VICTORIAN
COMPREHENSIVE
CANCER CENTRE

NEW FRONTIERS IN CANCER RESEARCH
2019

VCCC Symposium

#VCCCpostdoc19

WELCOME

Welcome to New Frontiers in Cancer Research 2019 - the third VCCC Postdoctoral Symposium.

This conference aims to highlight the exciting research of early- and mid-career scientists from across Victoria, and to work together to find innovative ways to progress our understanding and treatment of cancer.

A very warm welcome to our three plenary speakers, who are leaders from three cutting edge fields of cancer research: cancer bioinformatics, treatment and immunology.

Our thanks to the VCCC, all of the sponsors, consumer advocates, presenters and delegates for their tremendous response to this meeting. Without their support and enthusiasm, this event would not be possible.

We wish you all a stimulating and rewarding meeting.

Regards,

The 2019 Organising Committee

2019 ORGANISING COMMITTEE

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ACKNOWLEDGEMENTS

We would like to acknowledge the Traditional Owners of the land in which this event is taking place, the land of the Wurundjeri people of the Kulin Nation and pay respect to their Elders and families past, present and emerging.

The Organising Committee acknowledge the generous support of the Victorian Comprehensive Cancer Centre, and all of our sponsors.

We would like to thank everyone who provided assistance along the way, including Michelle Barrett, Erin Turner and the VCCC Communications team, the Peter Mac Postdoc Society (PMPS), Caroline Owen, the volunteer judges, and the consumer advocates.

Finally, thank you to the keynote speakers, presenters and all the delegates who have contributed to this meeting.

PROGRAM

VCCC Level 7 - Lecture Theatre B

Time	Event	Topic	Speakers
8.30am	REGISTRATION – Level 7 Foyer		
8.45am	Welcome	Organising Committee	
SESSION 1	Sponsored by Thermo Fisher Scientific		
8.50am	Keynote 1	Discovering cancer drivers with RNA-seq	Assoc. Prof. Alicia Oshlack
9.20am	Oral 1	High expression of p120ctn correlates with increased metastasis and poor survival in neuroblastoma patients	Dr Pamali Fonseka
9.35am	Oral 2	Predicting radiation-induced immune trafficking and activation in localised prostate cancer	Dr Simon Keam
9.50am	Oral 3	XenoSplit: A solution to the challenges in transcriptional profiling of circulating tumour cells	Dr Goknur Giner
10.05am	Flash Talk 1	Identifying inhibitors to minor class splicing as new cancer therapeutics	Dr Stephen Mieruszynski
10.10am	Flash Talk 2	Diverse somatic mutation profiles drive common patterns of transcriptional rewiring related to loss of multicellularity in 10,000 cancer patients	Dr Anna Trigos
10.15am	Flash Talk 3	The role of RNA sequencing in providing comprehensive molecular characterisation of patients' cancer	Dr Jacek Marzec
10.20am	Flash Talk 4	Pro-survival protein BFL-1 is a promising therapeutic target in melanoma	Dr Lahiru Gangoda
10.25am	MORNING TEA – Level 7 Foyer		
SESSION 2	Sponsored by Australian Biosearch		
10.55am	Oral 4	A novel murine model to study melanoma derived lymph node metastases	Dr Katharina Hochheiser
11.10am	Oral 5	Establishment of a CRISPR base-editing platform to identify tumour-driving mutations in vivo	Dr Martin Pal
11.25am	Oral 6	IL-11 suppresses anti-tumour CD4+ T cells to support tumourigenesis	Dr Jennifer Huynh
11.40am	Flash Talk 5	The Fanconi Anaemia pathway as a synthetic lethal drug target for BRCA negative tumours	Dr Michael Sharp
11.45am	Flash Talk 6	Caspase-2 protects against ferroptotic cell death	Dr Swati Dawar
11.50am	Flash Talk 7	Innate Lymphoid Cells in Melanoma: Promising New Targets for Cancer Treatment	Dr Nicolas Jacquelot
11.55am	Flash Talk 8	Is Fn14 a regulator of altered tumour glucose metabolism in cancer cachexia?	Dr Ingrid Burvenich
12.00pm	Keynote 2	Unravelling the molecular complexity of pancreatic cancer: from mechanism to therapy	Dr Marina Pajic

PROGRAM

Time	Event	Topic	Speakers
12.30pm	LUNCH – Level 7 Foyer		
	Poster presentations | Sponsored by Abcam		
SESSION 3	Sponsored by STEMCELL Technologies		
2.00pm	Oral 7	"*The benefit that comes from my existence*": Patients, bereaved family members, and the CASCADE team members' experiences of conducting a cancer rapid autopsy program	Dr Laura Forrest
2.15pm	Oral 8	Response and resistance to catalytic P300/CBP inhibition and multiple myeloma	Dr Simon Hogg
2.30pm	Oral 9	Peripheral Blood Biomarkers that Predict Response to Radiotherapy and Pembrolizumab Combination in Metastatic Breast Cancer	Dr Harini de Silva
2.45pm	Flash Talk 9	Utilizing Smac mimetics to induce necroptosis may avoid apoptosis-induced mutagenesis	Dr Mark Miles
2.50pm	Flash Talk 10	CIS inhibits NK cell turnover, differentiation and priming during development	Dr Rebecca Delconte
2.55pm	Flash Talk 11	Feasibility of an upright imaging technique for radiotherapy in an upright position	Dr James Korte
3.00pm	Flash Talk 12	Differentiation therapy by HDAC inhibition in t(8;21) acute myeloid leukemia requires immune cell mediated type I interferon production.	Dr Pilar Dominguez Rodriguez
3.05pm	AFTERNOON TEA – Level 7 foyer		
SESSION 4	Sponsored by BD		
3.35pm	Oral 10	Enhancing CAR T cell therapy by enabling their interaction with antigen presenting cells	Dr Clare Slaney
3.50pm	Oral 11	Understanding the role of a unique population of T-helper 17 cells in the pathogenesis of infection induced colon cancer	Dr Ka Yee Fung
4.05pm	Oral 12	Tissue-resident memory T cells mediate melanoma surveillance and are targets of immunotherapy	Dr Simone Park
4.20pm	Keynote 3	Enhancing CAR T cell function in solid tumours	Dr Paul Beavis
4.50pm		Awards, Conclusion of speaker program	Organising Committee
5.00pm	NETWORKING EVENT – Drinks and nibbles – Level 7 Atrium | Sponsored by Millennium Science		
7.00pm	Close		

KEYNOTE SPEAKERS

Associate Professor Alicia Oshlack

Head of Bioinformatics

Murdoch Children's Research Institute

Associate Professor Alicia Oshlack is the head of Bioinformatics at the Murdoch Children's Research Institute and an NHMRC Career Development Fellow (level 2). Oshlack has been working in the field of Bioinformatics for nearly 15 years and is best known for her body of work developing methods for the analysis transcriptome data. Oshlack has also built an extensive collaborative network with many national and international research groups uncovering molecular mechanisms of development and disease using a variety of genomic approaches. Oshlack is regarded as a worldwide expert in the field of RNA-seq analysis and has several projects utilising the analysis of single-cell transcriptomes. She also works in the areas of clinical diagnostics from DNA sequencing to epigenetics. She has received several awards including the Millennium Science award from the Lorne Genome conference, the Australian Academy of Science Ruth Gani medal for human genetics (2011) and the inaugural Georgina Sweet award for women in quantitative biomedical research (2016).

https://www.mcri.edu.au/users/dr-alicia-oshlack

Dr Marina Pajic

Laboratory Head, The Kinghorn Cancer Centre

Garvan Institute of Medical Research

Dr Marina Pajic completed her PhD (2008) in the laboratory of Prof Michelle Haber at the Children's Cancer Institute (CCI), University of New South Wales, where she investigated chemoresistance mechanisms in childhood cancers, supported by a University Postgraduate Award and a CCI Postgraduate Scholarship. During her post doc in the renowned group of Prof Piet Borst (Netherlands Cancer Institute), she established mouse models to study mechanisms of chemoresistance in breast cancer (PNAS 2007), characterising key therapeutic targets (Cancer Research 2009, Clinical Cancer Research 2017). In 2010, Dr Pajic moved back to Australia to join the Pancreatic Cancer Research Team and the Australian Pancreatic Cancer Genome Initiative (APGI), at the Garvan Institute for Medical Research. During this time, Dr Pajic developed substantial novel infrastructure of patient-derived models of pancreatic cancer, and unique personalised medicine projects for pancreatic cancer. She has independently run her group since 2013 as Group Leader, having been promoted to Garvan Faculty in 2018. She has made major advances in our understanding of tumour progression and metastasis, with 45 publications in the past 5 years in this field (65 career total; >6500 citations on Scopus). This includes world's first functional validation of tailored therapies for pancreatic cancer (Gut 2018, Science Translational Medicine 2017), delineating complex resistance mechanisms (Cancer Research 2018, Clinical Cancer Research 2017), and high-tier collaborative papers in cancer genomics and clinical research (Nature x7, Nature Comms, J Clin Oncology), and authoritative reviews for leading journals (Gastroenterology 2018). She is the current NHMRC Career Development Fellow and CINSW Career Development Fellow, with project funding from the NHMRC, Cancer Australia, CCNSW, CINSW, Avner Pancreatic Cancer Foundation supporting her research.

https://www.garvan.org.au/research/cancer/personalised-cancer-therapeutics

Dr Paul Beavis

Group Leader

Peter MacCallum Cancer Centre

Dr. Beavis completed his PhD at Imperial College London in 2010 and joined Peter Mac shortly thereafter to work in the Cancer Immunology Program. Since his recruitment to Peter Mac, Dr. Beavis has established a vibrant program of research focussing on the molecular and biological processes involved in tumour-induced immunosuppression, in particular the role of CD73 and adenosine receptor signalling. He also has a significant interest in developing novel CAR T cell technology to enhance their effectiveness in solid cancer. He is a fundamental cancer researcher and immunologist but his work clearly has a strong translational focus and his collaborative research studies with industry partners and with internal collaborators such as Prof. Sherene Loi have provided the foundation for clinical application of his work. Paul holds a career development fellowship from the NBCF and an NHMRC Project Grant as CIA and his team consists of himself, a post-doctoral researcher, two research assistants and two PhD students.

https://www.petermac.org/users/dr-paul-beavis

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ORAL PRESENTATIONS

High expression of p120ctn correlates with increased metastasis and poor survival in neuroblastoma patients

Pamali Fonseka
La Trobe Institute for Molecular Science

Shivakumar Keerthikumar, Paul Timpson, Di Giannatale Angela and Suresh Mathivanan

Neuroblastoma (NBL) is the most common extracranial solid tumor in children. Whilst advances in treatment has significantly improved the survival rate of low-risk NBL patients, the overall survival rate of high-risk NBL group, especially N-Myc amplified patients, is poor. Hence, there is an urgent need to understand the molecular mechanisms that regulate treatment resistance and develop therapeutic strategies to improve the survival rate of high-risk NBL patients. In this study, p120ctn was identified as one of the key players in regulating treatment resistance and highlighted a novel avenue to target cell lineage to treat NBL.

RNA-Seq data analysis and immunohistochemistry was performed on 206 NBL patients. RNAi-based knockdown, CRISPR/Cas9-based knockout, immunofluorescence, xenograft models, quantitative proteomics, co-immunoprecipitation, flow cytometry and phosphoproteomics analysis were performed to uncover the role of p120ctn in NBL pathogenesis.

RNA-Seq and immunohistochemistry analysis highlighted the mesenchymal-like signature of NBL tissue samples. It was revealed that high expression of p120ctn positively correlated with metastasis and poor outcome. Knockdown of p120ctn activated Wnt signaling and reduced the expression of the transcription factor N-Myc. Contrary to the function of p120ctn as a tumor suppressor, depletion of p120ctn induced mesenchymal-to-epithelial transition, attenuated angiogenesis, migration, invasion and sensitized the NBL cells to doxorubicin. Coimmunoprecipitation and phosphoproteomics revealed the pro-invasive role of p120ctn in regulating RhoA activation and Twist phosphorylation. Interestingly, induction of epithelial lineage reduced the expression of p120ctn and sensitised the NBL cells to doxorubicin.

Overall, this study highlights cell lineage alteration as an alternative to treat high-risk NBL patients.

Predicting radiation-induced immune trafficking and activation in localised prostate cancer

Simon P. Keam
Peter MacCallum Cancer Centre

Heloise Halse, Thu Nguyen, Catherine Mitchell, Franco Caramia, David Byrne, Sue Haupt, Georgina Ryland, Phillip K Darcy, Shahneen Sandhu, Piers Blombery, Ygal Haupt, Scott G Williams, Paul J Neeson

Prostate cancer is frequently cured with high-dose rate brachytherapy as a front-line treatment. However, a significant number unfortunately develop intrinsic resistance. Although considered to be an immune-excluded tissue, immune responses are implicated in driving tumour-eradication in prostate cancer. This has not been proven, and yet is used as the rationale for numerous clinical trials combining radiation and immunotherapies. We hypothesise that there is a predictable but differential relationship between radiation and the immune responses in prostate cancer that could be used to fulfil a clinical need - identifying patients that would benefit from immune intervention in conjunction with radiation.

We present here the results of comprehensive immunological profiling of a cohort of world-unique pre- and post-radiation tissues from 24 patients (RadBank cohort). These were assessed using pathological classification, tissue segmentation (cancer/surrounding stroma), multiplex IHC, gene expression profiling, T-cell receptor sequencing, and spatial computational analysis.

Our data resolved three classes of prostate cancer tissue based on immune infiltrate level, immune-activation and -checkpoint gene signatures, spatial clustering and T cell clone sequencing: (i) infiltrated and activated, (ii) non-infiltrated and low activation, and (iii) highly infiltrated yet possessing an inactive phenotype. A similar level of heterogeneity was found in irradiated tissue, with four subcategories corresponding to either maintenance (low or high) or directional change (increased or decreased) in infiltrate density.

We have begun to resolve clear patient classifiers based on immune responses to radiation, and identified patients groups likely to benefit from immune therapy alongside radiation. Importantly, these classifications are associated with baseline gene expression profiles that may be used for pre-clinical stratification and more sophisticated treatment paradigms.

ORAL 3:

XenoSplit: A solution to the challenges in transcriptional profiling of circulating tumour cells

Göknur Giner
Walter and Eliza Hall Institute

Delphine Merino, Aaron T. L. Lun, Jean Berthelet, Bhupinder Pal, Sepideh Foroutan, Aliaksei Holik, Melissa J. Davis, Jane E. Visvader, Gordon K. Smyth

Circulating tumour cells are cells in transit between primary tumours and metastases. The enumeration and characterisation of these cells have critical implications for the monitoring and treatment of patients with cancer. Despite ongoing efforts, isolation and profiling of these cells remain as a technical challenge, due to their rarity and fragility. Addressing these needs, we propose a new approach to study the transcriptome of circulating tumour cells in patient-derived tumour xenografts. Our approach not only integrates a cell size based filtering with Screen Cell to increase the library sizes of circulating tumour cell samples but also introduces a fast and accurate open-source method (XenoSplit, https://github.com/goknurginer/XenoSplit) that enables unprecedentedly precise detection of the true origin of RNA-Seq libraries. Furthermore, examining several datasets, including RNA spike-in control and breast cancer RNA-Seq genomes, we demonstrate that XenoSplit outperforms its counterparts in terms of precision and computational efficiency, and leads to the discovery of hundreds of biomarkers to characterise CTCs in the subsequent statistical analysis. On the other hand, cell size based filtering is unique as it does not rely on antibody detection, as well as being cost-effective and cell-friendly.

FLASH TALK 1:

Identifying inhibitors to minor class splicing as new cancer therapeutics

Stephen Mieruszynski
The Walter and Eliza Hall Institute

Karen Doggett, Kate Jarman, Chuan Tian, Helene Jousset, Joan Heath

The overarching aim of this project is to develop new cancer therapeutics targeting minor class splicing. During gene expression efficient minor class splicing is required for the removal of ~0.3% of introns in the human genome. Although a small subset, these unique introns are enriched in genes that participate in processes that are all essential for cancer cell growth and division, including the cell cycle, MAPK signalling, DNA replication and DNA damage repair. Using a variety of genetically engineered animal models of cancer, we have demonstrated that disruption of minor class splicing causes a reduction in tumour burden in hepatocellular carcinoma, lung and gastric adenocarcinoma, lymphoma and acute myeloid leukemia. These outcomes were due to a combination of reduced cancer cell proliferation and increased cancer cell death, most likely due to a reduction in the expression of minor class intron-containing transcripts. Importantly, these anti-tumour effects produced no toxicity to normal tissues. Thus, our cancer model work suggests that targeting minor class splicing may provide a broad-spectrum approach to cancer therapy. To pursue this idea, we developed a cell-based, high-throughput assay capable of distinguishing between inhibitors of minor class and major class splicing (which removes the remaining 97.7% of introns) or all splicing. This assay was miniaturised and automated to a 1,536-well plate format. At the time of writing, a successful pilot screen of ~4,000 compounds has been undertaken, and a screen of ~215,000 diverse, drug-like compounds is expected to be completed by mid-2019. Following the high throughput chemical screening, we will embark on target engagement and medicinal chemistry to generate lead compounds for pre-clinical validation.

Diverse somatic mutation profiles drive common patterns of transcriptional rewiring related to loss of multicellularity in 10,000 cancer patients

Anna Trigos
Peter MacCallum Cancer Centre

Richard Pearson, Anthony Papenfuss, David Goode

Introduction

Many hallmarks of cancer can be explained as a disruption of transcriptional networks shaped during the emergence of multicellularity, leading to tumours relying on cellular processes that date back to unicellular ancestors (e.g., cell replication, glycolysis). However, how profiles of genomic alterations that are unique to each patient contribute to large transcriptional changes that converge to these common hallmark phenotypes is currently not well understood.

Methods

We identified modules of genes with highly correlated expression in over 10,000 patients of 30 solid tumour types from The Cancer Genome Atlas. Modules were stratified by the predominant time of emergence in evolution of the genes in each module. We determined the role of somatic mutations in disrupting these modules by quantifying the change of localization (peripheral vs. central) of the mutated gene in regulatory networks in tumours compared to their initial localization in networks of normal tissues.

Results

We found modules dominated by either unicellular or multicellular genes were preserved between tumours and matched normal tissues, whereas modules with a mix of unicellular and multicellular genes were largely tumour-specific, and were often associated with specific recurrent point mutations and copy-number aberrations. Amplifications often led to the creation of new hubs around which tumour-specific modules formed, whereas deletions and point mutations tended to disrupt signalling between unicellular and multicellular genes in normal samples, leading to the fragmentation of modules in tumours.

Discussion

Our results reveal how genomic alterations in cancer affect gene expression by rewiring transcriptional control modules that developed during the evolution of multicellularity. This approach uncovers previously unappreciated patterns in the wide-spread transcriptional rewiring found across tumour types, and links it with the complete set of mutations in patients.

FLASH TALK 3:

The role of RNA sequencing in providing comprehensive molecular characterisation of patient's cancer

Jacek Marzec
University of Melbourne Centre for Cancer Research

Sehrish Kanwal, Lavinia Gordon, Oliver Hofmann, Sean Grimmond

Introduction

Precision oncology is becoming a standard approach in cancer patients care, with cancer molecular characterisation through genome sequencing being the major focus. In addition, there is growing evidence showing that patients transcriptome profiling can contribute to our knowledge of individual cancers by revealing additional layers to the disease biology. In this work we develop a pipeline for using cancer patient's RNA sequencing (RNA-seq) data to complement genome-based findings and aid therapeutic targets prioritisation.

Method

We use bcbio-nextgen RNA-seq pipeline (https://bcbio-nextgen.readthedocs.io) to process the RNA-seq read data from patient's tumour, followed by gene fusions prioritisation, per-gene read count data normalisation and transformation into standard scores to address challenges associated with analysing data from a single-subject. In addition, we build an internal reference cohort using a set of in-house high-quality tumour samples to assure input material and data processing compatibility. Finally, we integrate transcriptome data with genome-based findings from patient's whole-genome sequencing (WGS) data and annotate results using public knowledge bases to provide additional evidence for dysregulation of mutated genes, as well as genes located within detected structural variants or copy-number altered regions.

Results

We developed a pipeline capable to process and analyse RNA-seq data from an individual patient's tumour. In addition, the inclusion of an internal reference set assures the input material and data compatibility. The results are visualised in an approachable html-based interactive report with searchable tables and plots, providing variant curators with a tool to verify and prioritise genome-based findings.

Discussion

RNA-seq technology holds great promise for the clinical applicability in molecular diagnostic standpoint. However, it is not straight forward to translate this technology into clinical practice, mainly due to its single-subject setting. We developed a pipeline for integrating information from both WGS and RNA sequencing approaches to provide additional clinically relevant information that can help prioritise variants for therapeutic intervention.

Pro-survial protein BFL-1 is a promising therapeutic target in melanoma

Lahiru Gangoda
Walter and Eliza Hall Institute

Lin Tai, Pacman Szeto, Mark Shackleton, Guillaume Lessene and Marco J. Herold

The aberrant expression of apoptosis-regulating pro-survival BCL-2 family members is often associated with cancer cell survival and enhanced therapy resistance. In melanomas the BCL-2 family protein, BFL-1 is expressed at unusally high levels, yet it's role is poorly understood.

To establish whether BFL-1 is critical for melanoma cell survival, we first tested the impact of acute BFL-1 knockout using an inducible CRISPR/Cas9 system in different human derived melanoma cell lines. Intriguingly, knocking out BFL-1 did not effect cell survival. Next we tested whether BFL-1 expression is related to melanoma resistance to currently available targeted therpies such as BRAF inhibitors and BH3 mimetics that block other BCL-2 family members. Although, BRAF inhibitors alone did not show enhanced killing of the BFL-1 knockout cells, the combination with BH3 mimetics enhanced the death in melanoma cells that lacked BFL-1 in comparison to the wildtype cells expressing BFL-1. This suggests that BFL-1 represents a resistance factor for melanoma cells.

To confirm these results in a more clinically relevant setting, we will establish organoids from patient derived melanoma tissues. So far, we have optimized the organoid culturing protocol using mouse melanomas. BFL-1 will be knocked out in patient derived organoids using CRISPR/Cas9 technology and treated with other currently available anti-cancer drugs used for the treatment of melanomas .

In summary, using melanoma derived cell lines we found that cell death can be induced by deleting BFL-1 and combining this with conventional or targeted therapy. Direct inhibition of BFL-1 represents an attractive strategy to increase the efficacy of current melanoma treatment regimes. BFL-1 targeting represents a promising therapeutic target for melanoma and other malignancies with aberrant BFL-1 expression and might overcome the frequently observed resistance to current therapies.

ORAL 4:

A novel murine model to study melanoma derived lymph node metastases

Katharina Hochheiser
Peter MacCallum Cancer Centre / Peter Doherty Institute for Infection and Immunity

David Gyorki, Thomas Gebhardt

Cutaneous melanoma is one of the most common cancers in people with fair skin, with Australia and New Zealand having the highest incidence in the world. While melanoma is often diagnosed at an early stage, with the potential for curative-intent surgery to the primary site, in some patients spread of tumour cells to the draining lymph nodes will have already occurred at the time of diagnosis. Patients with microscopic deposits of melanoma identified in the sentinel lymph node (SLN) have a higher risk of melanoma re-occurrence and cancer-specific mortality. However, the majority of patients with a positive SLN biopsy (SLNB) will not relapse, which may indicate immune-mediated tumour cell control. Why this control suffices to prevent recurrence in some patients and fails in others, remains unclear.

We have recently developed a murine model of cutaneous melanoma, that not only shows tumour formation with variable growth kinetics and penetrance but also spontaneously metastasises to draining lymph nodes, resembling melanoma in humans. In order to better understand the mechanisms underlying metastasis development and immune control, we surgically excised progressively growing primary tumours in mice, modelling curative-intend surgery in patients. Utilising melanoma cells genetically modified to express firefly luciferase, we were able to monitor metastasis progression in those operated mice in a longitudinal fashion by bioluminescent in vivo imaging (IVIS). We found that mice genetically deficient for perforin developed lymph node metastases with higher incidence and faster progression compared to wildtype animals, indicating a role for this soluble mediator in metastasis control.

Our novel epicutaneous melanoma and surgery model sets the stage for investigating the molecular mechanisms and identifying critical mediators in metastasis control, with the ultimate goal of refining SLN assessment and preventing immune escape in the clinic.

ORAL 5:

Establishment of a CRISPR base-editing platform to identify tumour-driving mutations *in vivo*

Martin Pal
Walter and Eliza Hall Institute

Maggie Potts, Lin Tai, Andrew Kueh, Marco Herold

Introduction

The generation of DNA double strand breaks has been fundamental to successful genome engineering in mammalian cells. In this regard the advent of RNA-guided programmable Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) nucleases has revolutionized gene-editing applications. However, DNA double strand breaks generated by nucleases are ultimately toxic to cells and most successful gene editing applications rely on DNA template integration. Here we describe the establishment of a novel CRISPR base-editing platform, which achieves direct and precise point mutations in the genome of mammalian cells without creating DNA double strand breaks, thereby facilitating the discovery of new tumour-driving mutations in vivo.

Materials and Methods

Our CRISPR base editors consist of a deaminase enzyme linked to a nickase variant of Cas9 (xCas9), which also exhibits increased protospacer adjacent motif (PAM) compatibility. In order to achieve all four base pair conversions, we integrated both a cytosine base editor (BE3), capable of converting cytidine to thymidine (C->T), and an adenosine base editor (ABE), converting adenine to guanine(A->G) into our platform. To ensure sufficient expression of those large DNA base editors in cells we are using the piggyBac transposase to shuttle our CRISPR base editors into the genome of cells.

Results

As a proof of principle, we designed sgRNAs to target p53 in B-cell lymphoma cells creating mutantp53, which is resistant to Nutlin-3a (an antagonist of p53's E3 Ligase MDM2) induced cell death. In addition, we designed sgRNAs to introduce an early stop codon in the Bim locus in human 293T cells, thereby rendering the Bim gene inactive.

Outlook

The future aim of using CRISPR mediated base editing is to discover tumour-driving mutations in haematopoietic stem cells in vivo , thereby identifying new drug targets for anti cancer therapy.

IL-11 suppresses anti-tumour CD4+ T cells to support tumourigenesis

Jennifer Huynh

Olivia Newton-John Cancer Research institute

Ashwini Chand, Matthias Ernst

Introduction

There has been a long standing link between inflammation and cancer. This link has been characterised in recent years demonstrating how cytokines promote cancer growth. Accumulating evidence alludes to a role for the cytokine, IL-11, in the development of colorectal cancer. However, the mechanisms underlying IL-11 as a tumour promoter remain largely unknown. We postulate that IL-11 could be a mode of immunosuppression that can be inhibited to mount an effective, immune-mediated anti-tumour response.

Methods

To validate IL-11 as a key molecular driver of tumourigenesis, we treated il11r$^{-/-}$ mice with the carcinogen AOM to induce sporadic CRC. The progression of distal colonic tumours was monitored by mouse endoscopy. To ascertain the role for IL-11 on the stromal and hematopoietic compartment during tumour development, we established MC38 and CT26 syngeneic allografts in il11r$^{-/-}$ mice. Mice were euthanised and the tumours were immune profiled by FACs. To ascertain the effects IL-11 elicits on T cell activation, murine CD4$^+$ T cells were stimulated with PMA/Iono in the presence of IL-11 for 4h, and T cell activation was assessed by qPCR and Multiplex ELISA. These findings were extrapolated in vivo, whereby MC38 tumour-bearing il11r$^{-/-}$ mice were treated with an anti-CD4-depleting antibody.

Results

In this study, we demonstrate that colorectal cancer growth was significantly attenuated in il11r$^{-/-}$ mice compared to WT hosts. Tumours from il11r$^{-/-}$ mice harboured higher numbers of activated T cells. Moreover, IL-11 was found to suppress the capacity for CD4$^+$ T cells to produce pro-inflammatory cytokines and mount an effective anti-tumour response *in vivo*.

Conclusions

Here, we characterise an unrecognised role for IL-11 in suppressing anti-tumour CD4$^+$ T cells to enable tumourigenesis. Overall, the findings from this study support IL-11 as a viable cytokine-based immunotherapeutic avenue for the treatment of colorectal cancer.

FLASH TALK 5:

SESSION 2

The Fanconi Anaemia pathway as a synthetic lethal drug target for BRCA negative tumours

Michael Sharp
St. Vincent's Institute

Sylvie van Twest, Vincent J. Murphy, Jennii Luu, Kaylene Simpson, Andrew J. Deans and Wayne Crismani

Introduction

The aim of our project is to identify synthetic lethal reactions between the Fanconi Anaemia (FA) and Homologous recombination (HR)/BRCA DNA repair pathways.

Loss of genome stability is a common trait of cancer and many malignant cancers have defects in DNA repair pathways. One of these pathways, the FA pathway, detects and repairs DNA inter-strand crosslinks (ICL). There are >20 genes involved in the FA pathway, FANCA-FANCV. Six of which form an E3 ubiquitin ligase complex (FA core complex). Central to the FA pathway is an essential mono-ubiquitination reaction performed by the core complex. It is believed that this monoubiquitination reaction stabilises stalled DNA replication forks and can increases a cells viability that is already deficient in in other DNA repair mechanisms, such as the HR repair pathway. At least 14% of triple negative breast cancers, and 50% of ovarian cancers lack effective HR because of the loss of key HR proteins e.g. BRCA1 or BRCA2. Targeting a compensatory pathway to HR, such FA, is an opportunity for tumour-specific cell killing (synthetic lethality). In turn, an inhibitor of the FA core complex may induce synthetic lethality in BRCA1/2 cancers.

Methods

We have reconstituted this critical step in the FA pathway by recombinantly generating the nine proteins needed to ubiquitinate FANCI and FANCD2 and miniaturised the reaction for high-throughput drug screening. We have also developed biochemical and cellular assays to further validate potential FA core complex inhibitors.

Results

We have screened over 50,000 compounds from multiple compound libraries for inhibitors of the FA core complex and are currently validating hits. Here we present the initial findings of our drug screen.

Discussion

We believe the FA core complex is viable target for a new class of tumour specific chemotherapeutics. Our biochemical assay demonstrates it is possible to screen for small molecules that inhibit a key step in the FA pathway. As the project is ongoing, validation and characterisation of inhibitors may lead to new breast and ovarian cancer therapies.

Caspase-2 protects against ferroptotic cell death

Swati Dawar
Peter MacCallum Cancer Centre

M Corrales, K Montgomery, M Yates, K Fujihara, W Phillips, N Clemons

Ferroptosis is a recently coined non-apoptotic cell death characterised by accumulation of redox-active iron, glutathione depletion and the onset of lipid peroxidation. Functionally, ferroptosis is suppressed by lipophilic antioxidants and iron chelators. Accumulated evidence has shown that activation of ferroptosis contributes to anticancer treatment for several forms of human cancer. However, the exact function of ferroptosis in cancer still remains elusive. We have previously demonstrated that accumulation of mutant p53 protein enhances ferroptosis.

Caspase-2, one of the most evolutionarily conserved of the caspase family, has been implicated in the maintenance of chromosomal stability and tumour suppression. Caspase-2 deficient ($Casp2^{-/-}$) mice develop normally but show premature ageing-related traits including enhanced oxidative stress and impaired antioxidant response. Therefore, we hypothesised that caspase-2 may play a role in ferroptosis. We investigated the effect of various ferroptosis inducing small molecules following caspase-2 depletion in isogenic p53-null/mutant-p53 cell line models. Our results demonstrate that acute silencing of caspase-2 dramatically enhanced ferroptotic cell death, particularly in mutant p53 cancer cells, which could be fully rescued by various inhibitors of ferroptosis.

Furthermore, our data show that depletion of caspase-2 results in downregulation of SLC7A11 expression, a key component of the glutamate/cystine exchanger (system xCT) thereby impairing cystine uptake and lowering the endogenous stores of the major cellular anti-oxidant, glutathione. Thus, in combination with caspase-2 depletion, mutant p53 cancer cells are exquisitely sensitive to cell death induced by various ferroptotic inducers. Our results provide direct evidence that caspase-2 protects against ferroptotic cell death and further work is ongoing to characterize whether catalytic activity of caspase-2 is essential for its function in limiting ferroptosis. Ultimately, a more comprehensive understanding of the molecular mechanism underpinning ferroptosis could allow utilization of this cell death pathway as an anti-cancer strategy.

Innate Lymphoid Cells in Melanoma: Promising New Targets for Cancer Treatment

Nicolas Jacquelot
The Walter and Eliza Hall Institute

Gabrielle Belz

The immune cell infiltration into a tumour is heterogenous. It is composed of multiple cell types that can participate in the response and thus critically dictate the outcome for the patient. Recently discovered innate lymphoid cells (ILCs) are rapidly responding immune cells located in all surface barriers. A key function of this family of cells is to protect our body against pathogens and to maintain tissue homeostasis. ILCs are classified in three main groups based on transcription factors and cytokines they express. We can distinguish IFNγ and TNFα expressing NK cells and ILC1, IL-5 and IL-13-producing ILC2 and IL17 and IL-22-expressing ILC3. This suite of cytokines allows them to play multiple roles by regulating critical biological functions, including wound healing, autoimmunity and, importantly, the removal of cancers. Naturally present in the skin, their involvement in melanoma development and progression is currently unknown. Using pre-clinical models, we assessed their role and functions in this pathology. Firstly, we found that several ILC subsets infiltrate melanoma tumours. Secondly, these cells are functional and produce multiple cytokines that allow them to be major players in shaping the tumour microenvironment. Thirdly, tumour infiltrated ILCs are proliferating cells that express a wide range of immune checkpoints including PD-1, TIGIT, ICOS and CTLA-4. These features suggest that ILCs are likely to be modulated by current immunotherapies and thus impact on melanoma prognosis and a patient's outcomes. They may also be promising targets for future development of therapies.

FLASH TALK 8:

SESSION 2

Is Fn14 a regulator of altered tumour glucose metabolism in cancer cachexia?

Ingrid Burvenich
Olivia Newton-John Cancer Research institute

Osellame LD, Johnston AJ, Rigopoulos A, Gong SJ,
O'Keefe GJ, Lee ST, Hoogenraad NJ, Scott AM

Introduction

Cachexia is a syndrome characterized by unintentional weight loss, progressive muscle wasting and loss of appetite, seen in up to 80% of cancer patients. The molecular mechanisms of cachexia are poorly understood. Recently, treatment with an antibody (002) that targets the TWEAK receptor (Fn14) has been shown to reverse the symptoms of cachexia in syngeneic mouse models and extend the life-span of mice by restoring their body weight. Here we investigated via live imaging the glucose changes in mouse models of cachexia, to explore whether Fn14 plays a role in the metabolic changes occurring during cancer cachexia.

Methods

^{18}F-FDG PET imaging was performed in non-cachectic MEF H-Ras V12 versus cachectic MEF H-Ras V12 hFn14 tumour-bearing Nod SCID gamma mice (NSG) expressing human Fn14. Secondly, ^{18}F-FDG PET imaging was performed in cachectic C26 tumour-bearing NSG mice treated with anti-Fn14 002 antibody (10mg/kg, twice weekly) versus vehicle control treated mice. In the C26 model, 002 therapy was commenced before (preventative therapy) or after (curative therapy) symptoms of cachexia were measurable.

Results

^{18}F-FDG PET imaging demonstrated increased glucose uptake over time in cachectic versus non-cachectic tumour-bearing mice. This was observed both in the MEF H-Ras V12 hFn14 model as well as in the C26 model. Targeting Fn14 with 002 was able to prevent increased ^{18}F-FDG uptake in C26 tumours but more importantly, tumours of cachectic C26 mice with high ^{18}F-FDG uptake, showed reduced ^{18}F-FDG after two days of therapy with 002.

Discussion

In the absence of cachectic signalling of Fn14 (MEF H-Ras V12 model or 002 therapy of C26 model), glucose uptake levels of ^{18}F-FDG were low, implying that the expression of Fn14 has a direct link with the cachectic phenotype and glucose uptake of the tumour.

Conclusion

Our results demonstrate that the Fn14 receptor is linked to the glucose metabolic pathway of tumours that induce cachexia.

ORAL 7:

"*The benefit that comes from my existence*": Patients, bereaved family members, and the CASCADE team members' experiences of conducting a cancer rapid autopsy program

Laura Forrest

Peter MacCallum Cancer Centre

Erin Tutty, Philomena Horsley, Sadie Dunne, Odette Spruyt

Introduction

CASCADE is an Australian-first cancer rapid autopsy program that enables genomic analysis of metastatic tissue. Patients with metastatic disease are consented to CASCADE by their clinician at the end of life when no further curative treatment options are available. The conduct of CASCADE creates an inimitable and profound situation for patients, their family members, and for the clinicians and researchers involved. Capturing these experiences is critically important to understand and evidence the practical, psychosocial, and ethical aspects of a rapid autopsy program and to inform future research.

Methods

Three qualitative studies were conducted using semi-structured interviews to collect data with: 1) patients who have consented to CASCADE; 2) bereaved family members of CASCADE participants, and 3) the clinicians/researchers who conduct CASCADE. Qualitative data for each study were thematically analysed and triangulated to increase analytical rigour.

Results:

Nine interviews have been conducted with CASCADE participants, 14 with bereaved family members, and 20 with CASCADE clinicians and researchers. The trust between patients and their clinicians was evident, acknowledged, and necessary to consent patient to a rapid autopsy. Patients were primarily motivated by altruism to consent to CASCADE. Irrespective of family members' personal comfort with the rapid autopsy process, they respected and honoured patients' autonomous decision to participate. Although rewarding, conducting CASCADE was burdensome for many clinicians and researchers, and several had adopted various mechanisms to cope with this oft "*confronting*" research.

Discussion

The success of CASCADE is due to the diligence and commitment of the clinical and research team, and altruism and trust that is engendered with patients and their family members. Opportunities to de-brief within the CASCADE team may alleviate some of the impact of conducting this research and enhance the team's communication.

ORAL 8:

Response and resistance to catalytic P300/CBP inhibition in multiple myeloma

Simon Hogg
Peter MacCallum Cancer Centre

Olga Motorna, Leonie A. Cluse, Jake Shortt, Stephin J. Vervoort, Ricky W. Johnstone

Multiple myeloma (MM) is an incurable hematological malignancy that is typified by the accumulation of malignant plasma cells within bone marrow. Oncogenic transcription factors (TFs) and de-regulated transcriptional programs are causatively implicated in the pathogenesis of MM. TFs require a permissive chromatin state often mediated by post-translational acetylation of lysine residues on both histone and non-histone proteins to drive robust expression of target genes. A recently developed first-in-class catalytic inhibitor of histone acetyltransferases P300/CBP showed efficacy in pre-clinical models of prostate cancer, however the activity has not yet been evaluated in hematological malignancies. Here we report that MM cell lines and primary patient samples are exquisitely sensitive to catalytic P300/CBP inhibition *in vitro* and *in vivo*. We used integrated epigenomic and transcriptomic analysis to demonstrate that catalytic P300/CBP inhibition is associated with rapid hypoacetylation of P300/CBP substrates and rapid suppression of RNA polymerase II-driven nascent transcription. Oncogenic TFs including PRDM1 (BLIMP-1), MAF, MYC and IRF4 that feature prominently in MM biology were potently downregulated by catalytic P300/CBP inhibition. These studies establish a rationale for clinical investigation of catalytic P300/CBP inhibitors in MM and provide motivation to preemptively identify mechanisms of resistance using CRISPR/Cas9-based gene editing. To derive treatment-resistant populations, a human MM cell line expressing Cas9 and a genome-wide sgRNA library were exposed to catalytic P300/CBP inhibitor. This genetic screen identified a transcriptional repressor complex whose loss mediates primary resistance to catalytic P300/CBP inhibition. Overall, these pre-clinical studies highlight the therapeutic potential of catalytic P300/CBP inhibition in MM and identify compensatory resistance mechanisms that may be used to predict drug efficacy.

Peripheral Blood Biomarkers that Predict Response to Radiotherapy and Pembrolizumab Combination in Metastatic Breast Cancer

Harini de Silva
Peter MacCallum Cancer Centre

Peter Savas, Pasquale Petrone, Michael Neeson, Daniela Tantalo, Shankar Siva, Mathias Bressel, Steven David, Paul J Neeson and Sherene Loi

Introduction

Stereotactic ablative body radiotherapy (SABR) is believed to convert the tumor micro-environment from non-inflamed to inflamed, where immunogenic death and release of tumor antigens drive tumor specific T cell responses. Based on the hypothesis that the efficacy of SABR could be improved with the addition of anti-PD1 blockade, this clinical trial aimed to evaluate the safety and immunological effects of SABR and Pembrolizumab combination in patients with oligometastatic breast cancer.

Methods

Patients with oligometastatic breast cancer (1-5 mets) received SABR (20Gy) followed by eight, 3-weekly cycles of Pembrolizumab. Blood samples collected at baseline (pre-SABR), pre-cycles 1, 3, 5 and 30 days post-last pembro were analysed for T cell activation markers (CD69), checkpoints (PD-1, LAG3, TIM3), regulatory T cells, memory T cells and CD4$^+$/8$^+$ ratios by flow cytometry. Plasma IFNγ concentration was assessed by cytokine bead array assays.

Results

A total of 15 patients (median age = 54) were enrolled between March 2016 and Nov 2017. The number of patients with 1, 2, 4 and 5 metastases treated with SABR was 9 (60%), 3 (20%), 2 (13%) and 1 (7%), respectively. There were 3 (20%) TNBC, 2 (13%) HER2$^+$ and 10 (67%) ER$^+$/HER2$^-$ (Luminal) breast cancer patients. At the time of reporting 14/15 patients had completed two years of follow-up, 7 (46%) had progressive disease (non-responders), and there were no deaths.

The median proportions of PD1$^+$ CD8$^+$ T cells were significantly different between responders and non-responders at pre-SABR (35% vs 19%; p=0.002), pre-cycle 1 (35% vs17%; p=0.01), pre-cycle 3 (27% vs 15%; p=0.006), pre-cycle 5 (27% vs 17%; p=0.01), and 30 days post-last pembro (29% vs 11%; p=0.02). There was a significantly higher median percent increase of CD8$^+$ CD45$^+$ CCR$^-$ (effector), and EOMES$^+$ CD8$^+$ T cells from baseline to pre-cycle 1 in non-responders compared to responders (p=0.04 and 0.01 respectively). Proportions of regulatory T cells, CD4$^+$/8$^+$ ratios or plasma IFNγ levels were not significantly different between the two groups at pre-cycle1 (p=0.07, 0.69 and 0.49 respectively) or any other time points.

Conclusions

SABR and Pembrolizumab treatment resulted in immune activation in this cohort. CD8$^+$ T cell PD-1 expression is a potential biomarker that can be used to predict treatment response. Further correlative analyses are ongoing.

FLASH TALK 9:

SESSION 3

Utilizing Smac mimetics to induce necroptosis may avoid apoptosis-induced mutagenesis

Mark Miles
La Trobe Institute for Molecular Science (LIMS)

Dr Tanmay Shekhar, Assoc Prof Christine Hawkins

DNA damaging anti-cancer therapies such as chemotherapy and radiotherapy can contribute to an increase in second malignancies in cancer survivors due to their oncogenic effects on non-cancerous cells. The risk of these "therapy-related cancers" may be elevated in patients with impaired DNA damage responses. Agents that activate apoptotic pathways (such as death ligands) may also possess oncogenic properties as cells surviving apoptotic stimuli can acquire mutations via the mis-repair of DNA strand breaks generated by the apoptotic nuclease CAD in cells experiencing sublethal caspase activity. Therapies that are non-mutagenic (and hence probably not oncogenic) are therefore needed to eliminate the risk of therapy-related cancers. Interestingly, Smac mimetic drugs that antagonize IAP proteins to activate apoptotic and/or necroptotic cell death pathways appear to lack mutagenic activity in vitro. This study aimed to elucidate why Smac mimetic treatment does not provoke mutations. Using CRISPR/Cas9 gene editing to eliminate expression of key components involved in apoptotic or necroptotic cell death, or DNA repair, we found that unlike chemotherapy, in which mutagenesis was exacerbated, Smac mimetics failed to stimulate mutations in cells with proficient or defective DNA damage response pathways. Furthermore, necroptotic death mediated by RIPK3 or MLKL did not damage the DNA of Smac mimetic-treated cells. These findings suggest that Smac mimetic drugs may reduce the risk of therapy-related cancers particularly in patients with defective DNA repair, and that necroptotic cell death may avoid the mutagenesis associated with apoptotic death.

CIS inhibits NK cell turnover, differentiation and priming during development

Rebecca Delconte
The Walter and Eliza Hall Institute

Guittard, G., Goh, W., Hediyeh-Zadeh S., Hennessy, R., Rautela, J, Goubard, A., Josselin, E., Castellano, R., Souza-Fonseca-Guimaraes, F., Davis, M.J., Vivier, E., Nunès, J. A., and Huntington, N. D.

The detection of aberrant cells by natural killer (NK) cells is controlled by the integration of signals from activating and inhibitory ligands and from cytokines such as IL-15. We previously identified cytokine-inducible SH2-containing protein (CIS, encoded by *Cish*) as a critical negative regulator of IL-15 signalling in NK cells under inflammatory conditions. The functional effect of *Cish*-deficiency in NK cells resulted in increased anti-tumour immunity and hyper-proliferation in response to IL-15, however, in steady-state, it remained unclear how CIS regulates NK cell biology. To further our understanding of NK cell regulation, we investigated the role of CIS in the homeostatic maintenance of NK cell numbers *in vivo*. Indeed, CIS-ablation promoted the terminal differentiation of NK cells as well as increased expression of cell cycle markers, suggesting that under steady-state conditions, CIS plays a role in maintaining IL-15 driven regulation of NK cells *in vivo*. This did not manifest in an accumulation of CIS-deficient NK cells, suggesting that the requirements for $Cish^{-/-}$ NK cells to exert their full proliferative capacity were not met *in vivo*. Instead, loss of CIS conferred a lower activation threshold, evidenced by the augmented production of inflammatory cytokines and cytotoxicity when stimulated *ex vivo* and their increased functionality on a per cell basis. In conditional *Cish*-deficient mice

($Cish^{fl/fl}Ncr1^{iCre}$), homeostatic numbers of NK cells were again maintained, however they showed no alterations in maturation or cycling. Despite this, $Cish^{fl/fl}Ncr1^{iCre}$ NK cells still displayed a lowered activation threshold upon ex vivo stimulation and $Cish^{fl/fl}Ncr1^{iCre}$ mice were more resistant to experimental tumour metastasis. We conclude that the increased anti-tumour function observed in *Cish*-deficient mice is not caused by homeostatic alterations or accumulation of NK cells, but is intrinsic to NK cells and an effect of the rewiring of several activation pathways.

FLASH TALK 11:

Feasibility of an upright imaging technique for radiotherapy in an upright position

James Korte
Peter MacCallum Cancer Centre

Nicholas Hardcastle, Sarah Everitt, Sulman Rahim and Tomas Kron

Introduction

Many patients receiving radiotherapy (RT) could benefit from treatment in an upright position, due to inability to lie on their back or stomach, which is the standard treatment position. An upright position also changes patient anatomy and has potential to deliver a lower radiation dose to organs outside the desired treatment area. A major challenge of upright RT however is the acquisition of 3D imaging for treatment planning and daily positioning of the patient. In this work we reprogram a conventional RT machine to rotate the patient while acquiring a set of upright 2D images with the on-board imaging system. A modified iterative algorithm is used to reconstruct an upright 3D image on which we investigate the accuracy of RT treatment planning.

Methods

To evaluate if an upright image is suitable for RT planning we compared a RT plan on an upright image and a standard image. Our test object was an adult female phantom, which is an anthropomorphic object composed of plastic with densities closely matched to human tissue. The female phantom was imaged twice, first lying down in a standard imaging system and then in an upright position on the RT machine. A treatment plan was created to deliver radiation beams to a simulated lung tumour on each of the 3D images. For each image the estimated radiation dose to the tumour and surrounding organs was calculated.

Results

The upright images were similar to the standard images but had some imperfections, such a bright central artifact. The distribution of radiation dose calculated on each image was very similar in the tumour, with minor dose inaccuracies in other regions that are attributed to imperfections in the upright image.

Discussion & Conclusion

These initial results demonstrate that the quality of upright images acquired on a conventional radiotherapy machine have potential to be used for RT treatment planning. Future work will reduce the image imperfections to make this technique clinically feasible.

FLASH TALK 12:

Differentiation therapy by HDAC inhibition in t(8;21) acute myeloid leukemia requires immune cell mediated type I interferon production

Maria del Pilar Dominguez Rodriguez
Peter MacCallum Cancer Centre

Jessica Salmon, Kym Stanley, Maddison Kelly, Lev Kats,

Kate McArthur, Benjamin KIle, Andrew Wei and Ricky

W. Johnstone

Introduction

The translocation t(8;21), which results in the AML1-ETO (A/E) fusion protein, is one of the most common chromosomal abnormalities in acute myeloid leukemia (AML). A/E recruits histone deacetylases, aberrantly repressing AML1-target genes. We previously demonstrated that treatment with the HDAC inhibitor panobinostat decreased tumor burden and enhanced survival in mice bearing A/E leukemias. We decided to further analyse the mechanisms underpinning the therapeutic effect of panobinostat.

Methods

We used an *in vivo* t(8;21) AML mouse model driven by the expression of a variant of A/E (A/E9a) and oncogenic Ras (AML1-ETO9a:NRASG12D). We treated the mice daily with panobinostat (LBH-589) or vehicle, administered intraperitoneally for 3 days, and analysed the tumor cell transcriptome. For survival experiments we treated the mice for 4 weeks with panobinostat or vehicle.

Results

We analysed the gene expression changes induced by panobinostat on tumor cells (GFP[+]) isolated from AML1-ETO9a:NRASG12D mice and found significant upregulation of genes associated with type I interferon (IFN) signalling pathway in panobinostat-treated mice compared to vehicletreated mice. Importantly, we found that expression of type I IFN receptor (IFNAR1) on tumor cells was essential for the anti-leukemia effect of panobinostat. *In vitro* treatment with panobinostat did not induce type I IFN production by leukemia cells, but increased the production of IFNβ by activated Flt3L-derived dendritic cells. Moreover, we found that panobinostat effect was partially mediated by STING on non-tumor cells. Finally, combinatorial treatment of AML1- ETO9a:NRASG12D mice with panobinostat and IFNa resulted in improved therapeutic efficacy.

Discussion

Our results suggest that immune cells, probably dendritic cells, produce type I IFN in response to panobinostat, activating the antigen presentation machinery in leukemia cells, which enhances the anti-tumor immune response.

Enhancing CAR T cell therapy by enabling their interaction with antigen-presenting cells

Clare Slaney
Peter MacCallum Cancer Centre

Jack D Chan, Phillip K Darcy and Michael H Kershaw

Responses of solid tumours to chimeric antigen receptor (CAR) T cell therapy are often minimal. This is potentially due to a lack of sustained activation and proliferation of CAR T cells when encountering antigen in a profoundly immunosuppressive tumour microenvironment.

We previously demonstrated that adoptive transfer of CAR T cells in combination with a vaccine can eradicate large tumours in mice (1, 2). In these studies, the extensive proliferation of CAR T cell was driven by their interaction with the major histocompatibility complex (MHC) on antigen-presenting cells (APCs), mediated by a vaccine-specific T cell receptor (TCR). However, major limitations for the widespread use of this approach include the need for a specific TCR and a specific vaccine for individual patients.

In the current study, we generated novel, yet safe, bispecific proteins to mediate the interaction between APCs and CAR T cells without the need for a specific TCR or vaccine. We termed these bispecifics "**Bispecific Engagers of APCs and T cells (BEATs)**". Various BEAT formats have been designed; all based on an ability to simultaneously bind the CAR T cells and APCs. CAR T cell proliferation and function was enhanced by BEATs in vitro and in vivo. Both murine syngeneic and human xenograft solid tumour growth was significantly inhibited when CAR T cells were administered in combination with BEATs. This study demonstrates that facilitating the interaction of CAR T cells with APCs can enhance their antitumor activity, and the potential of using BEATs with CAR T cells in treating solid cancers.

1. Slaney CY, et al. (2017) Dual-specific chimeric antigen receptor T cells and an indirect vaccine eradicate a variety of large solid tumors in an immunocompetent, self-antigen setting. Clin Cancer Res.

2. Westwood JA, et al. (2017) An ultrastructural investigation of tumors undergoing regression mediated by immunotherapy. Oncotarget 8(70):115215-115229.

Understanding the role of a unique population of T-helper 17 cells in the pathogenesis of infection-induced colon cancer

Ka Yee Fung
Peter MacCallum Cancer Centre

Abdirahman S, Preaudet A, Burstroem L and Putoczki TL

Introduction:

A number of gastrointestinal (GI) pathogens can increase the risk of colorectal cancer (CRC). However, the mechanism underpinning this association has only recently gained attention. In mice, GI pathogens have been shown to trigger Interleukin (IL)-17 mediated Signal Transducer and Activator of Transcription 3 (STAT3) activation in epithelial cells resulting in enhanced tumour progression. The primary source of IL-17 is thought to be a subset of CD4-positive T cells called T-helper (Th)-17 cells, which reside in the lamina propria. We aim to establish if a unique Th17 cell population can inadvertently drive CRC progression.

Method:

We have identified a unique population of Th17 cells *in vitro* through gene expression analysis. We have utilised multiple animal models to validate the pathogenicity of this cell population *in vivo*, ranging from a classic Th17 disease model of experimental autoimmune encephalomyelitis (EAE) to GI infections with mouse pathogens that trigger colitis. To further understand the role of this unique Th17 cell population in CRC, we have coupled genetic mouse models of CRC with C. rodentium infection, which triggers early CRC onset.

Results and Conclusion:

It is becoming increasingly accepted that viruses and bacteria can directly mutate epithelial cells, secrete mutagenic products, or trigger prolonged inflammation. Th17 cells are part of an inflammatory response to infection, with their numbers increasing with tumour stage, and this increase is positively associated with poor prognosis in human CRC. We present the first evidence of a unique sub-population of Th17 cells that are present in the GI tract. Coupled to the knowledge that an estimated 20% of all cancers are caused by infectious agents, and the increasing epidemiological link between infections and CRC, there is an immediate need to better understand the host response to bacterial infections.

ORAL 12:

Tissue-resident memory T cells mediate melanoma surveillance and are targets of immunotherapy

Simone Park
Peter Doherty Institute

Olga Motorna, Leonie A. Cluse, Jake Shortt, Stephin J. Vervoort, Ricky W. Johnstone

Non-recirculating tissue-resident memory T (T_{RM}) cells reside permanently in epithelial tissues where they are known to protect against infection. T_{RM} cells have now been identified in various human cancers where they correlate with improved patient outcomes, but the mechanisms through which they may mediate protective tumour immunity remain unclear. Using a novel mouse model of transplantable cutaneous melanoma, we show that tumour-specific T_{RM} cells form spontaneously in the epithelial tumour microenvironment where they protect against cancer development independently of circulating memory T cells. A proportion of epicutaneously inoculated mice remained free of macroscopic tumours long after inoculation and intravital imaging revealed that these mice harboured dormant melanoma cells in their skin that were dynamically surveyed by tumour-specific T_{RM} cells. Depletion of T_{RM} cells from these mice triggered melanoma escape, indicating that T_{RM} cells suppress tumour progression by promoting cancer-immune equilibrium and implying that T_{RM} cells could be targeted to improve cancer treatments. In line with this, we found that pre-activated T cells transferred to tumour-bearing mice in a model of adoptive cell therapy gave rise to T_{RM} cells that inhibited the growth of established melanoma. These tumour-specific T_{RM} cells retained their capacity to execute effector functions and protect against tumour growth despite expression of various inhibitory molecules typically associated with T cell dysfunction. Combined, our findings reveal T_{RM} cells to be durable mediators of anti-tumour immunity and suggest that targeting T_{RM} cell responses could serve as a novel immunotherapeutic strategy to drive elimination of solid tumours.

POSTER PRESENTATIONS
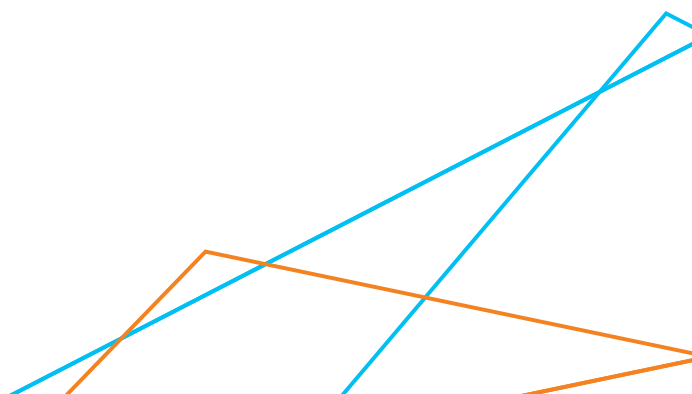
POSTER 01

The role of polarity protein PAR3 in haematopoietic system

Farzaneh Atashrazm

Peter MacCallum Cancer Centre

Alysha Dew, Metta Jana, Sarah Ellis

Cell polarity is a critical feature of multiple aspects of cell biology e.g. cell division and differentiation. Disrupted polarity network is well established in epithelial cancers where the polarity regulators act as tumour-suppressors or oncogenes. In the haematopoietic system, polarity proteins regulate asymmetric cell division (ACD) of haematopoietic stem cells (HSCs). ACD is central to the maintenance of normal haematopoiesis, ensuring the retention of self-renewal and differentiation potentials of HSCs. Disrupted ACD is associated with leukaemia transformation as leukemic cells lose the ability to differentiate and instead gain the ability to self-renew. Considering this as well as the role of polarity proteins in ACD, understanding how they regulate HSC ACD or contribute to leukaemia is of major importance. We examined the role of polarity protein PAR3 in acute myeloid leukaemia (AML).

To study the role of PAR3 loss in haematopoiesis, we utilised a *Par3* conditional knockout mouse model. For the human model, Par3 was knocked out in AML cell lines using CRISPR/Cas9.

PAR3 is differentially expressed in haematopoietic progenitors and their more differentiated progeny. PAR3 loss in a pre-clinical model of murine AML led to a significant increase in the proportion and number of granulocyte/macrophage progenitors, which are the hyperproliferative cancer stem cells. This was associated with deregulation of MAPK and HIPPO signalling pathways in Par3 KO animals. To study the cellular and molecular mechanisms of PAR3 in human AML, we have successfully knocked out the Par3 in several human AML cell lines. The role of PAR3 in asymmetric cell division is being studied by examining the cell cycle status (using FUCCI staining and live cell imaging), cell differentiation, polarised inheritance of cell fate determinants, mitotic spindle positioning, and the Notch signalling pathway; a major pathway in haematopoietic cell selfrenewal.

PAR3 acts as a tumour suppressor in AML.

POSTER 02

Investigating Primary Melanoma Immune Escape

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Peter MacCallum Cancer Centre

Simon Keam, Jessica Li, Paul Neeson

Introduction

Tumour infiltrating lymphocytes (TILs) number and localisation within the melanoma microenvironment influences clinical outcome. Recently, melanoma-associated T cells have been described by their density and spatial distribution. From this, tumours can be broadly classified as T cell inflamed, immune excluded and immune desert. We hypothesized that melanoma patient outcome is also influenced by immune cell network signalling, and a lack of 'T cell inflamed' gene signature represents an immune escape mechanism.

Methods

We accessed melanoma tissue biopsies (primary disease and wide local excision) from 11 patients. From FFPE sections, we performed multiplex IHC (mIHC), RNAseq and Nanostring (NS) immune gene expression profile (GEP) (with the pan cancer immune gene set). We assessed the density and spatial distribution of the T cells by mIHC; the RNAseq and NS immune GEP generated differentially expressed genes (DEG) and active immune signalling pathways.

Results and Discussion

From mIHC data, we stratified 5 samples into the T cell inflamed subset whilst 5 samples were considered immune-excluded and 1 sample classified as an immune-desert. cDNA derived from FFPE-RNA by the Clontech pico kit yields high transcript count and expression profiling efficiency. RNAseq samples stratified by tumour vs healthy have a distinct DEG profile with tumours expressing high levels of SOX10 and MLANA as expected. Stratification of samples to TIL-hi/lo results in heterogenous clustering. However, GEP of immune cells by NS analysis stratified by TIL-hi/lo show 2 TIL-hi samples cluster together with TIL-lo samples whilst possessing a DEG profile like TIL-lo cells suggesting that they may not be fully functional.

Conclusions

Our results indicate that immune cell density and localisation should also be assessed in conjunction with GEP to determine the functional capacity of the immune infiltrate and may represent molecular pathways exploited by melanoma to evade the immune system.

POSTER 03

Deciphering the immune suppressive function of regulatory T cells in tumours

Jonas Blume
Peter Doherty Institute

Axel Kallies

Regulatory T cells (Tregs) balance immune responses and are critical to prevent auto immunity. However, in the context of cancer, this is undesired as Tregs interfere with a desired immune response towards the tumour. Furthermore, Tregs actively contribute to exhaustion of CD8s by providing inhibitory receptors. Recent research has shown that Tregs are recruited into the tumour, making them an key player in the undesired immune escape observed in many cancers.

Our lab is interested in effector differentiation of Tregs, leading to migration and adaption to non-lymphoid tissues. Hence, we want to understand the way Tregs adapt to the tumour micro environment and inhibit and interfere with tumour killing lymphocyte. Furthermore, we aim to understand how Tregd react towards checkpoint inhibition, as emerging auto-immunity is a downside to checkpoint inhibition.

To this end, we employ several mouse models allowing us to track effector differentiation of Tregs and modulate Treg function by targeted deletion of critical transcription factors or cytokine signalling components.

POSTER 04

Repurposing of MDR1 inhibitors in AML: Combination with targeted Smac-mimetic therapy kills leukaemia stem cells, overcomes resistance and prolongs survival

Gabriela Brumatti

The Walter and Eliza Hall Institute

Emma L. Morrish, Anthony Copeland, Donia M. Moujalled, Jarrod J. Sandow, Natasha Silke, Kate E. Jarman, Gregor Ebert, Jessica A. Beach, Elizabeth L. Christie, Liana Mackiewicz, Giovanna Pomilio, Karla C. Fischer, Laura MacPherson, David D.L. Bowtell, Andrew I. Webb, Marc Pellegrini, Mark A. Dawson, Andrew H. Wei and John Silke

High levels of inhibitor of apoptosis (IAP) proteins have been linked to cancer progression and treatment resistance, leading to the development of small molecule inhibitors called Smac-mimetics (SMs). The clinical SM birinapant has been trialed for the treatment of leukemias and solid cancers, and despite the high tolerability and specificity of this drug, resistance of cancer cells to birinapant based therapies has limited the progression and success of clinical trials. Using a library of >5,700 bioactive compounds we identified inhibitors of multidrug resistance protein 1(MDR1/ABCB1) that overcome birinapant resistance in AML. We show that clinical MDR1 inhibitors tariquidar and zosuquidar, synergise with birinapant and other SMs to kill acute myeloid leukemias (AMLs). Inhibition of MDR1 in SM resistant leukemias increased intracellular levels of birinapant and efficiently kill leukemic stem cells (LSCs). More importantly, our in vivo studies show that the drug combination is well-tolerated and effective in treating leukemias. This study identifies MDR1 as a biomarker of SM therapy and reveals a new clinically-relevant personalised treatment for AML patients.

POSTER 05

The local microenvironment drives the identity of tissue-resident lymphocytes

Susan Christo

Peter Doherty Institute

Maximilien Evrard, Dane Newman, Simone L. Park, Julia E. Prier, Francis R. Carbone, Florent Ginhoux, Axel Kallies and Laura K. Mackay

Tissue-resident memory T (T_{RM}) cells are a population of non-recirculating lymphocytes that permanently reside in non-lymphoid organs. Together with other tissue-resident lymphocytes, these cells are critical for controlling infection and more recently, have been implicated in better prognosis of solid cancers. Although T_{RM} cells possess a common molecular signature distinguishing them from their circulating counterparts, the transcriptional identity of these cells differs vastly between organs. We found that organ-specific gene signatures are conserved between different subsets of resident lymphocytes within a given tissue, partly reflective of imprinting by local cytokines, including TGFβ and IL-15. We show that whilst T_{RM} cells in different tissues exhibit differential cytokine and molecular requirements for their development, locally-derived factors cooperate to globally suppress tissue-egress genes in resident cells across all organs. Collectively, our data demonstrate the adaptation of T_{RM} cells to specific tissue microenvironments. Exploiting such commonalities and differences in T_{RM} cell regulation may inform new immunotherapy strategies designed to enhance these cells in a site-specific manner.

POSTER 06

Natural killer cell function in patients with Multiple Myeloma in response to immunomodulatory therapy

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Introduction

Multiple myeloma (MM) is characterised by malignant plasma cells that accumulate in the bone marrow with poor survival outcome. Malignant plasma cells are known to be susceptible to Natural killer (NK) cell killing. Immunostimulatory agents such as Lenalidomide are known to enhance NK cell cytoxicity. In this study, newly diagnosed (ND) and refractory relapse MM (RRM) patients were provided induction therapy with lenalidomide and dexamethasone and only newly diagnosed MM patients underwent autologous stem cell transplants. NK cell function and phenotype was studied to determine if post induction treatment and autologous stem cell transplantation (ASCT) affected NK cells and to identify a timepoint that MM patients would benefit from antibody mediated therapy that enhance NK cell cytotoxicity.

Methods

In this study, flow cytometry based assays were used for phenotypic analysis of NK cells from peripheral blood of ND MM patients in comparison to healthy individuals both before and after transplant and cytotoxicity function was evaluated using CD107a degranulation and cell cytotoxicity assays. Gene expression analysis of NK cells from RRM patients was also performed to identify differences in NK cell function.

Results

The data suggests that NK cells are dysfunctional as disease progresses. NK cells from MM patients have an imbalance of activating and inhibitory receptors as compared to healthy individuals, with an increase in inhibitory receptors and show a decrease in antibody dependent cell cytotoxicity (ADCC). There was an accumulation of terminally differentiated CD57 positive NK cells and gene expression analysis revealed a fundamental difference between HD NK cells and MM NK cells.

Conclusion

Although NK cells are progressively dysfunctional in MM, the data demonstrates that patients receiving lenalidomide therapy may benefit from NK cell targeted monoclonal antibody therapy post induction as they showed better ADCC responses as compared to post ASCT.

POSTER 07

Repurposing clinically approved drugs to improve stem cell transplant outcomes

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Introduction

Allogeneic haematopoietic stem cell transplantation (alloSCT) cures cancer, but is associated with significant side effects such as graft versus host disease (GVHD) and infection. In order to improve patient outcomes, we need to reduce pre-transplant conditioning, promote donor cell engraftment and limit GVHD. Natural killer (NK) cells are important for controlling donor cell engraftment, and are reliant on BCL2 and JAK1/2 pathways for survival. We investigated if NK cells could be depleted from the immune system prior to alloSCT, using the clinically approved BCL2 inhibitor Venetoclax, and the JAK1/2 inhibitor Ruxolitinib.

Methods

We used mouse models of acute myeloid leukaemia (AML) to explore the ability of Venetoclax or Ruxolitinib to deplete NK cells in wild-type alloSCT recipients, immediately prior to transplant. AML-bearing mice were treated with drug for 2 days, then given a reduced dose of irradiation, and alloSCT from a MHC-mismatched donor. Mice were monitored for GVHD, donor cell engraftment, and anti-AML response.

Results

Pharmacological inhibition of BCL2 or JAK1/2 prior to alloSCT in mice with Venetoclax or Ruxolitinib respectively resulted in rapid depletion of recipient NK cells. A significant proportion of alloSCT recipient mice pre-treated with either drug developed full donor cell engraftment after reduced intensity conditioning, did not develop GVHD, and retained potent anti-tumour effects against pre-established AML.

Conclusions

BCL2 and JAK1/2 inhibition in alloSCT recipients, in combination with reduced intensity conditioning was:

1) well-tolerated

2) associated with low rates of GVHD

3) resulted in long-term donor haematopoietic cell engraftment

4) retained anti-tumour responses in an AML mouse model

Therefore, repurposing clinically-approved drugs to inhibit recipient NK cells may represent a means by which to deliver alloSCT more safely.

IL33 signalling and mast cells as new therapeutic targets against gastric cancer

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Christine Dijkstra, Frederik Masson and Matthias Ernst

Introduction

Cytokine-mediated inflammation is a driver of gastric tumorigenesis. Interleukin 33 (IL-33) regulates inflammatory responses but only recently, a role of IL-33 in cancer starts to emerge. Depending on the cancer stage or type IL-33 can provoke either pro- or anti-tumoral responses. Here we study the function of IL-33 signalling, mast cells and macrophages in gastric cancer.

Methods

We utilise gp130^{FF} mutant gastric cancer mice, compound mutants lacking IL33 signalling (ST2$^{-/-}$), mast cells (c-kit$^{W-sh/W-sh}$), macrophages (Csfr1$^{-/-}$) or drug treatments to interrogate the role of IL-33 and innate immune cells in the growth of gastric cancers.

Results

IL33 signals through its receptor ST2, which we found abundantly expressed in gp130^{FF} tumours. Deficiency of IL33 signalling (ST2$^{-/-}$) diminished gastric tumour growth, and was associated with a decrease in tumour-adjacent mast cells and tumour-associated macrophages (TAM) as well as reduced angiogenesis. Indeed, mast cell and macrophage numbers are elevated in the gp130^{FF}-tumours compared to wild type stomachs. Genetic depletion or pharmacological inhibition of mast cells and macrophages reduced tumour burden, again associated with decreased angiogenesis. Mechanistically, we show that tumour-produced IL33 can activate gastric gp130^{FF} mast cells, which in turn recruit pro-tumoral and pro-angiogenic macrophages to the tumour through release of chemo-attractants like Ccl2, Ccl3 and Ccl71.

Discussion

We conclude, that tumour-derived IL33 promotes gastric cancer growth through tumour- associated mast cells and TAMs. The results of our genetic and pharmacological experiments in mice suggest that either the IL33/St2 signalling node or mast cells and macrophages may represent novel therapeutic targets against inflammation-associated gastric cancer.

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POSTER 09

Unlocking somatic SNVs and CNAs from RNA-Seq

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Ian Majewski, Alicia Oshlack

The advent of high throughput sequencing has revolutionized cancer research by allowing us to detect somatic mutations genomewide. The analysis of high throughput sequencing data is challenging though. Researchers are limited to what classes of mutations bioinformatics methods can detect from the used sequencing technologies.

Whole genome sequencing and exome sequencing are commonly used for genome-wide identification of somatic mutations, and provides sensitivity for point mutations and copy number alterations. RNA-Seq is a cheaper option and provides data on gene expression, fusions and isoform usage that is not available from DNA-sequencing. However, RNA-Seq is traditionally not used for detecting point mutations in the DNA, and almost never for copy number alterations.

I will present superFreq: the first publicly available method capable of calling absolute and allele-sensitive copy numbers from RNA-Seq data, even without a matched normal sample. Accuracy and sensitivity is comparable to exomes for large scale copy number alteration, with decreasing sensitivity for focal alterations covering fewer than 100 genes, corresponding to 12Mbp. SuperFreq also calls somatic point mutations from RNA-Seq, with high sensitivity in transcribed regions of expressed genes.

There are tens of thousands of public RNA-Seq cancer samples, of which only a small fraction has been analysed for point mutations, and almost none for copy number alterations. SuperFreq unlocks reanalysis of these cohorts, potentially revealing new links between point mutations, copy number alterations and expression profiles with phenotypical properties.

As a proof of concept, I will reproduce known results on copy number alterations and point mutations from the TCGA CRC cohort using only the RNA-Seq data. Most importantly, I will invite the wide range of experts across multiple cancer types at Lorne Cancer to identify and re-analyse existing data sets within their area of expertise.

POSTER 10

Overcoming tumour heterogeneity by engaging host immunity to enhance CAR T cell therapy of solid cancers

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Adoptive cell therapy (ACT) using chimeric antigen receptor (CAR) T cells is a form of immunotherapy that involves the genetic engineering and re-infusion of a patient's own T cells to specifically target cancer. While CAR T cells have had a significant impact in some haematological malignancies, its efficacy in solid cancers have been limited to date. A major factor limiting its success is the heterogeneity of tumours and immune escape of antigen negative variants[1]. Dendritic cells (DCs) are professional antigen presenting cells specialised in the priming and activation of T cells, leading to their expansion and increased effector function. We previously show that DCs play a crucial role in immune checkpoint blockade therapy[2], and that DC activation is associated with enhanced responses to CAR T cell therapy[3]. We hypothesised that engaging the endogenous immune system through enhancing host DCs will improve T cell responses and overcome the challenges of tumour heterogeneity in ACT. To this end, we engineered T cells to secrete Fms-like tyrosine kinase 3 ligand (FL), a growth factor critical for the development of DCs. Mice treated with FL-secreting T cells showed expanded DC and precursor populations, and increased recruitment of host T cells to the tumour and draining lymph nodes (dLN). Strikingly, combination of FL-secreting T cells with adjuvants resulted in inhibition of tumour growth and improved survival in two mice models of ACT for breast and colorectal tumours. This was associated with an increased number of activated DCs and functional host T cells in the dLN following combination therapy, suggesting that this therapy is capable of inducing epitope spreading and eliminating tumours that are negative for the antigen targeted by the CAR. Our data suggest that enhancing endogenous DCs is a promising strategy to augment the efficacy of CAR T cells in solid cancers and may help combat the clinical problem of antigen negative tumour escape following therapy.

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POSTER 11

Evaluation of genomics changes in ductal carcinoma in situ as potential biomarkers of recurrence risk

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Dorothea Lesche, Hugo Saunders, Sakshi Mahale, Siobhan Hughes, Islam M Miligy, Jia-Min Pang, Andrew R Green, Emad A Rakha, Stephen B Fox, Ian G Campbell, Kylie Gorringe

Breast disease screening using mammography has seen an increased detection of breast cancers but also of pre-invasive lesions such as ductal carcinoma in situ (DCIS). The clinical management of DCIS is challenging due to a lack of accurate prognostic and predictive tests. Thus, accurate estimation of disease recurrence risk is currently not feasible, leading to over-treatment of patients with low risk disease. The aim of this study was to identify shared genomic copy number variation or sequence variants that can predict DCIS cases at increased risk for later recurrence.

Sections from FFPE blocks from primary DCIS cases and their subsequent recurrences were obtained from 45 patients and primary DCIS cases from patients without recurrence from 41 patients from the Nottingham City Hospital (UK). Representative sections were reviewed by two pathologists to identify areas for microdissection. Tumour epithelial cells were needle-microdissected and DNA was isolated from cell suspensions. Sequencing libraries were prepared using SureSelect (Agilent Technologies) recommended protocols using a custom capture panel of 110 breast cancer-related genes. Sequencing was performed on the Illumina HiSeq2500 system. Sequencing data was processed on an in-house bioinformatics pipeline to detect and filter for high confidence somatic variants. Copy number profiles were obtained from off-target reads using CopywriteR.

Copy number profiles and sequence variants will be assessed to confirm the clonal relationship of primary DCIS tumours and later recurrences. Genetic variants in matching clonal cases will be compared to variants in tumours without reported recurrence. Preliminary data analysis shows that genetic variants in the tumour suppressor TP53 are more common in tumours with recurrence compared to those without.

Such genetic variants characteristic for later recurrence will be used to subsequently build a model to predict individual recurrence risk.

POSTER 12

The EHF and CDX1 transcription factors co-ordinately regulate differentiation status, metastasis and tumour burden of colorectal cancer

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Allan Burrows, Laura Jenkins, Nicholas Clemons, Robin Anderson and John Mariadason

Colorectal cancer (CRC) is a leading cause of cancer related deaths for which there is an urgent need to develop novel treatment paradigms. The loss of differentiation is a fundamental characteristic of CRC, which is associated with increased metastatic propensity, reduced sensitivity to conventional chemotherapy and worse overall survival. However, the molecular basis for differentiation loss is not well understood. Ets Homology Factor (EHF) is a member of the E26 transformation specific (ETS) family of transcription factors, which is highly expressed in normal colonic epithelial cells. We observed that expression of EHF, along with several other intestinal specific transcription factors were significantly downregulated in poorly differentiated CRCs. The aim of this study was to determine the direct role for EHF, alone and in combination with other transcription factors, in regulating the differentiation status of CRC.

Our results indicate that knockdown or re-expression of EHF alone failed to alter the differentiation status of CRC cell lines. We therefore postulate that EHF cooperates with other intestinal transcription factors to regulate differentiation. Systematic knockdown of EHF with other transcription factors downregulated in poorly differentiated CRC cell lines demonstrated that the combinatorial knockdown of EHF and

CDX1 significantly downregulates the expression of differentiation markers and disrupted the glandular structure of moderately differentiated CRC cell lines. Conversely, stable re-expression of EHF and CDX1 in poorly differentiated CRC cells significantly induced differentiation marker expression and induced some features of glandular formation. Furthermore, the re-induction of EHF and CDX1 significantly inhibited tumour burden and metastasis in vivo. Collectively, these results suggest that EHF and CDX1 co-ordinately regulate the differentiation status and in turn, growth and metastasis of CRCs.

POSTER 13

Loss of Bcl-G predisposes the development of inflammation-associated colorectal cancer

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BACKGROUND

The gastrointestinal (GI) tract is lined by a single layer of epithelial cells, with defects in these cells implicated in the pathogenesis of inflammatory bowel disease and colorectal cancer (CRC). BCL-G is a member of the Bcl-2 family of proteins and is highly expressed by GI epithelial cells. Unlike the other members of the BCL-2 family, no clear pro- or anti- apoptotic role has been attributed to this protein. We examined the role of BCL-G in CRC cancer.

METHOD

Using BCL-G deficient mice, we investigated the role of this protein in tumour development in two colorectal cancer models: the AOM/DSS model of colitis-associated cancer, and the Apc^{MIN} model of sporadic CRC. We examined tumour burden in these mice and performed immunohistochemistry to determine proliferation and apoptosis. Additionally, a label-free quantitative proteomics analysis was performed on naïve and DSS treated mice to determine potential proteins which may be associated with BCL-G function.

RESULTS

We demonstrate that Bcl-G is highly expressed in the epithelial cells of the GI tract of mice during development through to adulthood, and show that its absence accelerates the progression of colitis-associated but not sporadic CRC. This tumour suppressive role for BCL-G was consistent with our observation that BCL-G expression was significantly reduced in the tumours of CRC patients. Quantification of proliferative, apoptotic, and inflammatory cells did not demonstrate a difference in BCL-G deficient mice. However, quantitative proteomics analysis of naïve and DSS treated mice demonstrated decreased expression of mucus-associated proteins including CLCA1, FCGBP, and MPTX1.

SIGNIFICANCE

Our results demonstrate that BCL-G contributes to tumour development in colitis-associated cancer in mice, potentially though interaction with mucus scaffolding proteins resulting in a change to the mucus layer that normally protects the epithelial cells and helps maintain barrier integrity.

POSTER 14

Tissue resident memory T cells in metastatic melanoma

Angela Pizzolla
Peter MacCallum Cancer Centre

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Pathogens recruit T cells to the affected peripheral tissues to fight off the infection. T cells that persist in the previously infected site after pathogen eradication show a memory phenotype and express tissue retention molecules: they are Tissue Resident Memory T cells (T_{RM}). Cytotoxic (CD8$^+$) TRM are present in several solid cancers and, in most, they correlate with longer disease-free survival and overall survival, better than CD8$^+$ T cells.

Our study characterizes the gene and protein expression, TCR sequence and functionality of CD8$^+$ T_{RM} in metastatic melanoma to understand their capacity to respond to cancer cells and to immune checkpoint inhibitors.

We analysed a rare case of mucosal metastatic melanoma, comparing the primary tumour and two temporally and physically distant metastases for immune cells infiltration via multiplex immunohistochemistry and we observed increasing CD8$^+$ T_{RM} frequency with time, which formed clusters at the edges of the tumour.

We sorted CD8$^+$ T_{RM} and other tumour infiltrating CD8$^+$ T cells subset from one metastasis and put them contact with tumour cells, in the presence or absence of one immune checkpoint inhibitor.

CD8$^+$ T_{RM} were more activated at contact with tumour cells and they produced more cytotoxic molecules and chemo-attractants compared to other tumour infiltrating CD8$^+$ T cells. CD8$^+$ T_{RM} were however less responsive to T cell receptor stimulation than other CD8$^+$ T cells, which was not reversed by one immune checkpoint inhibitor, suggesting a state of higher activation threshold, reflected by their higher expression of immune checkpoint molecules.

The TCR sequences of CD8$^+$ T_{RM} partially overlapped with other CD8$^+$ T cells subsets and with CD8$^+$ T_{RM} from the second lesion suggesting tumour infiltration of T cells of similar specificity, in agreement with similar mutational profile of the two metastases.

These preliminary data suggest that CD8$^+$ T_{RM} are enriched with tumour-specific T cells compared to other CD8$^+$ T cells subsets.

POSTER 15

PROTACs as a New Frontier. Kinetic studies into the activity of the BRD4 degrader MZ1

Michael Roy
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Sandra Winkler, Scott J Hughes, Claire Whitworth, Michael Galant, William Farnaby, Klaus Rumpel, Alessio Ciulli

There is growing excitement surrounding small molecule protein degraders, including **Pro**teolysis-**Ta**rgeting **C**himeras (PROTACs), as a modality for development of new therapeutics.[1]

PROTACs are bifunctional small molecules, consisting of a binder for a target protein of interest and a ubiquitin E3 ligase, joined by a linker. E3 ligases play a normal cellular role in protein recycling; facilitating proteins to be tagged with ubiquitin chains to flag them for degradation via the ubiquitin proteasome system (UPS). PROTACs hijack this system, acting as a bridge to bring the target protein and E3 ligase into close spatial proximity, thus 'tricking' the cell into ubiquitinating and degrading the target protein of interest.[2]

Due to their different mode of action, PROTACs offer potential to target proteins hitherto classed as 'undruggable', lacking for example a targetable active site, or proteins for which development of selective inhibitors is particularly challenging.[3]

The Bromodomain and Extra-Terminal motif (BET) protein family of epigenetic readers (including BRD2, BRD4, BRD3) are of therapeutic interest in cancer, however close homology within the family has made it difficult to develop selective small molecule inhibitors. [4] The PROTAC MZ1, however, has been shown to selectivity degrade BRD4.[5]

In this talk I will briefly overview the unique opportunities and challenges of PROTACs as a therapeutic modality. I will then describe recent work using biophysical methods to better understand the mode of action and selectivity of MZ1 towards BRD4 and to guide future PROTAC development. [6]

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POSTER 16

BCL-XL inhibition by BH3 mimetics induces apoptosis in EBV-associated T/NK cell lymphoma

Nenad Sejic
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Catherine Chang, Rosemary J. Tierney, Ping Lan, Guillaume Lessene, Heather M. Long, Andreas Strasser, Claire Shannon-Lowe and Gemma L. Kelly

Extranodal NK/T cell lymphoma (ENKTL) is a highly chemoresistant malignancy with a median overall survival of only 7.8 months. ENKTL is consistently associated with Epstein-Barr virus (EBV) infection - a human gamma herpesvirus contributing to 2% of the global cancer burden. However, the rarity of ENKTL has limited our understanding of the contribution of EBV to pathogenesis, and the mechanisms behind evasion of conventional treatments.

A panel of ENKTL cell lines have been previously established from human tumours. We have assayed the expression of EBV proteins and of members of the intrinsic apoptotic pathway in these cell lines. Furthermore, we have investigated the chemosensitivity of the tumour cell lines to conventional chemotherapeutics and to BH3-mimetic drugs, that can trigger the intrinsic apoptotic pathway, either as single agents or in combination with one another.

Reflecting primary tumours, ENKTL cell lines exhibit an EBV gene expression pattern known as latency II, defined by expression of the viral oncogenes LMP1 and LMP2. Diverse expression levels were observed across the cell line panel with regards to the BCL-2 family proteins where high LMP1 inversely correlated with the tumour suppressor pro-apoptotic proteins, BIM and BID. However, high LMP1 levels were not associated with increased BCL-2 expression as reported in non-T and NK cell tumours. Conventional DNA damaging drugs such as etoposide and methotrexate did not induce apoptosis in ENKTL cell lines. However, apoptosis could be induced to varying degrees with BH3-mimetics drugs that target BCL-XL and most effectively when combined with compounds targeting MCL-1. The BCL-XL-targeting compound A-1331852 successfully delayed tumour latency in NSG mice xenotransplanted with the SNK6 cell line.

Taken together, these data suggest that BH3-mimetics drugs could be a much needed therapeutic avenue for ENKTL.

POSTER 17

Exploring novel targets for CAR-T Therapy for Prostate Cancer

Joe Zhu
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Deborah Meyran, Jeanne Butler, Daniela Tantalo, Michael Neeson, Michael H Kershaw, Joseph A Trapani, Phillip K Darcy and Paul J Neeson

Introduction

Prostate Cancer affects one in six men. Treatment options for men with localized disease include radical prostatectomy or brachytherapy. Unfortunately, patients may also present with advanced disease, which is lethal due to treatment resistance. There is an unmet demand to develop new therapies that improve outcome. Harnessing the immune system offers great potential in this regard.

In this study, we identified novel targets for immunotherapy in men with prostate cancer. These include the carbohydrate antigen Lewis Y, and the glucose-regulated protein 78 (GRP-78). Lewis Y is highly expressed in prostate cancer, particularly in higher grade primary tumors and in metastatic disease. GRP-78 is also expressed in prostate cancer in men with aggressive disease.

Method

We generated a second-generation Chimeric Antigen Receptor T cells (CAR-T) targeting either Lewis Y or GRP-78 with a truncated CD34-tag for purification. The differentiation and phenotype of the CAR-T product may have critical impact on the post-infusion antitumor effect and persistence. Therefore, we investigated the phenotype of CAR-T with different cytokines, i.e. IL-2, IL-7 and IL-15, to generate cells with early differentiation phenotypes. After production, we also investigated the function of CAR-T cells both *in vitro* and *in vivo*.

Results

In the optimized protocol, starting from 3×10^7 peripheral blood mononuclear cells, we're now able to produce 109 bulk CAR-T cells or 5×10^8 purified CAR-T cells with over 98% purity, within 7-10 days. Furthermore, most of these cells remained early naïve or memory-like phenotype. These engineered T cells are capable to eradicate prostate cancer tumor cells in vitro and secrete TNFα and IFNγ. The *in vivo* study also showed that infused cells can reduce tumor burden and persist post infusion.

Discussion

Our results demonstrate that the novel CAR-T cells exhibited specific and efficient tumoricidal effect against prostate cancer cells. Furthermore, by introducing the CD34-tag, with the optimized protocol, we're now able to produce CAR-T cells with high purity and more early differentiation stage in a relatively shorter time. This may further benefit the clinical application of CAR-T therapy, by reducing the total number of the infused cells and shortening the waiting period for CAR-T production.
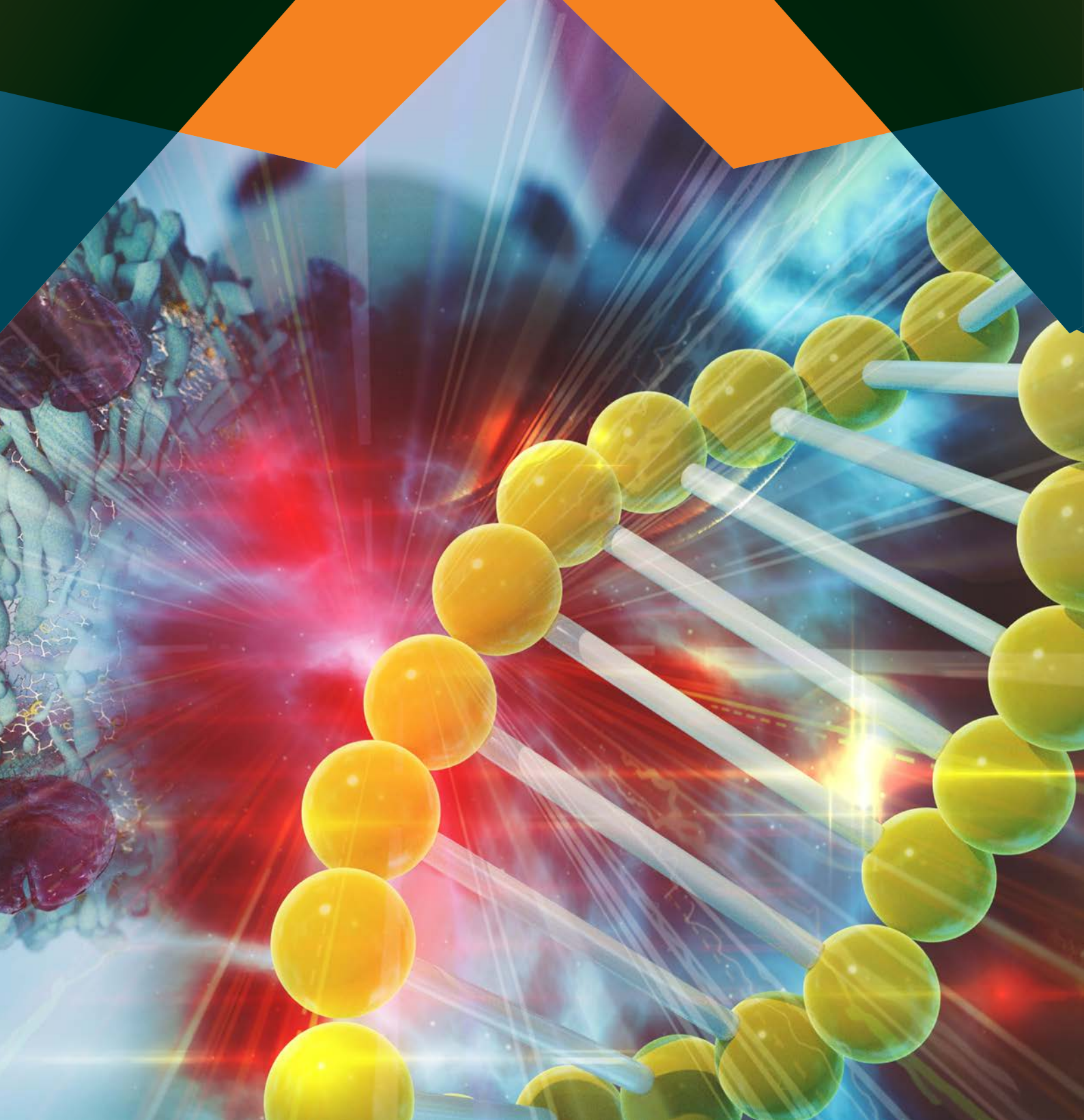
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