KCA Symposium
ECR & Postgrad Events

31st Oct - 1st Nov 2019 | Australian Maritime Museum
Darling Harbour, Sydney

GRANT WRITING WORKSHOP

Thursday 31st Oct, 9-10am

PROF ROGER REDDEL
Director of Children's Medical Research Institute, Westmead; Sir Lorimer Dods Professor, Sydney Medical School, University of Sydney

Prof Reddel is an internationally renowned expert on cancer cell immortalisation. His workshop will cover his recommendations for preparing grant applications as efficiently as possible. He is very cautious about making personal admissions around grant writing (he enjoys writing grant applications!).

EXPERT PANEL

"The Power of a PhD: A Panel Discussion Exploring Career Pathways beyond Academia"

Friday 1st Nov, 9-10am

A/PROF KATHERINE JANEWAY
Director of Clinical Genomics,
Dana-Farber Cancer Institute & Children's Cancer & Blood Disorders Center
Harvard Medical School

A/PROF ABBY ROSENBERG
Division of Bioethics and Palliative Care
Department of Paediatrics, University of Washington School of Medicine

DR JANELLE BOWDEN
Founder and CEO
Research4Me

DR CLEOLA ANDERIESZ
General Manager, Service Development and Clinical Practice,
Cancer Australia

DR STEPHANIE BLOWS
Principal Policy Officer
Office for Health and Medical Research, NSW Ministry of Health

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Oral Presentation Finalists

Thurs 3:15-4:15pm

Presenter	Institution	Title
Aaminah Khan	PhD Student, Children's Cancer Institute	Targeting the polyamine pathway as a novel therapeutic treatment against Diffuse Intrinsic Pontine Glioma
Victoria Prior	PhD Student, Kids Research, Children's Hospital Westmead	3-dimensional in vitro and cerebral organoid models to investigate high-grade glioma responses to an EphA2-targeting chimeric antigen receptor (CAR) T cell therapy
Janith Seneviratne	PhD Student, Children's Cancer Institute	Malignant neuroblasts differentiate between adrenergic and mesenchymal cell states through a transitional phenotype in peripheral neuroblastic tumours
Christopher Nelson	Post-Doctoral Researcher, Children's Medical Research Institute	Identification of the polo-like kinase 1 (PLK1) as a substrate of the cancer-associated tyrosine phosphatase EYA4
Sam Rogers	Post-Doctoral Researcher, Children's Medical Research Institute	Understanding the telomere proteome across the cell cycle

Rapid Fire Presentation Finalists

Students-Thurs 2:15 pm and ECR-Fri 2:25pm

Presenter	Institution	Title
Ane Kleynhans	PhD Student, Children's Cancer Institute	Therapeutic targeting of ubiquitin-specific protease 5 and unanchored polyubiquitin in MYCN-driven neuroblastoma
Chelsea Mayoh	PhD Student, Children's Cancer Institute	Whole transcriptome sequencing improves actionability in children with high-risk cancers
Sujanna Mondal	PhD Student, Children's Cancer Institute	The super enhancer-driven long noncoding RNA PRKCQ-AS1 promotes neuroblastoma tumorigenesis
Alice Salib	PhD Student, Children's Cancer Institute	An oncogenic role for splicing associated proteins in MYCN-driven neuroblastoma
Patricia Sullivan	PhD Student, Children's Cancer Institute	Identifying splice-altering mutations as drivers of high-risk paediatric cancers
Emmy Fleuren	Post-Doctoral Researcher, Children's Cancer Institute	Precision medicine for high-risk paediatric and AYA sarcomas
Vivian Kahl	Post-Doctoral Researcher, Children's Medical Research Institute	Telomere length measurement by DNA fibre analysis
Zsuzsanna Nagy	Post-Doctoral Researcher, Children's Cancer Institute	ALYREF is a novel therapeutic target and co-factor for MYCN-driven oncogenesis
Ruby Pandher	Post-Doctoral Researcher, Children's Cancer Institute	Efficacy of arginine depletion by BCT-100 in mouse models of neuroblastoma
Tiffany Tang	Research Assistant, Children's Cancer Institute	Novel chimeric antigen receptor (CAR) T-cell therapies for cancer

Oral Presentation Abstracts

Targeting the polyamine pathway as a novel therapeutic treatment against Diffuse Intrinsic Pontine Glioma

Aaminah Khan[1]

Co-Authors: Laura Gamble[1] , Denise Yu[1] , Swapna Joshi[1] , Dannielle Upton[1] , Laura Franshaw[1] , Mark R. Burns[2] , Murray Norris[1] , Michelle Haber[1] , Maria Tsoli[1] , David S. Ziegler[1,3]

1. Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney Australia
2. Aminex Therapeutics, Inc.
3. Kid's Cancer Centre, Sydney Children's Hospital, Randwick, NSW, Australia

Diffuse intrinsic pontine glioma (DIPG) is an aggressive paediatric brainstem tumour, with a median survival of less than 1 year. Polyamines are small intracellular polycations that control important aspects of cell biology and can be found upregulated in cancer. Difluoromethylornithine (DFMO) is an FDA-approved inhibitor of the enzyme ornithine decarboxylase (ODC1) which is a key driver of polyamine synthesis. This study aimed to investigate the efficacy of polyamine pathway inhibitors as a therapeutic strategy against DIPG. By qPCR and western blotting, high expression levels of key players of the polyamine pathway were observed in DIPG samples. Using alamar blue cytotoxicity and soft-agar clonogenic assays, we found that DFMO inhibited the proliferation of DIPG neurospheres. However, DIPG cells compensated for DFMO inhibition by increasing expression of the polyamine transporter SLC3A2. Addition of polyamine transporter inhibitor AMXT-1501 to DFMO showed synergistic inhibition of DIPG proliferation. Western blotting and flow-cytometric analysis of Annexin V-stained cells showed that combination treatment, enhanced apoptosis. Consistent with the in vitro results, the combination of DFMO and AMXT-1501 significantly prolonged the survival of mice bearing DIPG orthografts. DIPG tumours displayed higher polyamine concentrations compared to healthy brain tissue, and when treated with the combination, exhibited significantly decreased levels of polyamines. This combination therapy significantly extended the survival of DIPG engrafted mice, with 6/9 mice surviving until the humane endpoint of 160 days. Examination of RNA expression levels in a cohort of high-risk childhood cancers showed that the polyamine transporter, SLC3A2, was significantly overexpressed in DIPG and other paediatric high-grade gliomas compared with all other highrisk childhood cancers. Together these results suggest that polyamine inhibitors may be particularly active in these paediatric brain tumours. AMXT-1501 is currently in clinical development, and following completion of an adult Phase 1 trial a clinical trial for DIPG patients is planned.

Oral Presentation Abstracts

3-dimensional *in vitro* and cerebral organoid models to investigate high-grade glioma responses to an EphA2-targeting chimeric antigen receptor (CAR) T cell therapy

Victoria Prior

Co-Authors: Hsu K[1], Middlemiss S[1], Kramer B[1], Galeano-Nino J[5], Biro M[5], Maksour S[3,4], Miellet S[3,4], Hulme A[3,4], Dottori, M[3,4], and O'Neill GM[1,5]

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4. Illawarra Health & Medical Research Institute, Wollongong, Australia
5. EMBL Australia, Single Molecule Science Node, School of Medical Sciences, University of New South Wales, Sydney, Australia
6. Discipline of Child & Adolescent Health, Faculty of Medicine & Health, The University of Sydney, Sydney, Australia

Currently, no targeted treatments exist for high grade gliomas (HGG), the most deadly brain tumours. The exciting success of chimeric antigen receptor (CAR) T cells in treating haematologic malignancies has stimulated interest in this approach for solid tumours. The cell surface antigen Ephrin type-A receptor 2 (EphA2) is an attractive potential CAR T target for HGG. However, *in vivo* tissue barriers are a potential impediment for CAR T efficacy against solid tumours. To model – and overcome – these barriers, we have therefore analysed HGG and CAR T cells embedded in matrices that resemble *in vivo* tissue barriers and have characterised a cortical organoid model co-cultured with patient HGG cells which we aim to treat with CAR-T cells.

T cells from healthy donors were modified to express an EphA2 targeting CAR. Flow-cytometry-based analyses confirm specificity and toxicity of the CAR Ts for target cells. Subsequently, EphA2-targeting CAR-T cells were co-cultured with cognate patient tumour cells in a collagen I scaffold and tracked by live time-lapse confocal microscopy and post-imaging analysis, revealing effective target 'seeking' and tumour killing behaviour.To further enhance our investigation and to assess CAR T cell homing and recruitment to HGG tumours in a more biologically relevant context, we developed a 3D *in vitro* model using human embryonic stem cell-derived GFP-expressing cortical organoids co-cultured with patient-derived HGG tumour models. Characterization through immunostaining demonstrates our model represents early fetal human brain tissue with mixed cell populations. Tissue clearing methods and multiphoton imaging reveals that HGG tumour models aggressively invade organoids, recapitulating disease hallmarks observed *in vivo*. Western blotting confirms the absence of EphA2 in organoid cultures.

These exciting preliminary findings indicate great promise for CAR T cell treatment for HGG. We hope to soon corroborate these findings using our established cortical organoid and tumour co-culture.

Oral Presentation Abtracts

Malignant neuroblasts differentiate between adrenergic and mesenchymal cell states through a transitional phenotype in peripheral neuroblastic tumours

Janith Severitane

Co-Authors: Daniel R. Carter[1,2,3], Anushree Balachandran[1,2], Shi-Bei Du[4], Xiao-Jun Yuan[4], Chao Zhang[5], Belamy B. Cheung[1,2,5] & Glenn M. Marshall[1,2,6]

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Neuroblastoma, ganglioneuroblastoma and ganglioneuroma are subtypes of peripheral neuroblastic tumours (PNT) which comprise 7-10% of all childhood tumours. The histological subtyping of malignant neuroblasts within these tumours is the strongest predictor of patient outcome. However, the molecular features of these malignant neuroblasts remain uncharacterised.

To characterise malignant neuroblasts in PNTs, we isolated 5309 viable single cells from tumours of 7 PNT patients after surgery using fluorescence-activated cell sorting. Single cell RNA-sequencing libraries were then generated using the Smart-Seq2 protocol, followed by sequencing and data analysis. To define the PNT tumour microenvironment we first clustered cells based on their gene expression profiles and identified cell types using canonical gene markers. We then separated malignant neuroblasts from non-malignant cells using inferred cell copy number variations. Differential gene expression analysis of neuroblast clusters revealed a continuum of gene expression between previously defined adrenergic and mesenchymal transcriptional states. Cell trajectory analysis of these cell states revealed a novel population of neuroblasts transitioning between the two states. Gene ontology analyses of differentially expressed genes in transitioning neuroblasts revealed a neurodevelopmental phenotype, marked by chromatin silencing and neurogenesis. We applied this gene signature to existing neuroblastoma cohorts and found patients expressing these genes had much poorer prognosis. These findings suggested that PNTs with cell state plasticity have the capacity to evade and survive anti-cancer therapies.

We discovered that neuroblastoma cell lines with this transitional phenotype displayed markedly distinct chromatin landscapes through analysis of H3k27ac ChIP-sequencing datasets. Transitional neuroblastoma cell lines lacked super-enhancers that typically defined adrenergic or mesenchymal cell states, suggesting an epigenetic basis for this transitional phenotype and the potential use of epigenetic therapies to target these cells. The transitional neuroblasts identified in this study may therefore have implications for PNT patient prognosis stratification and the use of epigenetic therapies to target these cells.

Oral Presentation Abstracts

Identification of the polo-like kinase 1 (PLK1) as a substrate of the cancer-associated tyrosine phosphatase EYA4.

Christopher Nelson[1]

Co-Authors: Sam Rogers[1], Alexander P. Sobinoff[1], Christopher G. Thomlinson[1], Eloise Dray[2], Anthony J. Cesare[1], Hilda A. Pickett[1]

1. Telomere Length Regulation Unit, Children's Medical Research Institute, University of Sydney
2. Health Sciences Centre, University of Texas

The Eyes Absent gene family (EYA1-4) encodes a group of developmentally essential transcriptional coactivators with tyrosine phosphatase activity. EYA protein levels are very low in adult tissues but is often increased during tumorigenesis where EYA tyrosine phosphatase activity has been shown to promote cancer cell phenotypes such as proliferation, migration and invasion. Additionally, while most protein phosphatases are difficult to chemically inhibit with specificity because they share a common cysteine-based reaction mechanism, EYA protein phosphatases use a unique aspartate-catalysed reaction leading several investigators to suggest that EYA proteins may be good drug targets.

However, to determine the biological implications and chemotherapeutic potential of EYA inhibition there is a need to determine the substrates of EYA, as few are currently known.

To address this problem, we first elucidated EYA4 interacting proteins using a BioID proximity proteomics approach, wherein fusion of EYA4 with a biotin ligase results in biotinylation of interacting proteins which we detected with LC-MS/MS. Next, using LC-MS/MS again, we detected proteins with enhanced tyrosine phosphorylation in EYA4-depleted cancer cells. By comparing these two datasets, we identified polo-like kinase 1 (PLK1), a master regulator of mitosis, which itself is an oncogene currently being targeted in clinical trials. We further confirmed that PLK1 is a substrate of EYA4 using overexpression of EYA4 or a phosphatase defective mutant of EYA4. Next, we examined PLK1 tyrosine phosphorylation through the cell cycle and found that EYA4 dephosphorylates PLK1 immediately prior to mitotic entry. Furthermore, preventing PLK1 tyrosine dephosphorylation by depletion of EYA4 caused cells to transition from G2 to mitosis much more quickly, to have an increased mitotic duration and to have greatly enhanced levels of mitotic cell death.

Overall, our results indicate PLK1 as an EYA4 substrate and suggest mitotic cell death as a potential chemotherapeutic mechanism of targeting EYA4 tyrosine phosphatase activity.

Oral Presentation Abtracts

Understanding the telomere proteome across the cell cycle

Sam Rogers[1,2]

Co-Authors: Sonja Frolich[3], David Van Ly[1], Scott G. Page[1], and Anthony J. Cesare[1,2]

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Telomeres are the terminal nucleoprotein structures that regulate DNA damage response and repair activities at chromosome ends. All telomere functions are enabled by transient cell cycle-dependent protein interactions. However, the specific cell cycle-dependent molecular mechanisms governing telomere functions remain difficult to discern. Here we present the development of novel APEX2 proximity biotinylation tools combined with mass spectrometry, which has overcome the experimental limitations restricting analysis of the telomere proteome. Directed application of these tools has generated the first cell cycle-specific quantitative atlas of telomere interacting proteins functioning in: 1) telomere extension by the Alternative Lengthening of Telomeres (ALT) mechanism which enables cancer cell immortalisation, and 2) non-canonical mitotic telomere deprotection that triggers cell death in response to mitotic poisons (e.g. Taxol, vinblastine).

In ALT, we observe two waves of interacting factors localizing to telomeres in S- and G2-phases. Stringent statistical testing using permutation-based FDR identified 59 high-confidence cell cycle-dependent ALT regulators, 35 of which are novel. siRNA screening of these novel ALT regulators using automated high-throughput microscopy has demonstrated that depleting putative ALT regulators affects cell cycle dynamics, and APB formation (a marker of ALT activity). Additionally, we identified 12 proteins that showed statistically significant association with telomeres during non-canonical mitotic deprotection. Notably, a novel interaction between the telomere and components of TAK1, and WICH complex signalling cascade were observed.

Cumulatively, we have developed powerful proteomics tools which have generated the largest telomere interactome across the cell cycle. This study has elucidated novel potential regulators of ALT activity and non-canonical telomere deprotection that may impact cancer cell immortalisation, or improve the therapeutic efficacy of mitotic poisons.

Rapid Fire Presentation Abstracts

Therapeutic targeting of ubiquitin-specific protease 5 and unanchored polyubiquitin in MYCN-driven neuroblastoma

Ane Kleynhans[1]

Co-authors: Rituparna Mittra[1,2], Zsuzsanna Nagy[1,2], Patric Kim[1], Olivia Ciampa[1], Shizhen Zhu[5], Daniel R. Carter[1,2,3], Belamy B. Cheung[1,2], Glenn M. Marshall[1,4]

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4. Kids Cancer Centre, Sydney Children's Hospital, Sydney, Australia
5. Department of Biochemistry and Molecular Biology, Mayo Clinic

Histone deacetylase (HDAC) inhibitors are effective in preclinical models of the childhood cancer, neuroblastoma (NB). An HDAC inhibitor, suberanoyl hydroxamic acid (SAHA) has shown clinical success in the treatment of cutaneous T-cell lymphoma but lacks efficacy as a single agent in many solid tumours. We performed a SAHA combination drug screen which identified a novel small molecule, SE486-11, which synergistically enhanced the cytopathic effects of SAHA in NB cells. Combination therapy inhibited NB tumour growth in two different animal models. SILAC (Stable Isotope Labelling by Amino Acids in Cell Culture) analysis of protein from NB cells treated with combination therapy, identified significantly reduced Ubiquitin-Specific Protease 5 (USP5) expression compared to untreated controls. High USP5 expression in primary human NB samples predicted poor prognosis in patients with amplification of the MYCN oncogene, a driver oncogene in the disease. This implicates USP5 as a potential oncogenic driver in neuroblastoma.

The aim of this current project is to determine the oncogenic role of USP5 in MYCN-driven neuroblastoma. Knock-down of USP5 by specific siRNAs in MYCN-amplified neuroblastoma cell lines, led to a significant decrease in MYCN protein expression, half-life and mRNA levels. Chromatin immunoprecipitation assays demonstrated enrichment of MYCN protein near the USP5 transcription start site. Doxycycline-induced repression of MYCN expression in SHEP-tet21N correlated with decreased USP5 protein and mRNA levels. Co-immunoprecipitation assays showed that USP5 and MYCN forms a protein complex. Genetic suppression of USP5 in MYCN-amplified neuroblastoma cell lines led to a significant reduction in cell viability, cell proliferation, colony size and numbers. Taken together, our findings suggest that USP5 and MYCN participate in a forward feedback expression loop, maintaining high-level expression of both proteins, driving progression of the disease. Our data suggest that inhibition of USP5 function in neuroblastoma cells using small molecule inhibitors could be a novel therapeutic approach for MYCN-amplified tumours.

Rapid Fire Presentation Abstracts

Whole transcriptome sequencing improves actionability in children with high-risk cancers

Chelsea Mayoh[1]

Co-authors: Marie Wong[1], Amit Kumar[2], Paulette Barahona[1], Alexandra Sherstyuk[1], Emily Mould[1], Dong Anh Khuong Quang[3], Loretta Lau[1,4], Michelle Haber[1], Vanessa Tyrrell[1], Paul Ekert[1,3] and Mark Cowley[1]

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In the context of paediatric cancer precision medicine, several groups are utilising whole genome (WGS) or targeted sequencing (whole-exome (WES) or panel) and transcriptome sequencing (RNA-Seq) to identify the molecular basis for a patient's cancer. Whilst the feasibility of using WGS/WES/panel for mutation detection is well established, most groups only use RNA-Seq in clinical context for fusion detection. The Zero Childhood Cancer (ZERO) program provides a comprehensive precision medicine approach to High-Risk paediatric malignancies (survival <30%) to improve treatment outcomes.

A bioinformatics pipeline was developed to increase the utility of RNA-Seq in precision medicine through identification of driver fusions, somatic mutations and over-/under-expressed genes. We have incorporated 3 fusion callers (STAR-Fusion, JAFFA and Arriba) which identify lowly expressed fusion events and those that arise from complex structural rearrangements which were challenging to resolve in WGS. RNA-Seq mutation analysis identified 59% of driver mutations and confirmed allele specific expression in 18%, confirming the pathogenicity. This provides an orthogonal validation of WGS with RNA-Seq thus reducing the need for further clinical testing. Gene expression outlier analysis is a potential valuable resource for actionability but presents significant challenges. We have developed an in-house database utilising ZERO for identification of over-/under-expressed outlier genes for each patient. We have reported an outlier gene in 54% of patients of which 52% are in the absence of another molecular aberration.

ZERO has currently enrolled 309 patients across the full range of paediatric cancer subtypes. The RNA-seq pipeline has expanded the targeted therapeutic options and through integration with WGS at least one recommendation has been made for 74% of patients and a driver mutation identified in 92%. One example is a patient with a Frantz Tumour whom only had RHEB over-expression and within 2 months had a complete metabolic response on an mTOR inhibitor Everolimus.

Rapid Fire Presentation Abstracts

The super enhancer-driven long noncoding RNA PRKCQ-AS1 promotes neuroblastoma tumorigenesis

Sujanna Mondal[1]

Co-authors: Matthew Wong[1], Andrew Tee[1], Nicholas Ho, David S. Ziegler[1], Glenn M. Marshall[1], Pei Y. Liu[1], Marcel Dinger[2] and Tao Liu[1]

1. Children's Cancer Institute, Sydney, Australia
2. School of Biotechnology and Biomolecular Sciences, UNSW Science, UNSW Sydney, Kensington, Australia

While *MYCN*-amplified neuroblastoma has been the focus of neuroblastoma research in the past three decades, most human neuroblastomas do not harbour *MYCN* oncogene amplification, and their tumorigenic factors are unknown. The expression of critical oncogenes is driven by transcriptional super-enhancers which are controlled by the BET bromodomain protein BRD4 and the transcription factor TFIIH-associated kinase CDK7. Long noncoding RNAs (lncRNAs) play important roles in cancer.

Aims: To determine the mechanism through which the novel lncRNA PRKCQ-AS1 is up-regulated in *MYCN*-non-amplified neuroblastoma; to determine the role of PRKCQ-AS1 in neuroblastoma cell proliferation, survival and tumour progression; and to identify the mechanism through which PRKCQ-AS1 exerts oncogenic effects.

RNA sequencing analysis identified PRKCQ-AS1 as one of the transcripts most dramatically over-expressed in *MYCN* non-amplified neuroblastoma cell lines and tumour tissues, and Kaplan Meier analysis showed that high levels of PRKCQ-AS1 expression in neuroblastoma tissues correlated with poor prognosis in 476 patients. Examination of published chromatin immunoprecipitation sequencing data revealed super-enhancers at the PRKCQ-AS1 gene locus only in *MYCN* non-amplified neuroblastoma cells. RT-PCR experiments showed that transfection with two independent BRD4 siRNAs or treatment with the BRD4 inhibitor AZD5153 or the CDK7 inhibitor THZ1 considerably reduced PRKCQ-AS1 expression and cell proliferation in *MYCN* non-amplified neuroblastoma cells. Alamar blue assays, cell cycle analysis and clonogenic assays revealed that PRKCQ-AS1 knockdown with siRNAs or doxycycline-inducible shRNAs in *MYCN* non-amplified neuroblastoma cells led to growth inhibition, apoptosis and dramatic reduction in clonogenic capacity. RNA-binding protein pull-down and RNA immunoprecipitation assays identified MSI as a binding partner for the PRKCQ-AS1 lncRNA; and RNA sequencing has identified BMX as the downstream target of PRKCQ-AS1. In addition, Balb/C nude mice were xenografted with doxycycline-inducible PRKCQ-AS1 shRNA *MYCN* non-amplified neuroblastoma cells. Knocking down PRKCQ-AS1 with doxycycline in food considerably suppressed tumour progression and improved mouse survival.

The data suggest that the lncRNA PRKCQ-AS1 is upregulated by super-enhancer activity in *MYCN* non-amplified neuroblastoma cells, that PRKCQ-AS1 up-regulation induces neuroblastoma cell proliferation, survival, clonogenic capacity and tumorigenesis, and that targeting PRKCQ-AS1 expression can be an effective therapeutic strategy.

Rapid Fire Presentation Abstracts

An oncogenic role for splicing associated proteins in MYCN-driven neuroblastoma

Alice Salib[1]

Co-authors: Zsuzsanna Nagy[1,2], Chelsea Mayoh[1,2], Daniel R Carter[1,3], Belamy B Cheung[1,2], Glenn M Marshall[1,4]

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3. School of Biomedical Engineering, University of Technology Sydney, Sydney, NSW, Australia
4. Kids Cancer Centre, Sydney Children's Hospital, Sydney, NSW, Australia

Neuroblastoma is a common solid tumour in early childhood with a 50% survival rate for high-risk patients. Amplification of the MYCN oncogene remains the single most important genetic predictor of poor prognosis. Many of the pro-tumorigenic functions of MYCN are attributed to its ability to regulate global gene expression programs. Splicing is an important regulator of gene expression and we hypothesise that abnormal differential splicing may be associated with the molecular heterogeneity exhibited within neuroblastoma tumours and diverse clinical outcomes. In this study, we aim to identify the molecular mechanism through which MYCN regulates alternative splicing in neuroblastoma. Through co-immunoprecipitation experiments we identified SNRPD3, a splicing associated protein, as a novel MYCN binding protein. siRNA knockdown of SNRPD3 reduced cell viability, proliferation and colony number in MYCN-amplified neuroblastoma cell lines. Further investigation using the MYCN-inducible knockdown, SHEP.tet21N cells showed that SNRPD3 knockdown exhibited a more pronounced lethality in the presence of MYCN. Multivariate analysis of high SNRPD3 expression in a 649 neuroblastoma patient cohort revealed SNRPD3 as an independent indicator of poor prognosis. SNRPD3 mRNA expression was also significantly increased in ganglia cells from homozygous *TH-MYCN*$^{+/+}$ neuroblastoma mice when compared to ganglia cells from wild-type mice. RNA-sequencing was performed in SHEP.tet21N cells with suppression of SNPRD3 by siRNAs to identify aberrantly spliced genes and signalling pathways involved in the regulation of alternative splicing in neuroblastoma. Based on these results, we hypothesize that MYCN-driven transcriptional up-regulation and physical interaction with SNRPD3 contributes to changes in alternative splicing that perpetuate MYCN-driven oncogenesis. The selective relationship exhibited between MYCN and SNRPD3 indicates that SNRPD3 could serve as a novel therapeutic target in MYCN-amplified neuroblastoma.

Rapid Fire Presentation Abstracts

Introme: Identifying splice-altering mutations as drivers of high-risk paediatric cancers

Patricia Sullivan[1]

Co-authors: Mark Pinese[1], Velimir Gayevskiy[3], Chelsea Mayoh[1], Sarah Beecroft[4], Marie Wong-Erasmus[1], Vanessa Tyrrell[1], Emily Oates[2] & Mark Cowley[1]

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Mutations that affect pre-mRNA splicing can have a substantial impact on the resulting protein. Beside the canonical splice acceptor and splice donor regions, predicting the impact of mutations on splicing is challenging, and their identification requires specialised pipelines. ZERO currently identifies a targetable aberration in ~73% of patients; however, the analysis methods used do not consistently identify splice-altering mutations. Here we present Introme to identify potentially targetable aberrations in a large cohort of paediatric cancers. Introme uses machine learning to integrate predictions from multiple splice detection and tools (SpliceAI, MMSplice, dbscSNV and SPIDEX), thereby, evaluating the likelihood of each variant to alter splicing. We applied Introme to 110 patients, analysing a subset of known cancer genes in both the germline and tumour whole genome sequencing results. We have systematically reviewed the literature of 150 papers to identify 802 splice-altering mutations and 455 variants with no impact on splicing, all with functional support in the form of minigene/cDNA/RNA studies. A training set of 75% of the identified variants was used to optimise a machine learning classifier (AdaBag), and the remaining 25% of variants were used to measure the performance of Introme against existing tools. At a false positive rate (FPR) of 2%, Introme achieves a true positive rate (TPR) of 0.82. At the same FPR, the best performing individual tools are SpliceAI (0.74 TPR) and MMSplice (0.65 TPR). Introme was used to analyse 110 patients in the ZERO cohort, identifying 118 splice-altering mutations in known cancer genes such as PIK3CA, PIK3R1 and NF1. These mutations have been confirmed to affect splicing using matched patient RNA. The application of Introme to the ZERO cohort uncovers targetable aberrations that were missed by previous analysis methods. Detecting these splice-altering mutations aids the identification of targetable aberrations and facilitates the recommendation of personalised treatment options.

Rapid Fire Presentation Abstracts

Precision medicine for high-risk paediatric and AYA sarcomas

Emmy Fleuren[1]

Co-authors: Loretta Lau[1], Paulette Barahona[1], Alexandra Sherstyuk[1], Chelsea Mayoh[1], Dong Anh Khuong Quang[1], Marie Wong[1], Jinhan Xie[1], Daniel Batey[1], Dylan GrebertWade[1], Patrick Strong[1], Jin Yi Lim[1], Shu-Oi Chow[1], Amit Kumar[1], Tim Failes[1], Greg Arndt[1], Emily Mould[1], Michelle Haber[1], Richard Lock[1], Toby Trahair[1], Glenn Marshall[1], David Ziegler[1], Vanessa Tyrrell[1], Mark Cowley[1], Paul Ekert[1]

1. Children's Cancer Institute, Sydney, NSW, Australia.

Despite intensive multimodal treatment, survival of paediatric and adolescent and young adult (AYA) sarcoma patients remains poor. These sarcomas are extremely heterogenous, rarely harbour actionable genomic driver aberrations and no targeted therapies are routinely available. Accurate identification of clinically actionable targets and associated drug (combinations) therefore remains a critical challenge.

We aim to investigate the utility of the Zero Childhood Cancer (ZERO) precision medicine platform, incorporating molecular genomic and transcriptomic profiling with in vitro and in vivo drug testing, for pinpointing targeted therapeutic agents for young, high-risk (HR; expected survival 13 different histologies. HR sarcomas are the second largest patient group within the ZERO National Clinical Trial (PRISM) (29%; n=71). Using WGS (tumour, germline DNA) and RNASeq analysis, comprehensive molecular profiles have been established for 56 sarcoma patients comprising >13 different histologies. Reportable somatic SNVs, fusions, CNVs and aberrantly expressed genes (RNA) were identified in 45%, 64%, 45% and 64% of patients, respectively. These molecular profiles, supplemented with functional drug profiles when available, resulted in a targeted therapy recommendation for 71% (n=40) of sarcoma patients. Recommendations most commonly included PI3K/mTOR, receptor tyrosine kinase (RTK), MEK, CDK4/6 and PARP inhibitors, mostly in rationally selected combinations. Excitingly, for 13 sarcoma patients where treatment recommendations were acted upon, partial responses (PR; n=3) and stable disease (SD, n=1) are reported using abovementioned classes of inhibitors. Additional follow-up is ongoing and 7 (54%) of these HR patients are still alive today.

This study is one of the most comprehensive clinical efforts ever undertaken in precision medicine in advanced paediatric and AYA sarcoma. It not only provides a unique resource to study biology and therapeutic opportunities in a diverse set of HR sarcomas; our interim results also highlight the feasibility and clinical utility of precision medicine in HR sarcomas.

Rapid Fire Presentation Abstracts

Telomere length measurement by DNA fibre analysis

Vivian Kahl[1]

Co-authors: Joshua A. M. Allen[1], Christopher B. Nelson[1], Hilda A. Pickett[1]

1. Telomere Length Regulation Unit, Children's Medical Research Institute (CMRI), Westmead NSW, Australia

Telomeres are DNA and protein structures that cap the ends of human chromosomes and maintain genome stability. Telomere length (TL) has been considered an indicator of cellular health in both clinical and research settings. However, current techniques lack sufficient sensitivity to provide the distribution of TLs and the specific length of individual telomeres in a cell population. This is particularly problematic as it is the proportion of short telomeres, rather than average TL, that triggers cellular senescence.

We have developed a novel technique called telomere fibre-FISH (TFF) that involves applying DNA fibres using a constant stretching factor of 2 kb/μm onto silanised microscope slides and using a telomere-specific fluorescently-labelled probe to measure individual TLs by fluorescence microscopy. We have validated TFF against qPCR, TRF, flow-FISH, and metaphase Q-FISH, the four main TL measurement tests currently in use worldwide. Assays were performed on two telomerase-positive cell lines, HeLa and HT1080, and two ALT cell lines, U-2 OS and IIICF/c.

TL correlates across the methods (R^2 = 0.80 – 1.00), but TFF provides higher resolution imaging and more detailed information regarding the distribution of individual TLs and the absolute TL. We then developed a pipeline using CellProfiler, an open-source image analysis software, for automated telomere fibre analysis. Preliminary data show that automation correlates with manual scoring of telomere fibre lengths for U-2 OS (R^2= 0.89) and HeLa (R^2= 0.83) cell lines.

Beyond its specific clinical application for the diagnosis of telomere biology disorders, TL testing is emerging as a more general prognostic marker for ageing and disease. Therefore, accurate and appropriate TL measurement techniques are an important requirement for ageing and disease management. TFF provides the means to measure individual TLs at a level of detail not previously possible.

Rapid Fire Presentation Abstracts

ALYREF is a novel therapeutic target and co-factor for MYCN-driven oncogenesis

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Although gain of distal 17q21-ter is the most powerful genetic predictor of adverse outcome for children with neuroblastoma (NB), thus far, no therapeutic target genes have been reported for that region.

We investigated the 17q21-ter amplicon and identified ALYREF as a key regulator of aggressiveness in NB. ALYREF was ranked as the top 17q21-ter gene predicting poor patient prognosis. High ALYREF expression correlated with poor overall survival in a large cohort of primary NB patient tumour samples (n = 498, SEQC dataset). Multivariate analysis showed that high ALYREF expression was independently prognostic compared with other clinical and laboratory features in NB. MYCN is a major driver of tumourigenesis in NB, and high ALYREF expression correlated with MYCN expression. Microarray analysis carried out on the ganglia tissues of homozygote TH-MYCN transgenic NB mice showed that ALYREF was significantly up-regulated at MYCN-driven tumour initiation. Mechanistic experiments revealed that MYCN, is functionally dependent on ALYREF and the two proteins act in a positive forward feedback expression loop to promote NB progression. ALYREF exerts its effect on NB progression by directly binding to the region between MB Box III and IV of MYCN in the nucleus. Stable genetic suppression of ALYREF expression reduced cell viability and colony formation of MYCN-amplified NB cell lines. Most importantly, ALYREF knockdown in MYCN amplified NB cells resulted in a significant inhibition of tumorigenesis in vivo. Transient overexpression of ALYREF in non-malignant normal fibroblast cells had no effect on cell viability. Genome-wide profile of ALYREF-chromatin interactions using ChIP sequencing revealed that ALYREF binds DNA near the ubiquitin-specific protease 3 (USP3) gene and increases USP3 expression.

Our findings suggest a crucial role for ALYREF in high risk NB through effects on MYCN ubiquitination, and suggest that chemical disruption of MYCN-ALYREF binding in MYCN amplified NB cells may have potent therapeutic effects.

Rapid Fire Presentation Abstracts

Efficacy of arginine depletion by BCT-100 in mouse models of neuroblastoma

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Neuroblastoma is the most common extracranial solid tumour of children and accounts for 15% of childhood cancer deaths. Although some progress has been made in treating the disease, fewer than 50% of children diagnosed with high risk disease will survive and this drops to as low as 10% for relapsed and refractory disease. Pegylated arginase (BCT-100) has recently been shown to significantly delay tumour development and prolong survival of neuroblastoma-prone *Th-MYCN* mice (Fultang et al., 2019). This study investigated the effects of arginine depletion therapy as single agent and in combination with chemotherapy in neuroblastoma.

We used the transgenic (*Th-MYCN*) neuroblastoma model to assess arginine depletion therapy using BCT-100 as a single agent delivered 2x/week or 4x/week in a prophylaxis setting, prior to overt tumour development. Our results showed that more frequent dosing of BCT-100 (4x/week) resulted in a significant delay in tumour development and dramatically increased the survival of the mice (p-value: 0.0001). Furthermore, targeted metabolite profiling of the plasma samples taken from these mice showed that plasma arginine levels were significantly depleted in the mice treated with BCT-100 2x/week (p-value: 0.017) and 4x/week (p-value: 0.0018) when compared with vehicle treated control mice. We further assessed the efficacy of BCT-100 as a single agent, following the establishment of a small palpable tumour (5mm), and also in combination with the chemotherapy backbone, Irinotecan/Temozolomide. Our results showed that there was a significant increase in the survival of mice treated with 4x/week BCT-100 (p-value: < 0.0001) when compared with vehicle treated mice. This survival benefit was significantly improved with the addition of the Irinotecan/Temozolomide (p-value: 0.0001).

These data show that arginine depletion therapy using BCT-100 combined with chemotherapy represents a potentially exciting new approach of treating high-risk neuroblastoma.

Rapid Fire Presentation Abstracts

Novel chimeric antigen receptor (CAR) T-cell therapies for cancer

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Adoptive cell therapy provides potentially curative treatments for leukemia and lymphomas. Patients' own T-cells are genetically modified to express anti-tumour chimeric antigen receptors (CARs). Clinical trials of CD19-specific CAR (CAR19) T-cells have demonstrated remarkable efficacy against relapsed and refractory B-cell malignancies, particularly in paediatric cohorts. Currently, CAR T-cells are manufactured using viral vectors to deliver CAR to patients' T-cells. Transposon-based systems offer simpler and more economical methods of generating CAR T-cells compared to virally manufactured CAR T-cells.

This study aims to validate the efficacy of a novel CAR T-cell therapy generated using a PiggyBat transposon system derived from brown bat genome.

CAR19 T-cells were generated from healthy donors by co-electroporation of two plasmids encoding the transposase and a CAR19 transposon plasmids. T-cells were co-cultured with irradiated feeder cells and cytokines. CAR19 T-cells were tested using patient-derived xenograft (PDX) mouse models of chemorefractory paediatric B-cell lymphoblastic leukemia (B-ALL). Leukemic cell growth and CAR19 T-cell expression were monitored using multiparameter flow cytometry. T-cells transduced with CARs using PiggyBat transposons exhibited strong anti-tumour activity and significantly improved event-free survival. While all untreated mice developed leukaemia within 4-8 weeks, CAR19 T-cell treated mice demonstrated robust complete response and remained in remission for over 11 weeks. PiggyBat CAR19T-cells completely eradicated leukaemia blasts from bone marrow and spleen. Infused T-cells rapidly expanded and peaked at 6-7 weeks post-infusion before gradual decline. Small numbers of effector memory T-cells remained detectable at week 11 in peripheral blood, bone marrow, and spleen of mice in remission. PiggyBat CAR19 T-cells were as effective as PiggyBac CAR19 T-cells in inducing long-term remission in the B-ALL PDX mouse model.

The high anti-tumour efficacy shown in our preclinical studies provides a strong rationale for potential clinical translation of PiggyBat CAR19 T-cells for B-cell leukemias and lymphomas.