Cas13d expression. Further, our findings suggest guide expression levels may limit gene silencing efficiency. Clonogenic assays revealed a dose-dependent reduction in colony-forming capacity upon Cas13d induction, independent of guide expression. Our results indicate that careful control of both guide and Cas13d expression is needed and strategies to mitigate Cas13d toxicity while enhancing guide efficacy are in progress.

In parallel, we are using CRISPR/Cas9 cell lines we have generated in combination with a unique pipeline we have developed to generate PDGFRA structural variants by targeting intronic regions adjacent to the deleted exons. This approach enables us to recreate these PDGFRA variants endogenously to further characterise their function.

In summary, the cell lines developed offer a valuable resource for investigating the molecular mechanisms driving paediatric cancers. Furthermore, they serve as a toolkit for identifying and exploring potential therapeutic vulnerabilities in paediatric cancer to select targeted and more effective treatments for patients.

Speaker: Wing Fuk Chan

Abstract Title: Reactivation of δ-globin via CRISPR in treating β-haemoglobinopathies

Authors: Wing Fuk Chan, James J. J. The, Bradon Rumler, Stephanie Anderson, Huimin Cai, Zane S. Kaplan, Andrew C. Perkins

Affiliations: 1Australian Centre for Blood Diseases, Monash University

Full Abstract: β-haemoglobinopathies are inherited blood disorders that affect haemoglobin in erythrocytes, with hundreds of thousands of affected new-borns each year. While sickle cell disease results from a single-nucleotide substitution, β-thalassaemia is caused by hundreds of different mutations, making the direct repair of mutations challenging. As such, a universal therapeutic approach is widely sought.

Much of the effort has been put into reactivating the foetal γ-globin by various means in the adult cells. As an alternative, δ-globin is expressed in adult red blood cells and shares a much higher sequence homology to β-globin. As such, δ-globin, and the resultant haemoglobin A2 (HbA2), is biochemically more similar and fully functional as the conventional adult haemoglobin HbA. However, δ-globin is expressed at a low level and HbA2 only constitutes 2-3% of the total adult haemoglobin. The low expression is believed to be due to mutations at the promoter that disrupt transcription factor binding. Nevertheless, overexpression of δ-globin transgene is able to rescue the sickling phenotype of red cell in a mouse model. As such, we propose reactivating the δ-globin by editing the promoter as effective alternative therapeutics of SCD and β-thalassaemia.

In this project we employed CRISPR gene editing techniques to install an KLF1 motif at the δ-globin promoter in the immortalised HUDEP-2 cell line. Through RNP transfection along with an HDR repair template, we were able to drive the expression of δ-globin to ~160 fold in the HUDEP-2 cells compared to the control. The upregulated δ-globin transcript level is around 60% of that of β-globin. Upon differentiation, we observed the HbA2 level is upregulated to 20% by HPLC. Using NGS, we demonstrated the editing is highly efficient with over 50% HDR events. Lastly, we showed that this upregulation of δ-globin is highly dependent on the insertion site at the promoter.

Speaker: Akash Srivaths

Abstract Title: Genome-wide CRISPR Screen Identify Menin as a Mediator of Encorafenib Plus Cetuximab Resistance in BRAF V600E Mutant Colorectal Cancer

Authors: Akash Srivaths (1), Tirta Mario Djajawi (1), Kristen Needham (1), Chloe Gerak (2), Liam Neil (1), Sarahi Mendoza Rivera (2), Bhupinder Pal (1), Stephin Vervoort (2), John Mariadason (1), Conor Kearney (1)

Affiliations: 1. Olivia Newton-John Cancer Research Institute, 2. Walter and Eliza Hall Institute of Medical Research

Full Abstract: Colorectal cancer is the second most commonly diagnosed cancer and one of the leading causes of cancer-related deaths in Australia. Mutations in the BRAF gene are frequently detected in several cancers including colorectal cancer. Up to one in five patients with metastatic colorectal cancer have a BRAF mutation, with the V600E substitution being the most prevalent. These patients generally have a poor prognosis. A combination of encorafenib plus cetuximab has significantly improved the overall survival of such patients, compared to standard chemotherapy regimens in clinical trials, establishing it as the new standard of care for colorectal cancer with BRAF V600E mutation. While this treatment strategy is promising, it does not eradicate the tumor completely as tumor-intrinsic resistance poses a challenge. We performed whole-genome CRISPR screens to identify such resistance-causing genes in response to encorafenib plus cetuximab. We identified that MEN1 (Menin) confers resistance to encorafenib plus cetuximab treatment in colorectal cancer with BRAF V600E mutation. Indeed, MEN1 knockout, or treatment with revumenib, a small-molecule inhibitor of the Menin-MLL interaction, augmented the cytostatic effect of encorafenib plus cetuximab in BRAF V600E mutant colorectal cancer cells, whereas re-expression of MEN1 into MEN1-null cells reversed this phenotype. We further demonstrated that the protective role of menin is dependent on its interaction with MLL1, a histone methyltransferase. Taken together, we identify Menin as a key factor in driving encorafenib plus cetuximab resistance, providing rational for targeting of Menin in combination with encorafenib plus cetuximab for improved treatment of colorectal cancers with BRAF V600E mutation.

Speaker: Oliver Sinclair

Abstract Title: High depth broad scale CRISPR screening to identify fundamental synthetic lethal targets to P300/CBP inhibition

Authors: Oliver Sinclair, Jesse Balic, Tim Somervaille, Mark Dawson

Affiliations: Peter MacCallum Cancer Centre, Cancer Research UK Manchester Institute

Full Abstract: Multiple myeloma (MM) and acute myeloid leukaemia (AML) are distinct haematopoietic malignancies which together provide a large unmet clinical need. While both are discrete they progress stepwise through premalignant states, and share dependency on aberrant transcriptional programmes that block differentiation. This dependency renders both diseases susceptible to inhibition of core transcriptional machinery, including the transcriptional co-activators p300 and CBP. These highly homologous acetyltransferases occupy (super)enhancer and promoter cis-regulatory elements to critically govern cell-type and disease-specific gene regulatory networks. In MM, p300/CBP activity sustains elevated IRF4 levels, driving proliferation and survival. In AML, p300/CBP supports MYC expression, promoting rapid growth.

This work investigates CCS1477, a novel p300/CBP bromodomain inhibitor in clinical evaluation. While early trials report responses in 67% of MM patients, relapse and resistance remain significant barriers. To address these challenges, we performed unbiased CRISPR screening across AML and MM cell lines to identify synthetic lethal targets and resistance mechanisms.

CRISPR screens were conducted in five cell lines across two doses and timepoints, with the hope of identifying fundamental sensitising hits to p300/CBP inhibition. Hits were ranked by average RRA scores, revealing the metazoan INO80 chromatin remodelling complex as a key sensitising target. Validation in both disease states and in screen-naïve cell lines confirmed these hits sensitize cells to CCS1477 and to alternative p300/CBP inhibitors targeting histone acetyltransferase activity.

Our findings establish the INO80 complex as a critical regulator of p300/CBP-driven transcription and a disease, cell line and inhibitor agnostic synthetic lethal target to enhance efficacy. These hits would be overlooked in standard individual screening approaches. Therefore, this study presents a robust CRISPR screening approach to prioritise synthetic lethal targets which outperforms standard single cell line approaches.

Speaker: Huw Morgan

Abstract Title: Identifying New Roles For Ubiquitination Machinery In Regulating Dendritic Cell Function

Authors: Huw Morgan1, Haiyin Liu1, Laura E Edgington-Mitchell1, Justine D Mintern1

Affiliations: 1Department of Biochemistry and Pharmacology, The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia

Full Abstract: Ubiquitination is a post-translational modification that regulates protein degradation and activity, such as in regulating inflammatory signalling or protein surface expression. A cascade of enzymes carries out ubiquitination of proteins. One key player is the E3 ubiquitin (Ub) ligases, which are involved in the addition of ubiquitin. This project aims to unveil novel roles for these enzymes in immunity, focusing on dendritic cells (DCs). DCs are antigen-presenting cells specialised in regulating T cells during the adaptive immune response, maintaining the balance between health and disease. Of the over 600 known and putative E3 Ub ligases, only a handful have a described role in DC function. This project aims to identify other E3 Ub ligases that participate in the regulation of DCs.

To identify E3 Ub ligases that participate in DC function, we employed an arrayed CRISPR/Cas9 screen of all known and putative E3 Ub ligases and scaffold proteins in the mutuDC line. The expression of surface markers involved in DC function were analysed by spectral flow cytometry to assess the impact of gene knockout on DCs. This screen identified Cul3 as a new regulator of DC function. The screen results were confirmed in bone marrow-derived dendritic cells (BMDCs) differentiated from gene-edited Cas9 transgenic haematopoietic progenitors. Knockout of Cul3 upregulated the expression of co-stimulatory molecules CD80 and CD86 in unstimulated BMDCs and upregulated the expression of the inhibitory molecule PD-L2 following TLR3 and TLR9 stimulation, relative to wild-type BMDCs.

Using a functional genomics approach, we have identified new roles for ubiquitination machinery in regulating the function of DCs. We identified a new role for Cul3, a gene with no known role in DC function. Further exploration of these pathways will gain insight into the complex regulatory network of DCs and highlight new ways to manipulate DC function in health and disease.

Speaker: Kevin Sek

Abstract Title: Tumour-site directed expression of adenosine receptor subtype 1 (A1R) enhances CAR T cell function and improves efficacy against solid tumours

Authors: Kevin Sek (1,2), Amanda Chen (1,2), Thomas Cole (1,2), Philip K. Darcy (1,2,6), Paul A. Beavis (1,2,6)

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Full Abstract: The efficacy of Chimeric Antigen Receptor (CAR) T cells against solid tumours is limited by immunosuppressive factors including adenosine, which suppresses CAR T cells through activation of the A2A receptor (A2AR). Deletion of the A2AR with CRISPR/Cas9 significantly enhances the in vivo efficacy of both mouse and human CAR T cells. Alternatively, CAR T cells were engineered to express A1R, a receptor that signals inversely to A2AR. Using murine and human CAR T cells, constitutive A1R overexpression was demonstrated to significantly enhanced CAR T cell effector function but at the expense of CAR T cell persistence. Through a novel CRISPR/Cas9 “knock-in” approach CAR T cells were engineered to express A1R upon CAR T cell activation, which led to enhanced anti-tumour efficacy concomitant with improved long-term persistence and was dependent on the transcription factor IRF8. These data provide a novel approach for enhancing CAR T cell efficacy in solid tumours and provides proof of principle for site-directed expression of factors that promote effector T cell differentiation.

Speaker: Ziyan Liu

Abstract Title: Improving neuroblastoma response to BH3-mimetic drugs with CRISPRi

Authors: Ziyan Liu, Shuai Huang, Grant Dewson

Affiliations: WEHI

Full Abstract: High-risk neuroblastoma patients face a poor prognosis and limited response to chemotherapy, with 50% of children relapse following aggressive chemotherapy regimens, and median overall survival after relapse is 11 months. Hence there is critical need to identify more effective therapeutic targets with limited toxicities. Targeted BH3 mimetic drugs such as VENCLEXTA®/Venetoclax/ABT-199 are changing the treatment landscape for certain blood cancers, but their use in neuroblastoma has been hindered by resistance. To address this challenge, this study leverages CRISPRi screening technology to identify targets that can sensitize neuroblastoma to venetoclax treatment and overcome resistance.

Investigating these targets and utilizing the ubiquitin system could uncover synergistic pathways, enhancing venetoclax efficacy in neuroblastoma. By elucidating the underlying molecular mechanisms, this project aims to advance targeted therapeutic strategies and improve outcomes for neuroblastoma patients.

Speaker: Emily Derrick

Abstract Title: Utilising CRISPR technologies and high-throughput screening to interrogate the biology of secreted factors: a CXCL9 and CXCL10 case study.

Authors: Emily B. Derrick, Phillip K. Darcy, Paul A. Beavis

Affiliations: (1) Peter MacCallum Cancer Centre, (2) The Sir Peter MacCallum Department of Oncology, University of Melbourne

Full Abstract: Immune checkpoint blockade (ICB) has revolutionised the treatment of numerous cancer types, including melanoma and non-small cell lung carcinoma. ICB targets immune-inhibitory molecules on the surface of T cells, unleashing their anti-tumour potential. Despite ICB's success, a high frequency of patients fail to respond to this therapy. A key limiting factor to ICB responses is the number of T cells that infiltrate the tumour microenvironment. T cell infiltration in the context of ICB has been shown to be dependent on chemoattractant molecules CXCL9 and CXCL10. We have previously demonstrated that these chemokines are predominantly produced by intratumoral macrophages. Therefore, we aimed to identify strategies to enhance CXCL9/10 production in macrophages and subsequently improve T cell infiltration in solid tumours. To screen for secreted factors, we utilised CRISPR/Cas9-mediated homology directed repair (HDR) to generate a cell line that expressed GFP and BFP as a bona fide readout of CXCL9/10 production. Using this cell line, we performed a whole-genome CRISPR/Cas9 screen to identify genetic regulators of chemokine production. We successfully identified numerous regulators, including PTPN2 as a negative regulator of CXCL9/10 in macrophages. Using the same cell line, we performed a flow cytometry-based drug screen to identify novel compounds that induce CXCL9/10 expression. From a novel library containing 20000 compounds, several drug candidates improved chemokine expression and are currently being validated. By utilising CRISPR-HDR to generate a reporter cell line, we were able to establish robust platforms to interrogate the biology of secreted factors, through both whole-genome CRISPR/Cas9 screening and flow cytometry-based drug screening. This work has identified new therapeutic strategies that improve CXCL9/10 expression, which can be utilised in combination with ICB to improve clinical outcomes for patients.

Speaker: Serena Kane

Abstract Title: Screening for novel regulators of GATA3 expression

Authors: Serena R. Kane1,2, Milevskiy MJG1,2, Fu N1,2, Lindeman G1,2,3,4, and Visvader JE1,2

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Full Abstract: The GATA3 gene is vital for mammary gland development and plays important roles in breast cancer. Breast cancers with high GATA3 expression are associated with better clinical outcomes and improved response to endocrine therapy compared to GATA3 low tumours. Increasing GATA3 expression may be clinically favourable in breast cancer. I aimed to identify novel regulators of GATA3 through whole genome CRISPR screening using GATA3 reporter breast cancer cell lines. Three reporter cell lines were established by inserting a fluorescent reporter at the end of the natural GATA3 gene. I performed whole genome CRISPR knock-out screens with these reporter cell lines, sorting the top and bottom 10% of cells based on reporter fluorescence. Multiple candidates were investigated, with many knockouts not altering GATA3 protein expression. A putative positive regulator of GATA3 expression was identified and validated in multiple cell lines. I then investigated other members of the complex that this candidate acts through. Knocking out other genes from this complex also decreased GATA3 expression. These results suggest that we have identified a novel mechanism that positively regulates GATA3 expression.

Speaker: Andrew Li

Abstract Title: Genome-wide CRISPR-Cas9 Screens on Tumour Cells under Vδ2+ γδ T Cell Immune Pressure

Authors: Andrew Li (1), Conor J Kearney (1), Lisa A Mielke (1), Stephin J Vervoort (2), Andreas Behren (1,3), Kok Fei Chan (1)

Affiliations: (1) Olivia Newton-John Cancer Research Institute, and School of Cancer Medicine, La Trobe University, Heidelberg, Victoria, Australia (2) The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia (3) Department of Medicine, University of Melbourne, Parkville, Victoria, Australia

Full Abstract: Gamma-Delta (γδ) T cells are a non-conventional subset of T cells which are able to recognize tumour cells independent of human leukocyte antigen (HLA) molecules. The predominant Vδ2+ subset residing in the peripheral blood has been found to be activated through recognition of a complex formed by BTN2A1/3A1 molecules, which upregulate in response to endogenous metabolic dysfunction. Previous studies have identified an anti-tumour role for Vδ2+ γδ T cells, alongside antigen presenting and immunomodulatory capabilities. Considerable interest has therefore been generated for their application in tumour immunotherapy treatments such as adoptive cell transfer, as the recognition of conserved moieties allows for allogenic transfer with minimal risk of graft-versus-host disease (GvHD). However, while treatments involving γδ T cells have shown prior success against haematological cancers, durable patient responses have yet to be achieved against solid malignancies including melanoma. To better elucidate the potential of Vδ2+ γδ T cells in adoptive cell therapy, we have employed the use of advance whole-genome CRISPR-Cas9 screens to target individual gene knockouts in human melanoma cells across >19000 genes. These screens investigate the mechanisms that allow melanoma cell survival despite immune pressure from Vδ2+ γδ T cells. Here we identify gene candidates that may contribute to tumour immune evasion from Vδ2+ γδ T cell-mediated killing which require further investigation.

Speaker: Sophie Morgan

Abstract Title: Modelling BRPF1-associated neurodevelopmental disorders

Authors: Sophie TB Morgan, Hannah K Vanyai and Marnie E Blewitt

Affiliations: Walter Eliza Hall Institute of Medical Research, The University of Melbourne

Full Abstract: Bromodomain and PHD finger containing protein 1 (BRPF1) is a chromatin reader which complexes with histone acetyltransferases KAT6A and KAT6B. The BRPF1 complex plays essential roles in neurodevelopment, with heterozygous BRPF1 loss leading to neurodevelopmental disorders (NDDs), primarily the BRPF1 haploinsufficiency disorder Intellectual Developmental Disorder with Dysmorphic Facies and Ptosis (IDDDFP). Indeed, heterozygous loss of KAT6A and KAT6B also leads to NDD's. These disorders are characterised by intellectual disability and global developmental delay.

The molecular mechanism of BRPF1, KAT6A and KAT6B has not been thoroughly investigated owing to issues in reliable detection methods and low endogenous expression. Using CRISPR-Cas9 editing via ribonucleoprotein nucleofection, we have successfully HiBiT tagged BRPF1, KAT6A and KAT6B, as well as BRPF family members BRPF2/BRD1 and BRPF3 in human neuroblastoma SH-SY5Y cells. Furthermore, we have introduced IDDDFP patient pathogenic variants into HiBiT-BRPF1 to directly investigate the molecular consequences of different variants.

For the first time, we have identified the genomic binding targets of BRPF1 through Cut&Run using the HiBiT antibody, and will extend this to the other targets. Additionally, using the HiBiT toolkit, we have detected BRPF1 and complex members via immunofluorescence microscopy, on-blot luciferase assay and luminescence plate-based methods. Furthermore, experiments carried out in BRPF1 patient pathogenic variant lines support that IDDDFP is a BRPF1 haploinsufficiency disorder, with patient variants resulting in a loss of function.

Speaker: Moe El Mohamad

Abstract Title: Developing CRISPR-Cas13 antiviral therapeutics for respiratory pathogens of pandemic potential

Authors: Moe EI Mohamad1,2,3, Wei Zhao1, Rob de Rose1,4, Stanislav Kan1, Wenxin Hu2,3, Joshua Casan2,3, Honglin Chen2,3, Paula Cevaal1, Ajantha Rhodes1, Danielle Fong1, Nadia Saraya1, Michael Roche1, Damian Purcell1, Joseph A Trapani2,3, Matthew McKay5, Mohamed Fareh2,3, Sharon R Lewin1,7

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Full Abstract: There is an urgent need for effective antiviral therapies, especially against respiratory viruses of pandemic potential. We previously showed that CRISPR-Cas13 inhibits SARS-CoV-2 replication in vitro. The study aimed to test CRISPR-Cas13 delivery as mRNA in lung-targeting lipid nanoparticles (LNP) intravenously and intranasally. We used a novel LNP formulation, LNP-X, to optimize CRISPR Cas13b delivery to Vero-GFP cells. We co-encapsulated mRNA encoding for Cas13b together with a guide RNA (crRNA) targeting the coding sequence in the gene for GFP, and achieved a 90% reduction in GFP expression with 250 ng mRNA (n=3). We then delivered mRNA encoding for Cas13b with a crRNA targeting the nucleocapsid gene in SARS-CoV2 using LNP-X. Following 24 hours of mRNA delivery to Vero cells, the cells were infected with either the ancestral SARS-CoV2 or Omicron BA.1. After 24 hours, we observed a 3.8 and 2.4-log reduction in infectious virus in supernatant respectively, compared to an identical LNP which carried a non-targeting crRNA (negative control). We also tested LNP-X with and without modifications in Calu-3 cells grown in air-liquid interface (ALI) cultures. We encapsulated the LNPs with a nanoluciferase tagged-CRISPR Cas13b mRNA (Cas13b-NanoLuc) and transfected the cells with 1000 ng mRNA. One LNP induced a 3-fold increase in Nanoluc expression compared to the other LNPs. For in vivo delivery, we encapsulated Cas13b-NanoLuc mRNA in an LNP that contains the cationic lipid DOTAP, known to target the lungs following intravenous administration. After administering 15 µg Cas13b-Nanoluc intravenously, lung Nanoluc expression was 9-fold higher than in the spleen and liver. Cas13b can be delivered as mRNA in vitro in cell lines and organoid, and in vivo using novel LNPs. In vitro, Cas13b mRNA and crRNA targeting nucleocapsid can potently inhibit SARS CoV2 replication. This approach could be a promising platform for therapeutics for respiratory pathogens of pandemic potential.

Speaker: Joshua King

Abstract Title: Harnessing Cas13 to target Multiple Myeloma

Authors: Joshua King, Joe Trapani, Ricky Johnstone, Mohamed Fareh

Affiliations: Peter MacCallum Cancer Centre, The University of Melbourne

Full Abstract: Cas13 is a Cas nuclease with RNase activity. It utilises a guide RNA (gRNA) to complimentarily base-pair, and subsequently cleave and degrade target RNA. Numerous groups have co-opted Cas13 for highly selective degradation of RNA molecules in a variety of cell lines. There has also been significant interest into harnessing Cas13 as a personalised therapeutic. By contrast, a number of groups have reported that certain Cas13 orthologues, upon cleaving target RNA, proceed to indiscriminately degrade bystander RNA, thereby adversely affecting host cell viability. It has been postulated that this collateral activity may be harnessed for selective cell elimination. We, and others, have therefore proposed that Cas13 may represent a promising Oncology therapeutic. We aim to exploit Cas13 to i) silence undruggable oncogenic RNA molecules and ii) induce of transcriptome destruction in selective cancer cells.

Multiple Myeloma (MM) is the most common haematological cancer in Australia. Approximately 15% of MM patients exhibit a translocation of chromosome 4 and 14 (t(4;14)) that leads to the overexpression of the epigenetic regulator NSD2. This enzyme drives global epigenetic rewiring and dysregulation of a plethora of oncogenic pathways implicated in MM pathogenesis. There are currently no approved therapeutics targeting NSD2 available to MM t(4;14) patients.

This project aims to characterise and exploit RfxCas13d activity for i) programmed on-target cleavage of RNA, and ii) collateral activity in human, multiple myeloma cell lines (HMCLs). We first seek to elucidate the determinants of RfxCas13d activity, and how it impacts HMCLs. We then aim to program RfxCas13d to target IgH-NSD2 fusion transcripts to drive selective death of t(4;14) HMCLs. Finally, we aim to develop lipid nanoparticles to deliver RfxCas13d and personalised gRNAs to patient-derived MM cells as a proof of principle therapeutic.

Session Three: Immunotherapy *(Sponsored by Scientifix/Revvity)*

Invited Speaker: Paul Beavis
Talk Title: CRISPR engineering of armored CAR T cells enables tumor restricted payload delivery with enhanced efficacy and safety

Speaker: Tirta Djajawi

Abstract Title: TAK1 protects tumour cells from combined CTL-derived TNF and IFN-y

Authors: Tirta M. Djajawi, Anne Huber, Akash Srivaths, Oliver Ozaydin, Sarahi Mendoza Rivera, Stephin J. Vervoort and Conor J. Kearney

Affiliations: Olivia Newton-John Cancer Research Institute, Melbourne, VIC

Full Abstract: Cancer immunotherapies, particularly immune checkpoint blockade, have demonstrated remarkable efficacy in some cancer types including melanoma. However, regardless of the clinical success of checkpoint inhibitor therapies, many patients do not respond or develop resistance, leading to relapse. Whereas some mechanisms of tumour immune escape are understood, the full spectrum of immune evasion routes are not well understood. Given the issue of high non-response rates and patient relapse, there is an urgent need to molecularly investigate tumour immune evasion tactics in order to develop improved immunotherapy approaches.

Here, we conducted a kinome-wide CRISPR-Cas9 screen in melanoma cells to identify factors that limit CD8+ T cell-mediated anti-tumour immunity. We identified that TAK1 suppresses combined CTL-derived IFNy- and TNF-induced cell death, thus protecting from CD8+ T cell-mediated killing. Indeed, TAK1 knockout or pharmacological targeting of TAK1 with the clinical inhibitor Takinib enhances CD8+ T cell-mediated cancer cell killing in a TNF and IFNy-dependent manner, while re-introduction of TAK1 in TAK1-deficient cells reverses this effect. Furthermore, through genome-wide CRISPR screening in a TAK1 deficient background, we identified STAT1, RIPK1 and Caspase-8 as key effectors of the combined TNF and IFNy-induced cell death. Taken together, we identify TAK1 as a negative regulator of anti-tumour immunity, unveiling TAK1 targeting as a novel immunotherapeutic approach or as an adjunct to existing immunotherapies.

Speaker: Phoebe Dunbar

Abstract Title: Generating CRISPR/CAS9 armoured TCR-T cells for the treatment of solid tumours

Authors: Phoebe Dunbar (1, 2), Amanda Chen (1, 2), Kah Min Yap (1, 2), Phil Darcy (1, 2), Paul Beavis (1, 2)

Affiliations: 1. Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, 3000, Victoria, Australia. 2. Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, 3010, Australia

Full Abstract: CAR T cell therapy has exhibited remarkable clinical success in the treatment of haematological malignancies, however, their efficacy in solid tumours is limited by antigen heterogeneity and immunosuppression imposed by the tumour microenvironment. To overcome these barriers, 'armoured' CAR T cells which secrete pro-inflammatory cytokines have been developed. However, toxicities related to the unrestricted expression of the armouring transgene has limited the application of these CAR T cells in the clinic. Our lab has developed a novel CRISPR/Cas9-mediated homology directed repair (HDR) strategy whereby we aimed to engage endogenous gene regulatory mechanisms drive transgene expression in a tumour-localised manner (in review, Nature). Genome-wide RNA-sequencing was used to identify genes in CAR T cells with tumour-specific expression. The promotor NR4A2 was identified as a key candidate due to its inhibitory role in T cells. This novel CRISPR HDR strategy was employed to knock in proinflammatory cytokine, IL-12 into NR4A2 promoter which supports the delivery of cytokines directly to the tumour site, leading to enhanced anti-tumour efficacy and long-term survival of mice in both syngeneic and xenogeneic models.

Although we have developed a novel technology that tightly restricts expression of pro-inflammatory cytokines to T cell activation, the application of this technology in a CAR T setting is limited by on-target toxicity in healthy cells where tumour antigen is expressed. I therefore hypothesise that the application of tumour neoantigen specific TCR-T cells would provide an even greater safety profile for this technology. I have generated T cells expressing an engineered TCR targeting the tumour neoantigen KRAS G12D and subsequently verified that the NR4A2 promotor effectively turns on within this neoantigen specific TCR-T cell setting. I have further engineered TCR-T cells to express an NR4A2/IL-12 knock in, which elicits improved anti-tumour efficacy and an improved safety profile in xenogeneic mouse models.

Speaker: Emily Anderson

Abstract Title: Robust Genomewide Application of Deactivated CRISPR-Cas9 Systems for Expression Regulation

Authors: Emily Anderson1, Clarence Mills1, Joanna Gawden-Bone2, Jera Law1, Abhijit Patil2, Matthew Brockman3, Brian Ziemba1, Andrew Riching1, Kevin Hemphill1, Simon Scrace2, Zaklina Strezoska1, and Josien Levenga1

Affiliations: 1. Revvity, Lafayette, Colorado, United States 2. Revvity, Cambridge, United Kingdom 3. Revvity, Mulgrave, Australia

Full Abstract: CRISPR-Cas9 has been engineered for modulating transcription and epigenetics; CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) systems act via a nuclease-deactivated Cas9 (dCas9) fused to modifiers and targeted to promoters by gene-specific synthetic guide RNAs (gRNAs). We have optimized CRISPR-based modulation for use in primary and difficult cells (requiring chemically synthesized gRNAs transiently delivered with dCas9 mRNA or ribonucleoprotein (RNP)). Synthetic gRNA delivery was also tested for streamlined high-throughput arrayed screening using stably-expressing Cas9 cell lines. Finally, high-titer All-in-one dCas9-effector lentiviral systems were produced allowing up to full genome pooled screens.

We demonstrate a simple, robust CRISPRa approach compatible with dCas9 fused to the VP64-p65-Rta tripartite activator (dCas9-VPR). Endogenous activation can be tuned/enhanced using multiple chemically modified synthetic gRNAs. Using the All-in-one dCas9-VPR lentiviral system, we have conducted a whole-genome CRISPRa screen, which faithfully recapitulates data from prior drug-resistance screening using the complex, three-vector Synergistic Activation Mediator (SAM).

The Dharmacon CRISPRi system is more efficient than first-generation CRISPRi. This novel dCas9 fusion uses domains from transcriptional repressors Sal-like protein 1 (SALL1)/Sin3 histone deacetylase corepressor complex component (SDS3). An unbiased protein interaction experiment demonstrates dCas9-SALL1-SDS3 interacts with key members of the histone deacetylase and Swi-independent 3 complexes (endogenous functional effectors of SALL1 and SDS3). dCas9-SALL1-SDS3 with chemically synthesized gRNAs can be used orthogonally to siRNA to verify downstream phenotypes. Finally, we performed the first genome-wide pooled screen with single lentiviral vector (All-in-one) dCas9-SALL1-SDS3 CRISPRi and observed enhanced essential gene dropout compared to first-generation single-vector dCas9-KRAB CRISPRi.

These CRISPR modulation systems were optimized for short-term, non-viral delivery or for pooled screening with high-titer lentiviral libraries. These formats enable simple, robust, and rapid gain- or loss-of-function experiments. Complex end-point assays are now accessible, and scale up to high throughput arrayed or pooled screening platforms is possible for multiple compounds, hypotheses, or cell types.

Session Four: Therapeutic Applications *(Sponsored by MaxCyte)*

Invited Speaker: Shivani Pasricha
Talk Title: TBA

Speaker: Ashleigh Geiger

Abstract Title: Cas9-NG: a new vision for autosomal dominant Retinitis Pigmentosa precision therapeutics

Authors: Ashleigh B Geiger (1,2), Fatwa Adikusuma (1,2), Laurence OW Wilson (3), Louise J Robertson (1,2), John PM Wood (1), Robert J Casson (1), Paul Q Thomas (1,2).

Affiliations: 1. Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia. 2. South Australian Health and Medical Research Institute Gene Editing Program, Adelaide, Australia. 3. CSIRO Digital Genome Engineering, New South Wales, Australia.

Full Abstract: Autosomal dominant Retinitis Pigmentosa (adRP) is an important cause of progressive, irreversible blindness. At least 12% of all non-syndromic adRP is due to the Pro23His mutation in Rhodopsin (RHO), a photoreceptor-specific GPCR that is essential for retinal function and health. The Pro23His mutation operates via dominant-negative and toxic gain-of-function mechanisms, rendering gene replacement strategies non-viable. Conversely, as RHO is partially haplosufficient, selective ablation of the mutant allele using CRISPR/Cas9 technology represents a tantalising prospect for treatment. However, guide RNA (gRNA) design is heavily restricted due to the unavailability of canonical SpCas9 5′-NGG-3′ PAM sequences at this locus. In this study, we leveraged alternative CRISPR/Cas9 platforms to target the RHO Pro23His mutation in vitro and in vivo. To initially assess disease-allele targeting activity, we generated a genetic cell model using Prime Editing. Candidate gRNAs were tested, and deep sequencing identified several which displayed highly efficient and selective targeting of the mutant allele. Encouraged, we tested our most promising candidates in a humanised preclinical RHO Pro23His mouse model, delivering AAV-CRISPR via intravitreal injection. 70 days post-injection, we assessed AAV-CRISPR delivery success via immunofluorescence, identifying robust evidence of Cas9 transduction in target cells of treated retinae. Excitingly, phenotype testing via dark-adapted electroretinogram revealed a treatment group with significantly improved photoreceptor activity compared with untreated controls, suggesting sustained retinal neuroprotection. To our surprise, this treatment utilised the engineered CRISPR variant Cas9-NG, which is currently absent from the CRISPR therapy literature due to perceived inefficiency and non-specificity. Importantly, these results are accompanied by molecular-level evidence of Cas9-NG activity against the human RHO Pro23His allele in treated murine retinae. Together, these data provide preclinical evidence that potentiates our allele-specific targeting approach as a candidate treatment for RHO Pro23His adRP, and expands the CRISPR therapeutic toolbox via the novel application of Cas9-NG.

Invited Speaker: Catherine Mills
Talk Title: TBA

Session One: Molecular Oncology

Invited Speaker: Susan Woods

Talk Title: CRISPR in action: engineering bowel cancer models and bacteria for tumour detection

Speaker: Christina Koenig

Abstract Title: Using cutting edge CRISPR base editor technology to explore development of lymphoma

Authors: Christina Koenig #1#2#3, Andrew Kueh #1#2#3#4, Sarah Diepstraten #3#4, Maggie Potts #1#2#3#4, Lin Tai #1, Lauren Whelan #3, Marco Herold #1#2#3#4

Affiliations: #1Olivia Newton-John Cancer Research Institute, Heidelberg, Melbourne, Australia. #2School of Cancer Medicine, La Trobe University, Bundoora, Melbourne, Australia. #3The Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Australia. #4Department of Medical Biology, University of Melbourne, Parkville, Melbourne, Australia.

Full Abstract: Neoplastic diseases result from the unrestricted growth of cells that have been transformed into a malignant state. Genetic defects mainly caused by single nucleotide variants (SNPs) in tumour suppressor genes or in proto-oncogenes allow cancer cells to acquire essential biological properties and deregulate several cellular processes, ensuring their survival and efficient growth. To model these SNPs and identify cancer-driving mutations, we employ the recently described CRISPR base editing (BE) technology.

We established experimental procedures using the CRISPR BE technology together with single guide (sg)RNA libraries specifically designed to introduce the top mutations in human cancer-causing genes into the mouse genome in vitro and in vivo. In vitro, we transduce tumour prone EμMYC cells (a model of B cell lymphoma) with lentiviral BE plasmids able to introduce base changes and a respective sgRNA library for targeting the individual mutations in the genes of interest. We then treat this pool of engineered cells with diverse chemotherapeutic drugs, such as DNA-damaging agents or BCL-2 family inhibitors. In a major in vivo approach, we isolate hematopoietic stem and progenitor cells (HSPCs) from E14.5 embryos of newly developed EμMYC/TRE-CBE double-transgenic animals (harbouring an inducible BE system) and transduce them with BE sgRNA libraries to reconstitute lethally irradiated recipient mice. To activate the base editing that will create the specific mutations in pre-leukemic cells, recipient animals receive doxycycline food. Tumours that arise at an accelerated pace will be isolated and the tumour promoting sgRNA and genetic mutation identified.

Using this approach will reveal critical mutations in tumour suppressor pathways and oncogenes involved in the transformation of haematological malignancies. This will enhance our understanding of malignant transformation and provide novel targets for anti-cancer therapies.

Speaker: Jasleen Rajpal

Abstract Title: Uncovering the Functions of Alternative Splicing in EMT using RNA-Targeting CRISPR Technology

Authors: Jasleen Rajpal1, Caroline A. Phillips1, John Toubia1, Katherine A. Pillman1, Gregory J. Goodall1,2 and Philip A. Gregory1,2

Affiliations: 1Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, SA, 5000, Australia 2Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, SA 5000, Australia.

Full Abstract: Epithelial-Mesenchymal Transition (EMT) is a tightly regulated cell differentiation process that plays crucial roles in embryonic development and cancer progression. During EMT, the RNA-binding protein Quaking (QKI) orchestrates widespread changes in alternative splicing (AS) which influences cell plasticity, migration, and invasion. Despite the importance of AS in EMT, the functional roles of most alternatively spliced proteins remain unclear due to challenges in manipulating AS. To address this, we are developing RNA-targeting CRISPR technology to facilitate precise manipulation of alternative splicing at the transcript level and enable large-scale functional studies. Our approach utilizes catalytically inactive "dead" CasRx (dCasRx) to bind specific RNA sequences, block splice machinery access, and induce exon skipping. Additionally, we have developed a dCasRx-QKI fusion protein to facilitate exon inclusion by targeting QKI to selected binding motifs. As a proof of concept, we show that dCasRx effectively blocked the inclusion of exon 3 in the transcription factor NFYA which results in increased cell proliferation. This CRISPR-based strategy will be applied to pooled single-cell screening to evaluate the functional consequences of hundreds of individual splicing alterations on cell proliferation, migration, EMT, and drug resistance.

Invited Speaker: Teresa Sadras
Talk Title: Using CRISPR technology to model complex chromosomal aberrations: a step towards better understanding leukemia pathobiology

Session Two: Environmental Applications

Invited Speaker: Karen Massel

Talk Title: TBA

Invited Speaker: Charles Robin

Talk Title: TBA

Day 2 Flash Talks

Speaker: Aqsa Mazhar

Abstract Title: Identification of the synthetic lethality for chemotherapy drugs to develop novel combination therapy for rhabdomyosarcoma through CRISPR technology

Authors: Aqsa Mazhar 1,2, Daenikka Ravindrarajah 1,2, Twishi Gulati 4, Glenn M. Marshall1,3, Belamy B. Cheung 1,2

Affiliations: 1.Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, NSW, Australia: 2.School of Clinical Medicine, UNSW, Australia : 3.Kids Cancer Centre, Sydney Children's Hospital, Randwick, NSW, Australia: 4.Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre Victoria Australia

Full Abstract: Rhabdomyosarcoma (RMS) is the most common childhood soft tissue sarcoma, comprising 4.5% of all childhood cancers. Alveolar tumours (ARMS) contribute to approximately one-third of RMS, and it is clinically more aggressive due to a propensity for metastasis and recurrence. Most ARMS expresses one of two oncogenic gene fusions: PAX3 or PAX7 with FOXO1, which act as a dominant-acting oncogene in driving tumorigenesis. To date, there is no inhibitors which directly bind to PAX3-FOXO1. Currently, CRISPR-Cas9 screens are an increasingly valuable method for determining synthetic lethality and drug-resistant genes across the entire genome in the present of chemotherapy drugs or target therapy drugs.

Objective: To identify drug resistant genes in alveolar RMS by CRISPR-Cas9 screen and develop the effective novel combination therapies with currently clinical used therapeutic drugs for alveolar RMS.

Methods and Results: We have successfully generated stable Cas9 expressing Rh41 cell line by transducing cells with lentiCas9- mCherry plasmid After determining the lentivirus titre of pXPR-011 construct using FACS analysis, we confirmed the success of transduction of Rh41 with Cas9-mCherry lentivirus by Western Blot assay. We have performed CRISPR KO screen and transduced cas9-Rh41 cells with sgRNA library (whole genome library) by using 0.3 MOI with irinotecan treatments by using IC30 and collected cell pellets at different time points. After extraction of genomic DNA and amplification of PCR, we have sent samples for sequencing to identify the candidate genes that play a role in drug sensitivity in alveolar RMS cell line. We are validating candidate genes obtained from CRISPR KO screen by performing in vitro phenotypic and molecular analysis to determine the efficacy and mechanism of synergy in the drug combinations for further in vivo study.

Conclusion: This study will provide new avenues for the treatment of alveolar RMS by identifying the drug-resistant mechanism and developing novel combination therapy

Speaker: Thomas Cole

Abstract Title: CRISPR editing of adenosine receptor expression in CAR T cells to enhance therapy of solid tumours

Authors: Kevin Sek, Paul Beavis, Phil Darcy

Affiliations: Peter MacCallum Cancer Center

Full Abstract: Tumour-infiltrating CAR-T cells face many immunosuppressive conditions within the solid tumour microenvironment, including the accumulation of hypoxia-driven metabolites such as extracellular adenosine. This metabolite acts primarily through the A2A receptor (A2AR) and suppresses adoptive cellular immunotherapies including CAR-T cells. We hypothesized that using CRISPR gene-editing technologies to modulate the adenosine receptor immunosuppressive axis could enhance CAR-T cell function in solid tumours.

The A1 receptor (A1R) is an alternative adenosine receptor that signals inversely to A2AR. Earlier work demonstrated that CRISPR homology-directed repair (HDR) to knock-in A1R into an endogenous T cell promoter achieved tightly tumour-restricted A1R expression by hijacking endogenous transcriptional regulatory mechanisms. This approach enhanced effector function, cytokine production, and prolonged persistence of CAR-T cells in human solid tumour models. Through this investigation we discovered novel transcription factors activated by A1R signalling in CAR T cells that are targetable with CRISPR KO to enhance therapy even further.

Subsequent testing was conducted in murine CAR-T cells in models that recapitulate the immunosuppressive tumour microenvironment. The use of the same promoter did not effectively overcome the suppressive influence of endogenous A2AR signalling. However, by using alternative promoters with stronger but less specific transgene expression, we improved CAR-T effector function in vitro, though at the expense of depleting the memory pool crucial for in vivo efficacy.

We consequently explored a dual CRISPR strategy: A2AR ablation combined with A1R HDR to synergistically enhance CAR-T cell function by strengthening the A1R bias. This approach resulted in amplification of the A1R transcriptional phenotype and further augmented CAR T cell effector function. In summary our study leverages CRISPR gene-editing to remodel the adenosine receptor profile of CAR-T cells, providing a promising strategy for enhancing their efficacy in solid tumours.

Speaker: Laura McCoullough

Abstract Title: Targeting the hepatitis B RNAs using CRISPR-Cas13b to suppress hepatitis B virus replication and protein expression in vitro and in vivo

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Full Abstract: Background: Bacterial CRISPR-Cas13b endonuclease has been repurposed to target RNA in mammalian cells by designing highly specific 30 nucleotide CRISPR RNAs (crRNAs) complementary to the target RNAs of interest, which reduces the possibility of off-target effects. Recent preclinical studies have used CRISPR-Cas13b as a novel antiviral to target viral RNAs such as SARS-CoV-2 and influenza RNAs to reduce viral replication. Hepatitis B virus (HBV) is a DNA virus that replicates through an RNA intermediate known as the pregenomic RNA (pgRNA). The pgRNA and viral mRNAs represent novel antiviral targets, which may be targeted by CRISPR-Cas13b. Here, in a world first study, we used CRISPR-Cas13b to target the HBV RNAs to reduce HBV replication and protein expression in vitro and in vivo.

Methods: Cas13b crRNAs were designed to target the HBV RNAs. Hepatoma cells were transfected with wildtype (WT) HBV of multiple genotypes, Cas13b and crRNA plasmids. A HBV stable cell line and HBV infection model were transfected with Cas13b and crRNA plasmids. The impact on HBV replication and protein expression was determined. WT HBV, Cas13b and crRNA plasmids were hydrodynamically co-injected into CBA mice and sera hepatitis B surface antigen (HBsAg) was measured. Cas13b mRNA and crRNA were delivered by lipid nanoparticles (LNPs) in a HBsAg-expressing stable cell line and secreted HBsAg was measured.

Results: Cas13b strongly suppressed HBV replication and protein expression in all cell lines tested. The effect was pan-genotypic. Sera HBsAg was reduced by ~50% in vivo. LNP-encapsulated Cas13b mRNA reduced secreted HBsAg by 87% in a HBsAg-expressing stable cell line.

Conclusion: CRISPR-Cas13b successfully targeted the HBV RNAs to significantly reduce HBV replication and protein expression in vitro and in vivo which, together with other studies that have used CRISPR-Cas13b to target viral RNAs, further demonstrates its potential as a novel antiviral.

Speaker: Ryan Lee

Abstract Title: Efficient CRISPR-Cas9 therapies for the correction of Duchenne Muscular Dystrophy amendable to exon 45 targeting

Authors: Ryan HB Lee1,2, Fatwa Adikusuma1,2, Paul Q Thomas1,2,3

Affiliations: 1. School of Biomedicine, University of Adelaide 2. South Australian Health and Medical Research Institute 3. South Australian Genome Editing Facility

Full Abstract: Duchenne Muscular Dystrophy (DMD) is a monogenic muscle-wasting disorder caused by mutations that disrupt Dystrophin production. Since no curative treatments are available, DMD is universally fatal around 30 years old. CRISPR therapies for DMD install loci-specific edits which could restore the production of a truncated yet functional Dystrophin, through exon skipping or reframing. Importantly, the long-lasting nature of these edits are potentially curative and overcome the lifelong administration required for current treatments. Streptococcus pyogenes Cas9 (SpCas9) remains the most utilised system for therapeutics due to its established efficiency. Smaller Cas9s like Staphylococcus aureus Cas9 (SaCas9) allow for single Adeno-associated virus (AAV) delivery, which holds promise in vivo as therapeutic benefit can be achieved with a lower AAV dosage.

This study aims to identify SpCas9 and SaCas9 gRNAs which efficiently target exon 45 of the human DMD gene and to validate the dual-gRNA approach we devised to maximise Dystrophin restoration. These therapies would be applicable to 9% of DMD patients harbouring exon 46-47, 46-51 and 44 deletions (Δ44). Here, we present a clonal Δ44 DMD model, generated through paired SpCas9 activity on wildtype immortalised, human myoblast and validated through PCR and Western blotting.

gRNAs were transfected in triplicate into this Δ44 model, and the resulting editing profile was analysed through Next Generation Sequencing (NGS). Dystrophin restoration post-treatment was also assessed through cDNA PCR and Western blotting.

Through NGS, several SpCas9 & SaCas9 gRNAs with mean on-target editing efficiencies exceeding 90% were identified, of which up to 55.8% of therapeutic editing was observed. In accordance with our dual-gRNA approach, the co-delivery of these gRNAs led to a significant enhancement of therapeutic exon skipping in RNA and Dystrophin restoration in protein.

Collectively, these experiments demonstrate the therapeutic promise of our CRISPR candidates, thus justifying future in vivo studies.

Speaker: Maria Faleeva

Abstract Title: Functional Mapping of PRC2 Mutations with Unbiased Base Editing.

Authors: Ali Motazedian, Maria Faleeva, Henrietta Holze, Kapil Mcinerney Mark Dawson

Affiliations: Peter Maccallum Cancer Centre VCCC

Full Abstract: Polycomb Repressive Complex 2 (PRC2) regulates gene expression through its histone methyltransferase activity. It consists of three core proteins: EZH2, SUZ12, and EED. EZH2 is the catalytic subunit that methylates histone H3 at lysine 27 (H3K27), crucial for gene silencing. SUZ12 provides structural support and interacts with accessory proteins, whilst EED recognises existing H3K27 methylation, allosterically boosting EZH2 activity to maintain repressive chromatin states. PRC2's structure and function are highly conserved across eukaryotes, highlighting its vital role in cellular processes. Mutations in PRC2 can cause both loss (LoF) and gain (GoF) of function, inducing either hypo- or hyper-methylating activity in various cancers and congenital disorders. The effects of DNA mutations on PRC2's activity and stability necessitate further research to provide insights into the evolutionary mechanisms that enable PRC2's role in epigenetic regulation.

This project aimed to systematically alter the coding sequence of PRC2 complex members using base editors (BEs) and characterise LoF and GoF mutations. We used five different BEs to perform A to G and C to T mutations, inducing random mutations across a significant portion of the gene for a comprehensive analysis of PRC2 function at single cell resolution. Experiments were conducted in K562 cells which tolerate GoF and LoF mutations without a discernible effect on growth and survival.

Our screen identified synonymous, stop, and missense mutations, several of which are hypothesised to be dominant negative. We identified LoF mutations in both structured and unstructured domains of PRC2 proteins. Guides and mutations frequently clustered in specific 'hotspots' within disordered domains of SUZ12 and EED, highlighting key regions not identifiable by conventional structure determination methods. Finally, transcriptome sc-RNA sequencing revealed whether PRC2 disruption has binary or variable effect on gene expression.

In summary, this BE-driven mutagenesis screen revealed diverse PRC2 LoF and GoF mutations undetectable by conventional methods.

Poster Session Two

Speaker: Matthew O'Neill

Abstract Title: Combating the rise in antimicrobial resistant Neisseria gonorrhoeae through the development of point

Authors: Matthew T O'Neill, BSc [Hons],1,2* Soo Jen Low, PhD,1* Janath Fernando, BSc [Hons],1 William J Kerry, MSc,2 Natasha Wild, BSc [Hons],2 Marcelina Krysiak, MSc,1 Simran Chahal [Hons],1 Jacqueline Prestedge, BSc [Hons],1,3 Francesca Azzato, BSc,1,3 Prof. Christopher Fairley, PhD,4,5 Eric PF Chow4,5, 6 Chuan K Lim, PhD,2,3 Catriona Bradshaw, PhD,4,5 Prof. Deborah A Williamson, PhD,1 Shivani Pasricha, PhD1,2

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Full Abstract: Combating the rise in antimicrobial resistant Neisseria gonorrhoeae through the development of point-of-care CRISPR-based diagnostics.Gonorrhoea and chlamydia, caused by Neisseria gonorrhoeae (NG) and Chlamydia trachomatis (CT), respectively, account for over 200 million new cases of sexually transmitted infections annually and can cause severe complications including infertility and chronic pain if left untreated. Timely treatment and prevention of transmission requires improved access to diagnostics via innovative point-of-care test (PoCT) development. We developed a multiplexed CRISPR-Cas12a/Cas13a test with isothermal pre-amplification for the detection of NG and CT with two biomarkers per pathogen, which can be reflexed to an additional test for detecting a mutation in the gyrase A gene associated with ciprofloxacin resistance in NG. Candidate primer and guide RNA sets were designed using an in-house bioinformatic pipeline, and the most sensitive sets were tested in combination for final selection of gene targets. Analytical sensitivity of the test was evaluated using serial dilutions of gDNA from target pathogens, and specificity assessed using a panel of clinically relevant viral and bacterial pathogens. The test detected single copies per µL of bacterial gDNA and showed no cross-reactivity to closely related species and relevant pathogens. Clinical validation was performed on genomic DNA (gDNA) from 600 clinical samples on a portable fluorimeter. The assay is fully integrated onto a PoC device, with a run duration of 40 minutes to result. Overall, we have advanced CRISPR-Cas multiplexing capability and developed a novel PoCT for the detection of NG, CT, and a mutation associated with antimicrobial resistance, which is anticipated to improve access to testing and antimicrobial stewardship.

Speaker: Jeralyn Wen Hui Ching

Abstract Title: Dissecting gene regulatory mechanisms at the autoimmune risk locus CD83

Authors: Jeralyn Wen Hui Ching, Viacheslav Kriachkov, Stephen Nutt, Hamish King

Affiliations: Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Full Abstract: More than 10,000 variants are linked to autoimmune diseases and over 90% of disease-associated genetic variants identified through genome-wide association studies reside in the non-coding genome, including distal regulatory elements (DREs). However, the molecular mechanisms underlying these DREs and any functional consequences of genetic variants within them remain largely untested and unclear. Here we observed an autoimmune risk locus, CD83, containing 3 fine-mapped SNPs linked with rheumatoid arthritis, found in open chromatin in B cells. Among the 3 risk variants, rs74405933 (G>A), is located on a CD83-regulating, CRISPR activation (CRISPRa)-responsive DRE and is predicted to disrupt a NF-KB transcription factor binding motif. To measure the impact of rs74405933 on CD83 expression, prime editing was used to introduce the risk allele A (rs74405933) into human B cells. A depletion in the frequency of the risk allele in the CD83 high-expression quantile suggests an association between SNP rs74405933 and reduced CD83 expression. Beyond rs74405933, other SNPs found in open chromatin regions within the locus must also be tested to uncover their contribution to CD83 regulation. To comprehensively map and quantify DREs in an unbiased manner, we have designed a tiled CRISPRa library spanning a 300 kb region, targeting a 200 bp window in primary human B cells. The enrichment of gRNAs in the top 20% of CD83-expressing cells will reveal CRISPRa-responsive regions corresponding to functional DREs. This facilitates further investigation into how these SNPs within the identified DRE regions influence phenotypes such as target gene expression. Next, we aim to establish a scalable toolkit for prioritizing non-coding genetic variants and reveal DRE mechanisms at high resolution, with potential applications to other loci and diseases.

Speaker: Felix Brown

Abstract Title: Identifying resistance factors to STING agonists in blood cancer

Authors: Felix Brown, Sarah Diepstraten, Eddie La Marca

Affiliations: The Walter and Eliza Hall institute

Full Abstract: Recently, the combination therapy of STING (Stimulator of Interferon Genes) agonists with BH3-mimetics was identified as effectively killing various blood cancers via intrinsic apoptosis, but independent of p53. While resistance to BH3-mimetics is a major clinical issue and has been heavily researched, resistance factors for STING agonists in blood cancers are entirely unknown. My honours project aims to predict resistance factors to STING agonists ahead of their clinical deployment by harnessing CRISPR activation (CRISPRa) technology in blood cancer cell lines. Using next-generation CRISPRa sgRNA libraries to upregulate genes of known function, while pressuring cells with STING agonist treatment, I will identify genes that confer resistance to STING agonist killing. This high-throughput CRISPRa screening approach mimics the mutational landscape of blood cancer patients, enabling the early detection of resistance pathways. These findings may one day assist in optimising STING agonist-based therapies when treating blood cancer patients.

Speaker: Nathan Chai

Abstract Title: Developing new-to nature de-novo design CRISPR-Cas13 inhibitor

Authors: Nathan Chai, Cyntia Taveneau, Jovita D'Silva, Rebecca S. Bamert, Rhys Grinter, Gavin J. Knott

Affiliations: Biomedicine Discovery Institute, Bio21

Full Abstract: CRISPR-Cas systems are revolutionary tools with practical uses ranging from genetic engineering to gene drives. A key aspect of utilizing CRISPR technology effectively is the discovery and development of anti-CRISPRs, which are phage-derived proteins that can strongly inhibit CRISPR functions. However, finding naturally occurring anti-CRISPRs is difficult, and many important Cas effectors lack inhibitors to control their activity. This study presents a method using de novo protein design to create novel proteins that regulate CRISPR-Cas activity. The research shows that AI-designed anti-CRISPRs (AIcrs) can specifically and powerfully inhibit the target protein, CRISPR-Cas13. The study includes thorough design validation and demonstrates the use of AIcrs in managing the anti-phage activity of CRISPR-Cas13 in bacteria. The ability to quickly design custom inhibitors for CRISPR-Cas systems will aid in the development of gene editors for clinical applications and help prevent their misuse.

Speaker: Ksenija Nesic

Abstract Title: Generation of a PARPi-sensitive homozygous BRCA1-methylated OVCAR8 cell line using targeted CRISPR gene editing

Authors: Ksenija Nesic1,2*, Sally Beard1,2*, Lijun Xu3, Olga Kondrashova3, Cassandra J. Vandenberg1,2, Alan F. Rubin1,2, Fan Zhang4, Alexander Dobrovic4^, Nicola Waddell3, Clare L. Scott1,2,5,6,7, Kristy Shield-Artin1,2#, Matthew J. Wakefield1,2,5#

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Full Abstract: Up to 17% of high grade serous ovarian carcinomas (HGSOC) harbour BRCA1 promoter methylation (meBRCA1), making them susceptible to treatment with targeted PARP inhibitor (PARPi) therapy. Unfortunately, meBRCA1 loss can be acquired following PARPi or platinum chemotherapy, resulting in BRCA1 re-expression and PARPi resistance. Our understanding of meBRCA1 stability in HGSOC is currently limited, in part due to a paucity of pre-clinical models with homozygous meBRCA1. Herein, we describe the generation of a several OVCAR8 cell line derivatives containing landing pad constructs, for future functional studies, and representing various BRCA1 states, including a homozygous meBRCA1 variant. Our PARPi resistant OVCAR8 has two methylated BRCA1 copies and one unmethylated copy, enabling BRCA1 expression. CRISPR-Cas9 gene editing was used to delete copies of the BRCA1 gene in landing pad-containing clones of this cell line (A6 and H4). We produced one variant with deletion of all BRCA1 copies (H4-53), and another with two copies deleted and only a single methylated gene copy remaining (A6-30 – validated further using nanopore long-read sequencing). These both lacked BRCA1 gene expression and were sensitive to PARPi treatment. The A6-30 line was transplanted into immunocompromised mice to generate a xenograft model that retained homozygous meBRCA1 and demonstrated some response to PARPi in vivo. Thus, using CRISPR gene editing we have created several novel isogenic HGSOC cell line models, including one with homozygous meBRCA1, that will support future studies of meBRCA1 stability and PARPi resistance.

Speaker: Ana Parra Nunez

Abstract Title: Manipulating sex ratios by targeting haplolethal gene wupA in Drosophila

Authors: Ana Parra Nunez, Simon Baxter, Charles Robin

Affiliations: University of Melbourne

Full Abstract: Y-linked editors (YLEs) are constructs inserted into the Y chromosome that cleave chromosomes required for female reproduction and survival. These edits cause dominant lethality or sterility in the progeny; therefore, the fitness of female progeny is reduced.

We have designed a CRISPR/Cas9-based approach to bias sex ratios by targeting the haplolethal wings up A (wupA) gene on the X-chromosome of Drosophila melanogaster. For most haplolethal genes, two copies are required for viability; however, dosage compensation mechanisms allow haplolethal genes to occur on the X chromosome of D. melanogaster, where males are XY. The disruption of wupA during spermatogenesis results in fewer viable female progeny, as they carry one functional copy of the gene, and there is no dosage compensation mechanism.

Three sgRNA sequences targeting wupA were inserted into a fly line, successfully disrupting wupA during spermatogenesis under a nosCas9 germline promoter. Our fly crosses resulted in 14% female F1 progeny, a significant deviation from Mendelian ratios. We screened surviving females for allelic resistance, tested the efficiency of our Cas9 and sgRNAs, and assessed the fitness of wupA-poisoning males.

YLEs that hinder the functionality of haplolethal genes in pests may ultimately lead to the decline of the target population through female elimination. Our current objectives include identifying ideal Y-chromosome insertion sites for sgRNA constructs and improving the sex-biasing efficiency. Our ultimate aim is to transfer this sex-biasing system into the agricultural pest Drosophila suzukii, whose Y-chromosome we have successfully assembled.

Speaker: Eddie La Marca

Abstract Title: Identifying transcription factors regulating haematopoietic differentiation using CRISPR activation in vivo

Authors: John E La Marca, Sarah T Diepstraten, Margaret A Potts, Gemma L Kelly, Marco J Herold

Affiliations: The Walter and Eliza Hall Institute of Medical Research, The Olivia Newton-John Cancer Research Institute, The University of Melbourne, La Trobe University

Full Abstract: Identifying the genes regulating the development of haematopoietic lineages has long been of great interest, due to the preponderance of blood-associated pathologies. However, identifying transcription factors (TFs) that positively promote specific lineage development is difficult, as it requires the generation of mice capable of upregulating that candidate gene specifically within the haematopoietic stem and progenitor cell (HSPC) compartment.

We hypothesise that CRISPR activation (CRISPRa) technology can be used to address these questions in an efficient manner. To facilitate such studies, we have generated an efficient dCas9 mouse model (Deng et al, 2022). By transducing HSPCs from our CRISPRa model with a library of TF-targeting sgRNAs, and transplanting cells into lethally-irradiated recipient mice, we aim to identify factors driving different haematopoietic lineage decisions.

To that end, we have first investigated how many sgRNAs are detectable after HSPC transduction and reconstitution in various haematopoietic tissues, and have established cell sorting protocols necessary to retrieve sufficient cell numbers from each blood compartment for next-generation sequencing of sgRNAs. Next, we will use these techniques to identify what TFs are enriched or depleted in certain haematopoietic lineages, and build our understanding of how haematopoiesis occurs and can be disrupted in disease.

Speaker: Lucas Newton

Abstract Title: Developing a pipeline for generating gene knockout and knock-in fluorophore fusion proteins using CRISPR-Cas9 to study T cell development in primary mouse thymocytes

Authors: Lucas M. Newton1,2, Mirren Charnley1,2, Sarah M. Russell1,2

Affiliations: 1.Organogenesis and Cancer Program, Cancer Research, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia. 2. Optical Sciences Centre, Swinburne University of Technology, Melbourne, Victoria, Australia.

Full Abstract: β-selection is a critical checkpoint during T cell development that determines the fate and functional competence of mature T cells. This process occurs in the thymus, where developing T cells undergo stringent selection based on their ability to successfully rearrange and express a functional T cell receptor (TCR) β-chain. β-selection not only ensures the generation of a diverse T cell repertoire capable of recognising a wide array of antigens but also plays a fundamental role in shaping immune tolerance and responsiveness. Our previous work has identified new regulators of β-selection using a transgenic Cre-LoxP system in mice to generate protein knockdown primary mouse thymocytes. However, this method is infamously leaky and requires the costly generation of new transgenic mouse lines for each gene of interest. Therefore, we are now working towards applying CRISPR-Cas9 technology to genetically manipulate primary mouse developing T cells and the mouse stromal cell line OP9-DL1/4, which represents the thymic niche. Our goal is to produce a pipeline to genetically manipulate human and mouse developing T cells and the stroma. Here, we present an update on our progress in generating CD5 knockout primary mouse thymocytes by applying CRISPR-Cas9 to foetal liver-derived hematopoietic stem cells prior to expansion and differentiation. In addition, we share our initial results into the generation of StayGold and mCardinal fluorescently tagged endogenous Numb and Bcl6, which both play significant roles in regulating asymmetric cell divisions during T cell β-selection. Furthermore, this work provides insights into our efforts to generate E-cadherin knockout OP9-DL1/4 stromal cells, which has proven problematic using conventional CRISPR-Cas9 approaches in mouse cells. We believe this work will prove helpful to those interested in genetically manipulating haematopoietic cell systems and we look forward to much needed feedback on our current achievements.

Speaker: Emily Hann

Abstract Title: Uncovering the design principles of CRISPR/Cas13d as an effective antiviral strategy

Authors: Emily Hann1,2*, Debolina Majumdar1, Daniel Layton1, Mark Ziemann3, David Cahill2, Beata Ujvari2, Mohamed Fareh4, Karel A Schat5, Arjun Challagulla1

Affiliations: 1Australian Centre for Disease Preparedness, CSIRO Health and Biosecurity, Geelong, VIC, Australia, 2School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia 3Burnet Institute, Melbourne, VIC, Australia 4Peter MacCallum Cancer Centre, Melbourne, VIC, Australia 5Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.

Full Abstract: The CRISPR/Cas13d is a programmable RNA endonuclease system that has been harnessed for facile and efficient targeting of cellular and exogenous RNAs, including RNA viruses such as influenza virus. Given the robust catalytic activity and specificity of Cas13d, coupled with the ease to design crRNAs, CRISPR/Cas13d has the potential to offer an advantage over conventional antiviral strategies by rapidly designing antiviral effectors. However, there is a considerable knowledge gap in our current understanding of the principles governing the effectiveness of crRNAs in the context of mutation-driven influenza virus evolution and emerging strains. In this study, we delineated the principles for the development of effective crRNAs by targeting a DsRed fluorescence reporter gene in chicken fibroblast DF1 cells. To systematically determine the optimal design for crRNAs, we designed multiple versions of crRNAs to investigate the minimum length of the crRNA, protospacer flanking sequence, degree of mismatch tolerance, and collateral effects. Our data revealed variable knockdown levels between crRNAs, in which a few crRNAs achieved over 95% DsRed knockdown. Other crRNAs exhibited moderate to no effects, although they targeted adjacent RNA locations. crRNAs showed a preference for length requirements and sequences with fewer than 21-nt failed to knockdown the reporter gene. We demonstrated that crRNAs exhibit a high degree of tolerance to single-nucleotide mismatches, regardless of the position at which the single-nt mismatch was introduced. However, 4-nt mismatches within crRNA significantly reduced targeting efficacy, and eight nucleotide mismatches completely diminished targeting efficacy. Finally, Cas13d induced collateral degradation of bystander RNA, suggesting that additional studies are necessary to understand its pleotropic effects. This study extends our understanding of the Cas13d targeting mechanism through multiple iterations of crRNAs, providing a roadmap to design crRNAs for improved effectiveness, which will be important against rapidly evolving viruses.

Speaker: Sarah Diepstraten

Abstract Title: Whole genome CRISPR/Cas9 screening to understand resistance to BH3-mimetic drugs in lymphoma

Authors: Sarah Diepstraten 1 2 , John E La Marca 1 2 3 4 ,Yexuan Deng 1 2 3 4 , Andreas Strasser 1 2 , Marco Herold 1 2 3 4 , Gemma Kelly 1 2

Affiliations: 1. Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia 2. Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia 3.Olivia Newton-John Cancer Research Institute, Heidelberg, Melbourne, Australia 4.School of Cancer Medicine, La Trobe University, Bundoora, Melbourne, Australia

Full Abstract: One hallmark of cancer is dysregulation of the intrinsic apoptosis, or cell death, pathway. This pathway is mediated by proteins of the BCL-2 family, which includes both pro-apoptotic and pro-survival factors, the levels of which are usually carefully maintained to determine whether a cell lives or dies. BH3-mimetics are exciting new anti-cancer drugs which directly bind and inhibit specific pro-survival proteins, tipping the balance of the apoptosis pathway towards favouring cell death. As testament to their efficacy, the BH3-mimetic drug venetoclax, which targets the pro-survival protein BCL-2, has been approved by many regulatory agencies worldwide for the treatment of particular blood cancers. New BH3-mimetics targeting MCL-1, an essential survival factor for a wide range of cancers, are currently in clinical trials for diverse haematological malignancies. However, emerging clinical data suggests that while BH3-mimetics are initially highly effective at killing cancer cells, drug resistance frequently develops over long-term treatment, resulting in patient relapse. We are using a systematic approach to explore how lymphoma cells can become resistant to BH3-mimetics targeting BCL-2 or MCL-1. We employ whole-genome CRISPR/Cas9 knockout and activation screens to identify factors which contribute to drug resistance in lymphoma. To enable these studies, we developed a faithful mouse model of aggressive double hit lymphoma, through use of our novel CRISPR-activation enabled mouse. From these screens, we recently characterised the tumour suppressor protein p53, among others, as an important mediator of the cellular response to BH3-mimetic drugs.

Speaker: Martin Pal

Abstract Title: Development of a diagnostic assay to detect Trichomonas tenax using CRISPR-Cas12a technology

Authors: Joshua Slattery1, Camilla Donnelly2, Anna Walduck1, Bernd Kalinna1, Martin Pal1,2,3

Affiliations: 1Rural Health and Research Institute, Charles Sturt University, Orange, NSW, Australia; Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW, Australia; 3School of Dentistry and Medical Sciences, Faculty of Science and Health, Charles Sturt University, Wagga Wagga, NSW, Australia

Full Abstract: Periodontal disease is the major cause of tooth loss in adults worldwide. In Australia, a third of adults are suffering from moderate-severe periodontitis and an additional third suffering the milder gingivitis. While previously bacteria have been thought to be responsible for the onset of this disease, recently the protozoan Trichomonas Tenax has been implicated in disease progression. However, suitable point-of-care detection methods aiding a causative role for T. tenax are lacking. Here, we describe the development of a novel diagnostic assay for the detection of T. tenax using CRISPR-Cas12a technology. For this, Cas12a protein was purified in-house and a 6kb region of the T. tenax genome was screened to identify potential guide RNA sequences utilising a TTTV-PAM sequence. A total of 170 potential guide RNA sequences were analysed by developing a ranking formula calculating a specificity score for each candidate guide RNA. Each sore was based on potential off-targets in the human genome as well as sequence overlaps with the closely related species T. vaginalis. Using this ranking, 14 guide RNAs were subsequently tested using synthetic T. tenax DNA and a 5'FAM- TTTTTTTTT-3'ZEN/BHQ fluorescent reporter. 12 of 14 guide RNAs demonstrated detectable fluorescence with on-target DNA and no fluorescence in negative controls, and a 4pM target DNA concentration was determined as detection limit. In summary, we have identified multiple unique sequences within the genome of T. tenax suitable for the design of a detection assay based on CRISPR-Cas12a technology. Future experiments are warranted to move this assay into a field-based point-of-care testing regime, and the use of patient-derived clinical samples will require the development of an isothermal Recombinase Polymerase Amplification (RPA) step to improve sensitivity prior to CRISPR target recognition.

Speaker: Tahmina Tabassum

Abstract Title: Novel CRISPR-Cas9 Fusion for Safer Gene Editing

Authors: Tahmina Tabassum1, Giovanni Pietrogrande1, Jake Bradford2, Dimitri Perrin2 & Ernst J. Wolvetang1

Affiliations: 1Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia 2Queensland University of Technology, Brisbane, Australia

Full Abstract: Efficiency and safety are key issues dominating the surge of new modified CRISPR tools designed to improve precise genome editing. CRISPR has been widely effective in gene disruption studies but successful application in gene correction or replacement studies is limited. Insertion of genetic material via homologous recombination (HR) is a challenge, making development of safer gene editors a growing research area especially for clinical and translational applications. DNA breaks are governed by complex DNA repair response pathways involving various regulators. Cas9 fusions with HR regulating proteins have successfully increased knock-in efficiencies in multiple studies. However, there has been no breakthrough for reducing off target effects in such editing practices. Using small molecules to inhibit the non-homologous end-joining pathway is the most common practice with the trade-off of cytotoxicity and global DNA repair dysregulation. In this study we have developed a novel CRISPR-Cas9 fusion protein for safer editing without the need for global dysregulation of repair mechanisms. Cas9 is fused to a motif which helps reduce indel formation through post-translational modifications at target site. We targeted HEK293 cells for gene correction of transgenic GFP with our fusion protein and wildtype SpCas9 where fluorescence data confirms comparable GFP correction by HR between both modalities. Interestingly, sequencing results reveal significant reduction of on-target indel mutations in cells edited with our fusion editor. This preliminary finding is promising and has guided optimisation of the editor to assess off-target implications in primary cells and apply in CAR-NK therapy.

Session Three: Emerging Technologies

Invited Speaker: Sandro Ataide

Talk Title: seekRNA: a new gene editing tool

Speaker: Jakob Schuster

Abstract Title: Novel CRISPR-Cas9 Fusion for Safer Gene Editing

Authors: Jakob Schuster, Matthew E. Ritchie, Quentin Gouil, Michael B. Clark

Affiliations: WEHI, The University of Melbourne (Department of Anatomy and Physiology)

Full Abstract: The analysis of sequencing data produced by high-throughput CRISPR screens can involve complex read processing tasks. Such tasks include validating the structure of oligos and identifying amplification artefacts and chimeras in guide RNA libraries, as well as assessing the editing efficiency of a guide design in targeted genomic DNA sequencing reads. Researchers often create custom software to perform such analyses, a process which can be time-consuming and bug-prone, and may not be compatible with changes in library design. There is an urgent need for read processing software that does not sacrifice speed or simplicity to support the plurality of sequencing data, enabling bioinformaticians to fluently work with FASTQ, FASTA and BAM/SAM files without writing their own tools from scratch.

To address this, we introduce Matchbox, a powerful and versatile read processing tool, which can be applied effectively to CRISPR data to perform such read-level analyses even when read structures are novel and analysis tasks are complex. By providing a rich language for error-tolerant pattern-matching and manipulation of reads, Matchbox enables flexible and fast processing of reads agnostic of the sequencing methods used to generate them. Users can write their own Matchbox scripts to tackle new bioinformatic problems, and pre-made Matchbox scripts are provided for both general and CRISPR-specific read processing tasks. We demonstrate that Matchbox achieves a fast speed comparable to that of existing flexible tools on common tasks, but addresses a broader range of bioinformatic needs, representing a new state-of-the-art in sequence processing. Matchbox can be used on its own to perform exploratory analysis, or incorporated into analysis pipelines.

Invited Speaker: Omer Gilan

Talk Title: Harnessing CRISPR screens to unravel the complexity of chromatin regulation

Speaker: Cyntia Taveneau

Abstract Title: De novo design of CRISPR-Cas13 inhibitors

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Full Abstract: CRISPR-Cas systems are transformative tools with real-world applications from genetic engineering to gene drives. Critical to the effective use of CRISPR biotechnology is both the discovery and development of anti-CRISPRs - phage derived proteins capable of potently inhibiting CRISPR functionality. However, the discovery of naturally occurring anti-CRISPRs can be challenging and many biotechnologically relevant Cas effectors have no inhibitors to tune or regulate their activity. Here, we present an approach that leverages de novo protein design and advanced in silico methods, such as RF Diffusion, to create new-to-nature proteins that control CRISPR-Cas activity. We demonstrate that these designs, referred to as AI-designed anti-CRISPRs (AIcrs), are capable of highly potent and specific inhibition of the target protein, CRISPR-Cas13. Comprehensive validation through biochemical assays and structural biology confirms their efficacy, and we demonstrate AIcr utility in controlling the anti-phage activity of CRISPR-Cas13 in bacteria.

The ability to rapidly design custom inhibitors of CRISPR-Cas machinery will contribute to the ongoing development of gene editors for application in the clinic and safeguard against their misuse. This work also underscores the revolution in protein design and highlights the urgent need to leverage these advancements for future biotechnological applications.

Session Four: Virology

Speaker: Priyank Rawat

Abstract Title: Suppression of HIV transcription via LNP-delivered mRNA-encoded CRISPR-Cas13: a novel latency promoting agent

Authors: Priyank Rawat (1), Stan Kan (1,2), Paula Cevaal (2), Ajantha Solomon (2), Kiho Tanaka (2), Joseph A Trapani (1), James McMahon (3), Sharon R Lewin (2,3,4), Michael Roche (2,5), Mohamed Fareh (1)

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Full Abstract: Background: Prolonged persistence of HIV latently infected cells on antiretroviral therapy (ART) in people with HIV (PWH) serves as a roadblock towards a cure for HIV infections. CRISPR-Cas13 is a programmable RNA-nuclease that can effectively degrade viral transcripts and could be exploited to eliminate viral reactivation after cessation of ART. We hypothesised that the CRISPR-Cas13 system could knock down the essential HIV Tat protein, thus locking HIV into deep latency. Methods: We used a proprietary lipid nanoparticle (LNP) formulation to deliver a Tat-targeting Cas13 mRNA to HIV latently infected cell lines, JLat A2 and ACH2. Tat expression was measured by RT-qPCR, western blotting and viral reactivation via flow cytometry-based assessment of GFP reporter and viral p24 expression. Additionally, we assessed the ability of Tat-targeting Cas13 mRNA to suppress viral expression in human CD4+ T cells infected with a replication-competent reporter virus via flow cytometry and digital PCR-based HIV transcription profiling.Results: In JLat A2 cells, LNP-delivered Tat-targeting crRNA and RfxCas13d mRNA led to 80%±5% reduction in Tat mRNA expression and 90%±9% reduction in GFP expression indicative of viral reactivation. In ACH2 latency model, Tat-targeting RfxCas13d mRNA resulted in reduction of 65%±10% in viral p24 expression. Similar findings were observed using the pspCas13b ortholog. Additionally, we observed a significant reduction in cell viability (40%±8) with RfxCas13d Tat-targeting mRNA relative to a non-targeting control, a consequence of collateral activity of RfxCas13d. Finally, in human CD4+ T cells infected with a replication competent HIV reporter virus, Tat-specific crRNA and Cas13 mRNA resulted in 50%±10% reduction in productive infection, consistent with an overall reduction in the viral RNA and DNA expression. Conclusions: Collectively, our results demonstrate that a Tat-targeting Cas13 mRNA delivered via LNP effectively suppresses Tat expression in HIV latency cell lines and primary CD4+ T cells infected with an HIV reporter virus.

Speaker: Zak Janetzki

Abstract Title: Editing of HBV DNA in vitro and in vivo using a CRISPR/Cas9 Base Editor approach

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Full Abstract: Introduction: Current hepatitis B virus (HBV) treatments do not target the HBV covalently closed circular DNA (cccDNA) minichromosome reservoir, nor do they target HBV integrated DNA. There is a desperate need to develop novel therapeutics that target both cccDNA and integrated DNA; to improve HBV cure rates. CRISPR/Cas9 base editors (BEs) 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. The aim of this project analyse single guide RNA (sgRNAs) targeting all HBV open reading frames (ORFs) and to test the efficacy of transient expression of BEs and sgRNAs in reducing HBV replication and protein expression in vitro and in vivo.

Methods: All sgRNAs introduce premature stop codons to reduce HBV protein expression and replication. The efficacy of sgRNAs were tested via plasmid transfection with HBV DNA and Cas9 BEs in HepG2 cells. HBV replication markers and proteins were measured and compared to a non-targeting control sgRNA.

Results: sgRNAs with different BEs targeting HBV ORFs achieved knockdown of HBV proteins and intracellular core-associated HBV DNA with varying efficacy. Further analysis of the impact on HBV RNA, DNA and protein expression in vitro will be performed. A combination of sgRNAs will also be tested. Different models will be used including HBV stable cell lines, an HBV infection system, and a murine model. BEs will also be delivered as mRNA packaged in lipid nanoparticles in vitro and in vivo, with and without current therapeutics.

Conclusion: These studies will determine the utility of Cas9 BEs for introducing specific base changes into the HBV cccDNA and integrated DNA, as an important first step towards developing this approach as a novel HBV therapeutic.

Speaker: Michael Moso

Abstract Title: CRISPR-activation for HIV latency reversal using lipid nanoparticle delivery

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Full Abstract: Background: The major barrier to HIV cure is the persistence of latently infected CD4+ T cells harbouring integrated HIV DNA. One major strategy for HIV cure is the 'shock and kill' approach, whereby latency reversal agents are used to upregulate HIV transcription and induce cell death through virus-mediated cytotoxicity or immune-mediated clearance. We sought to assess CRISPR activation (CRISPRa) for HIV latency reversal using the synergistic activation mediator (SAM) system and lipid nanoparticle (LNP) delivery. Methods: LNPs were synthesised using an optimised lipid mix (LNP-X) combined with CRISPRa mRNAs and HIV-targeting gRNAs (gRNA-L, O, 1, 2) (CRISPRa-LNPs). Potency of CRISPRa-LNPs with single or multiplexed gRNAs was assessed in the Jlat-A2 T-cell line, which contains a GFP reporter gene under the control of the HIV promoter. Mismatch tolerance of CRISPRa was evaluated using single- or double-base mutations in gRNA-L. CRISPRa-LNP activity in primary T cells was assessed using a gRNA targeting the host gene, CD25. Ex vivo CRISPRa activity was assessed in CD4+ T-cells from people with HIV on antiretroviral therapy (n=8) and measured using digital RT-PCR to quantify induction of HIV transcripts. Results: HIV-targeting CRISPRa-LNPs led to potent HIV expression in Jlat-A2 cells, with highest potency observed using multiplexed gRNAs L+O. Single base mutations from position 1-10 and sequential double mismatches up to positions 3,4 retained >50% activity, indicating a degree of mismatch tolerance. CD25-targeting CRISPRa-LNPs led to 3.2-fold increase in CD25 expression in resting CD4+ T-cells, demonstrating successful delivery of CRISPRa to primary cells. CD4+ T-cells treated ex vivo with CRISPRa-LNPs demonstrated median 2.0-fold increase in cell-associated HIV transcripts, indicating increased HIV transcription. Conclusion: LNP-X can deliver CRISPRa machinery to primary T cells and reactivate transcription of latent HIV. Further optimisation of delivery of CRISPRa is being undertaken to improve potency of this approach in resting CD4+ T-cells.