Embracing Research and Impact

Birmingham | 24-25 November 2025

CHILDREN WITH CANCER UK

Embracing Research and Impact

Birmingham | 24-25 November 2025

CHILDREN WITH CANCER UK

Welcome to the Children with Cancer UK Research Conference 2025

As Chair of the Scientific Advisory Panel, I am delighted to introduce this year's Book of Abstracts, which showcases the breadth and depth of research dedicated to improving outcomes for children and young people with cancer.

These abstracts reflect the passion, innovation, and collaborative spirit that drive our community forward.

I hope they inspire meaningful dialogue, spark new ideas, and reinforce our shared commitment to transforming the future of paediatric oncology.

Chris

Chris Halsey
Chair of SAP

Agenda Day 1

Children with Cancer UK Research Conference 2025
Dates: 24 – 25 November 2025
Location: Edgbaston Park Hotel & Conference Centre – Birmingham

Bridging the Gap – Making Research Real

10:00 – 10:05 | Welcome
Speaker: Nick Vaughan-Williams (Provost and Vice-Principal, University of Birmingham)

10:05 – 10:10 | Children with Cancer UK
Speaker: Gavin Maggs (CEO, Children with Cancer UK)

10:10 – 10:20 | Welcome & Vision
Speaker: Professor Bruce Morland and Professor Pamela Kearns, University of Birmingham

10:20 – 11:00 | SMPaeds Update
Speaker: Dr Sally George (Group Leader: Developmental Oncology, Institute of Cancer Research)

11:00 – 11:30 | Refreshment Break

11:30 – 12:50 | Popcorn Research: Lightning Talks
Short talks by early-career researchers

12:50 – 14:00 | Lunch

14:00 – 14:30 | Industry Spotlight: The Future of Childhood Cancer Research
Speaker: Dr Frank Mussai (University of Birmingham)

14:30 – 15:15 | International Benchmarking of Childhood Cancer Survival by Stage (BENCHISTA Project) – Clinical and Family Perspectives
Speaker: Emeritus Professor Kathy Pritchard-Jones (UCL) & Dr Angela Polanco (NIHR / PPIE Lead for BENCHISTA Project)

15:15 – 15:40 | Refreshment break

15:40 – 16:00 | Smoothing the Edges: Early phase clinical trial participation – a Research Nurses perspective
Speaker: Tracey Crowe (Lead Nurse for Paediatric Oncology Research, Royal Marsden Hospital)

16:00 – 16:30 | Transforming Potential into Progress: What Fellowship Support Makes Possible
Speaker: Professor Zoë Walters (Professor in Translational Epigenomics, University of Southampton)

16:30 – 16:50 | SAP Update + Open Forum
Speaker: Scientific Advisory Panel

16:50 | Closing Remarks

Evening Activities

17:00 – 18:00 | Drinks Reception and Poster Exhibition

19:00 – Late | Evening Dinner and Entertainment

Agenda Day 2

Collaboration, Connection, and Real-World Change

09:30 – 09:40 | Welcome Back
Speaker: Professor Bruce Morland

09:40 – 10:25 | INSTINCT-MB: Discovery, development, and delivery of effective combination therapies for high risk medulloblastoma
Speaker: Professor Steven Clifford (Chair of Molecular Paediatric Oncology & Director, Newcastle University Centre for Cancer)

10:25 – 10:55 | Projects for All: Research Showcase – Bespoke preclinical models to improve outcomes for Ewing sarcoma patients
Speaker: Dr Elizabeth Roundhill (Research Fellow, University of Leeds)

10:55 – 11:30 | Refreshment break

11:30 – 12:30 | Behind Every Grant: Our Partners
Topic: Acknowledging supporters and collaborators.
Cancer Research UK – C-Further, Tommy Rennison
Tessa Jowell Brain Cancer Mission, Nicky Huskens
Azaylia Foundation, Dominik Byrne
CCLG: The Children & Young People's Cancer Association, Ashley Ball-Gamble

12:30 – 13:00 | Projects for All: Research Showcase – Developing novel immunotherapies for paediatric T-cell acute lymphoblastic leukaemia
Speaker: Professor Marc Mansour (Clinical Professor of Paediatric Haemato-Oncology and Honorary Consultant, UCL)

13:00 – 14:00 | Lunch & Posters

14:00 – 14:30 | Research Update – CSF microRNAs for the diagnosis of CNS germ cell tumours.
Speaker: Professor Matthew Murray (Professor in Cellular and Molecular Pathology, University of Cambridge)

14:30 – 15:15 | Research Update – The unsolved challenge of CNS leukaemia
Speaker: Professor Chris Halsey (Head of School / Professor of Paediatric Haemato-Oncology, University of Glasgow)

15:15 – 15:45 | Projects for All: Research Showcase – The Paediatric Hepatic International Tumour Trial – PHITT
Speaker: Sarah Pirrie (Principal Biostatistician, Cancer Research UK Clinical Trials Unit, University of Birmingham)

15:45 – 16:00 | Closing Reflections
Speaker: SAP Members

The Scientific Advisory Panel

Professor Christina Halsey

Professor of Paediatric Haemato-Oncology & Head of School of Cancer Sciences, University of Glasgow, and Honorary Consultant Paediatric Haematologist, Royal Hospital for Children, Glasgow.

Chris is a clinical academic paediatric haematologist and Head of the School of Cancer Sciences in Glasgow where she leads >400 staff and postgraduate students in their mission to carry out patient centred science to transform cancer outcomes.

Chris also leads her own highly translational research programme – supported by a Cancer Research UK Programme Foundation Award. Her particular research focus is on central nervous system (CNS) involvement in children and young people with acute leukaemia. Current treatments for CNS leukaemia are toxic and there are a lack of novel therapies in this area. This represents a major unmet clinical need.

Chris and her lab are using advanced technologies and sophisticated laboratory models to gain a deeper scientific understanding of how leukaemia cells survive in the CNS. They hope to apply this knowledge to improve treatment by designing better diagnostics, new drugs and immunotherapies, and ways to reduce neurotoxic side-effects. She also leads several international research efforts to improve our scientific understanding of childhood cancers and to provide evidence-based clinical guidelines. Chris is particualrly intersted in supporting team science initiatives, early-career researchers and patient and public engagement in research.

Dr Olga Slater

Dr Slater has been Consultant Paediatric Oncologist at Great Ormond Street Hospital in London since 2010. Dr Slater works full time and has an extensive patient cohort. Therefore she works only at Great Ormond Street in her NHS and private patient capacity.

Dr Slater is the Lead for Sarcoma Services and Clinical Lead for Langerhans Cell Histiocytosis (LCH). Apart from the clinical interest, Dr Slater is also the educational and clinical supervisor for clinical fellows/ junior doctors providing supervision and training of the new paediatric workforce.

Dr Slater has clinical and academic research interest especially in the area of sarcoma and Langerhans Cell Histiocytosis.

The Scientific Advisory Panel

Professor Sue Burchill

Professor Sue Burchill graduated with a BSc in Pharmacology (1982) and PhD in Medicine (1986) from the Sunderland School of Pharmacy and the University of Newcastle upon Tyne respectively, before pursuing a translational research career. After post-doctoral research in the University of Newcastle upon Tyne, England and The University of Arizona Cancer Centre, USA she moved to Leeds in 1992 to establish the Children's Cancer Research Group at St James's University Hospital (SJUH) in Leeds, where she remains the Scientific Director. Sue is also the CHORAL Director of translational laboratory research for young people and Deputy Head of Molecular Medicine in the Leeds Institute of Medical Research, SJUH.
Sue has made major contributions to medical research in the field of cancer in children and young people, her leadership in multi-disciplinary collaborative groups includes shaping international strategies for the evaluation of novel circulating biomarkers, identification and advancement of targeted treatments to improve cure rates and minimise treatment associated morbidities.

Her own research is focussed on strategies to detect and eradicate the metastatic drug-resistant disease that is responsible for progression and relapse in neuroblastoma and bone cancers that affect young people.

Professor Petra Hamerlik

Petra Hamerlik is an accomplished researcher in primary pharmacology and brain cancer research. Her professional career has been a blend of academic and industry roles, including positions at the Danish Cancer Society, Cleveland Clinic, and AstraZeneca. In September 2022, Petra was recruited to the University of Manchester as the Inaugural Brain Tumour Charity Chair of Translational Neuro-Oncology. Her current work is focused on identifying novel treatments and early detection of intracranial tumours in children and adults.

The Scientific Advisory Panel

Professor Matthew Murray

Matthew Murray is a University Professor and Honorary Consultant Paediatric Oncologist, working at both the Department of Pathology, Cambridge University and Department of Paediatric Haematology and Oncology, Cambridge University Hospitals NHS Foundation Trust, United Kingdom. His research programme studies the clinical and molecular aspects of childhood cancers, particularly germ cell tumours (GCTs), which includes testis cancer, one of the commonest malignancies affecting young men. He was the first to demonstrate the utility of specific circulating microRNAs for diagnosis, disease-monitoring, and early relapse detection in this disease. He has published >140 manuscripts to date and has attracted >£6.5M of grant funding to date as a Principal and Co-Investigator. Professor Murray has key national and international leadership roles in cancer biology, including:

- 'MaGIC: Malignant GCT International Collaborative' (https://magicconsortium.com/), a UK-US led initiative developing international trials of extracranial GCTs in children, adolescents & young adults, including embedded biological aims; Member 2009-. Biology Co-Lead and Executive Committee Member; 2011-2023
- International Society of Paediatric Oncology (SIOP-Europe) Brain Tumour Group – GCT Subgroup; Member 2009-; Chair 2017-2022
- Children's Cancer and Leukaemia Clinical Studies Group, GCT Subgroup; 2010-
- 100,000 Genomes Project (GeCIP) Testis Cancer Domain Co-Lead; 2015-

Declaration of Interests: Nil

Professor Julie Irving

Professor Julie Irving is a scientist at Newcastle University with more than thirty years of experience in the field of leukaemia and is a member of national and international childhood leukaemia groups and steering committees. Her team focuses on childhood acute lymphoblastic leukaemia and they have successfully translated their experimental findings into the clinic for the benefit of children.

The Scientific Advisory Panel

Professor Zoë Walters

Professor Zoë Walters is Professor of Translational Epigenomics in the School of Cancer Sciences at the University of Southampton, where she co-leads the Innovation for Translation Research Group (ITRG) with Professor Tim Underwood. The ITRG is committed to improving the outcomes of patients with solid tumours of unmet clinical need by delivering better, less harmful and more precise treatments, via a combination of clinical and scientific excellence. Central to this mission is the Strategic Oversight Team (SOT), a group comprising patients, carers and relatives of patients, and a high-performance coach, who work closely with the ITRG at all stages of research to ensure that patient experience, priorities and perspectives remain at the heart of the programme.

Prof Walters' research focuses on the genomics and epigenomic regulation of paediatric and adult solid tumours, with a particular emphasis on paediatric and adult soft tissue sarcomas, and oesophageal adenocarcinomas. She leads multiple national and international collaborations that integrate molecular biology, tumour microenvironment analysis, and machine learning to identify new therapeutic vulnerabilities and improve clinical decision-making. Her programme of research spans epigenetic therapies, immunotherapy development, spatial biology, and computational modelling of high-risk cancers, and has contributed to the development of preclinical strategies now informing translational research and clinical trial design.

Alongside her research, Prof Walters plays an active leadership role within the University of Southampton as Deputy Head of School (Education) and serves on numerous strategic committees. She also contributes widely to the cancer research community through her service on grant

Dr Madeleine Adams

Madeleine Adams is a Consultant Paediatric Oncologist at the Children's Hospital for Wales in Cardiff. She has a clinical interest in sarcoma and paediatric cancer predisposition and a research interest in quality of life and patient reported outcomes.

The Scientific Advisory Panel

Dr Lynley Marshall

Dr Lynley Marshall completed her undergraduate medical training in Johannesburg, South Africa. She specialised in paediatrics and then paediatric oncology in the UK, training in the Southwest, Bristol, Oxford and The Royal Marsden. She undertook her PhD at The Institute of Cancer Research, University of London in the area of novel targeted therapeutics for paediatric high-grade glioma.

Dr Marshall is Paediatric Clinical Research Lead at The Royal Marsden Hospital (RMH) and The Institute of Cancer Research (ICR), London, co-leading the Children and Young People's Cancer Centre with Prof. Chris Jones. She leads the Paediatric and Adolescent Oncology Drug Development Team, focusing on cancer drug development and early phase clinical trials of experimental therapeutics for high-risk, poor- prognosis malignancies, specifically solid tumours and neuro-oncology.

Dr Marshall chaired the UK's NCRI Children's Group Novel Agents Subgroup for 6 years. She is Deputy Lead for the UK's Paediatric Experimental Cancer Medicines Centre (ECMC) Network and Clinical Trials Theme Co-Lead. She has served on the Clinical Trials Committee of the Innovative Therapies for Children with Cancer (ITCC) European Early Phase Clinical Trials Consortium since 2015, on the ITCC Solid Tumour Steering Committee since 2021, and is a member of the Scientific Steering Committee and Fostering Age Inclusive Research (FAIR Trials) Working Group of the SIOPE-ITCC ACCELERATE multi-stakeholder platform committed to accelerating new drug development for children and adolescents with cancer via collaborative partnership between academia, clinicians, patient advocates, industry and regulators.

Dr Marshall is UK co-ordinating investigator on a number of academic and commercial paediatric/TYA early phase clinical trials, including the E-SMART European platform trial. She is paediatric/TYA lead on the CRUK-sponsored DETERMINE tumour and age-agnostic clinical trial.
She is passionate about training and mentoring early career researchers and helping them develop their own careers in the field of early phase clinical trials.

The Scientific Advisory Panel

Dr Vincenzo D'Angiolella

Vincenzo D'Angiolella is Charles and Ethel Barr Chair of Cancer Research at The Institute for Genetics and Cancer within the University of Edinburgh. He previously worked as Professor of Molecular Oncology at the University of Oxford where he served as director of the Master Course in Radiation Oncology within the Department of Oncology. His research group focuses on understanding the role of the ubiquitin system in cancer pathogenesis and response to treatment with chemo- and radiotherapy. He is currently holding two programme grants from the Cancer Research UK (CRUK) and Medical Research Council (MRC), focused on the role of Cullin Ring ubiquitin Ligases (CRLs) in brain cancers (medulloblastoma and glioblastoma) pathogenesis. Studies from his laboratory have uncovered the mechanism of action and function of Fbxl17 in medulloblastoma (Raducu et al., 2016) and highlighted a novel synthetic lethal interaction between cyclin F loss and Chk1 kinase inhibition (Burdova et al., 2019). More recently, the laboratory has identified that mutations occurring on the E3 ligase KBTBD4 in medulloblastoma change substrate specificity driving the recognition of the chromatin remodelling complex CoREST (Chen et al., 2022). This work describes the first neomorphic mutation in an E3 ubiquitin ligase and elucidates a novel mechanism of tumorigenesis in medulloblastoma. In addition, research from his group uncovered novel targets in glioblastoma through CRISPR screen, elucidating an axis required for DNA repair in mitosis (Yang et al., 2024).

Vincenzo has a Medical Degree (MD) from the University of Naples "Federico II" and completed his PhD at the same University in the field of General Pathology. During his PhD utilising X. Laevis as a model system, he investigated the process of mitosis and spindle checkpoint (D'Angiolella et al., 2001; D'Angiolella et al., 2003; D'Angiolella et al., 2007)

Following the completion of his studies, he worked as a postdoctoral fellow at the New York University School of Medicine in the USA in the laboratory of Professor Michele Pagano. During the postdoctoral studies, he elucidated the mechanism-of-action and function of cyclin F, the founding member of the F-box proteins. He uncovered that cyclin F does not partner with Cyclin Dependent Kinases (CDKs) like other cyclins do, but acts as an E3 ubiquitin ligase, forming a canonical CRL1 (D'Angiolella et al., 2012; D'Angiolella et al., 2010).

Embracing Research and Impact

Birmingham | 24-25 November 2025

CHILDREN WITH CANCER UK

Oral abstracts

Overcoming Inotuzumab Ozogamicin resistance in B-ALL by targeting the tumour microenvironment with the novel amino acid degrader Cysteinase

Authors and Affiliations
Samantha Atkinson
Imperial College London

Scientific Abstract
The CD22-targeted antibody drug conjugate Inotuzumab Ozogamicin (IO) induces remission in ~80% of patients with relapsed/refractory B-cell Acute Lymphoblastic Leukaemia (B-ALL). However, IO rarely leads to long-term remissions, despite deep molecular responses in most patients. Interestingly, CD22 expression correlates poorly with IO-response and CD22-negative relapse is uncommon. The bone marrow microenvironment, including mesenchymal stromal cells (MSC), are increasingly recognised as important in treatment resistance in B-ALL. Unpublished work from our lab suggests IO-resistant B-ALL cells, reside in a niche with MSC.

We sought to characterise the transcriptional and metabolic profile of IO-resistant B-ALL cells to identify targetable vulnerabilities. We used in vitro and in vivo models of B-ALL to characterise IO-resistant cells with flow cytometry based metabolic assays, bulk RNASeq and targeted and untargeted metabolomics by mass spectrometry.

We used an in vitro niche model co-culturing 5 B-ALL cell lines and 6 patient derived xenografts (PDXs) with MSC cell lines/primary MSCs compared to B-ALL cells in mono-culture to demonstrate that B-ALL cells are protected by MSCs from IO-induced death. We predicted protection from oxidative stress and mitochondrial transfer may be important given our previous findings with DNA-damaging chemotherapy (Burt, 2019) and IOs reliance on oxidising the anti-oxidant glutathione (GSH) to induce DNA damage. Using a CellROX™ dye we confirmed that IO increases reactive oxygen species (ROS) in B-ALL cells in mono-culture and this effect is abrogated when B-ALL cells are co-cultured with MSCs.

Using a Mitotracker© dye transfer assay we also confirmed that MSC transfer mitochondria in a dose responsive manner after exposure to IO. These results were validated with a mixed species model where murine mitochondrial DNA was detectable by PCR in sorted human B-ALL cells after co-culture with the murine MSC cell line MS5.

To investigate MSC-mediated reduction in ROS in B-ALL cells, we performed targeted metabolomics of B-ALL cells and media in mono-culture versus co-culture with MSCs. The most striking difference was a 2-4 fold increase of cysteine in the co-culture media, the rate limiting amino acid for GSH production. Subsequent heavy carbon-labelling studies confirmed MSC were secreting cyst(e)ine, which B-ALL cells took up to produce GSH.

The novel amino acid degrader Cysteinase overcame MSC-mediated protection of B-ALL cell lines and PDXs in vitro by degrading cyst(e) ine in the media and inducing oxidative stress and ferroptosis in the B-ALL cells. IO synergised with Cysteinase in B-ALL killing in the niche model.

In a high-risk KMT2A-AF4 SEM human xenograft murine model of B-ALL, Cysteinase significantly reduced cystine in the peripheral blood and bone marrow serum, leading to a significant reduction in B-ALL disease burden and improvement in survival of the mice. Ongoing work is exploring the combination of IO and Cysteinase in the KMT2A-AF4 human xenograft model and high-risk PDX models.

In summary, we propose a model where surviving B-ALL cells after IO are ‘rescued’ from oxidative stress by MSC secretion of cyst(e)ine and transfer of mitochondria. This protection can be overcome by Cysteinase and our preliminary results suggest IO and Cysteinase may be a promising therapeutic combination.

Enhancing Rhabdomyosarcoma immunotherapy: targeting cytokine-induced immune evasion pathways

Authors and Affiliations
Alex Look[1], Rosa Gomes Alves Martins[1], Christina Putnam[1], Edd James[1], Timothy J Underwood[1,2], Ali Roghanian[2,3], Zoë S Walters[1,2]

1 School of Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
2 Institute for Life Science, University of Southampton, Southampton, UK
3 Antibody and Vaccine Group, Centre for Cancer Immunology, School of Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

Scientific Abstract
Rhabdomyosarcoma (RMS) is the most common paediatric soft tissue sarcoma and is characterised by a marked capacity to evade immune surveillance, even in the presence of infiltrating immune effector cells within the tumour microenvironment (TME). Understanding how RMS responds to inflammatory signals is therefore essential for developing effective immunotherapeutic strategies. Interferon-gamma (IFN-γ), secreted primarily by activated T cells and NK cells, is a key mediator of antitumour immunity. It enhances antigen presentation and cytotoxic immune responses yet paradoxically can also induce immune-evasive mechanisms in tumour cells through activation of regulatory signalling pathways, most notably the JAK-STAT axis.
To investigate how RMS responds to inflammatory cytokines, we employed functional in vitro assays, including CyQUANT proliferation assays and multiparametric flow cytometry to evaluate surface marker expression. We studied both the fusion-negative RMS subtype (RD) and the more aggressive fusion-positive subtype (Rh30). IFN-γ exposure was directly cytotoxic to both RD and Rh30 cells, leading to reduced proliferation and viability. However, this effect was accompanied by a significant upregulation of the inhibitory ligands PD-L1 and HLA-E on the tumour cell surface. This upregulation occurred both after direct cytokine stimulation and in the context of $CD3^+$ T cell co-culture, highlighting the dual role of IFN-γ in driving both tumour clearance and immune escape. Fusion-positive Rh30 cells consistently displayed higher basal and inducible levels of these ligands compared with RD. Beyond IFN-γ, we explored the role of tumour necrosis factor-alpha (TNF-α), another cytokine abundant within inflamed TMEs. TNF-α robustly upregulated the expression of PD-L1, HLA-E, and HLA-ABC. The latter is of particular interest, as HLA class I upregulation could increase recognition by $CD8^+$ T cells but simultaneously enhance inhibitory interactions with NK cells. Importantly, the convergence of TNF-α and IFN-γ underscores the complexity of cytokine signalling in RMS, where proinflammatory cues intended to strengthen antitumour immunity can simultaneously establish potent immunosuppressive barriers.

Taken together, our data highlights a central paradox: cytokines such as IFN-γ and TNF-α, while critical for effective immune attack, also prime RMS cells to resist elimination by increasing inhibitory ligand expression. These insights provide a strong rationale for combinatorial therapeutic strategies in RMS. Specifically, integrating cellular immunotherapies (e.g. CAR-based approaches) with checkpoint blockade targeting PD-L1 and HLA-E may overcome these barriers and enhance therapeutic efficacy.

Retinal Organoid Screening Reveals ABT-737 and Luminespib as Selective Agents Against Tumorigenic Cone Precursors in Retinoblastoma

Authors and Affiliations
Joe Kelk
Newcastle University

Scientific Abstract
Retinoblastoma (Rb) is a rare paediatric cancer of the developing retina, primarily caused by biallelic inactivation of the RB1 tumour suppressor gene located on chromosome 13q14, or by MYCN amplification in a susceptible retinal cell type 1. The incidence of Rb is approximately 1 in 15,000 live births. It accounts for 7–17% of all tumours diagnosed during infancy and 4–6% of all cancers in children under the age of 15 2–4. Globally, an estimated 8,600 to 9,000 children are affected each year, with around 40–50 new cases diagnosed annually in the UK alone 5. Retinoblastoma (Rb) is primarily driven by proliferating cone precursors, particularly those in the G2/M phase, as revealed by recent single-cell transcriptomic studies. In the current study, we utilized patient $RB1^{-/-}$ retinal organoids to evaluate the cytotoxic efficacy and selectivity of candidate therapeutics targeting these tumour-initiating cells.

A primary screen of 37 compounds identified 11 with significant cytotoxicity against Rb retinal organoids, which was subsequently refined to 6 candidates exhibiting selective activity against proliferating cone precursors. Among these, ABT-737 and luminespib emerged as lead compounds, demonstrating dose-dependent depletion of $Ki\text{-}67^{+}/RXR\gamma^{+}$ cone cells and strong apoptotic induction, evidenced by Caspase-3 activation and Annexin V/7-AAD flow cytometry, without inducing necrosis. Single-cell RNA sequencing confirmed that both agents selectively targeted the Rb-like proliferating cone precursors. Ocular pharmacokinetic modelling using pluripotent stem cell-derived RPE monolayers revealed limited permeability across the outer blood-retinal barrier for both drugs; however, luminespib showed moderate translocation, likely due to its lower molecular weight. In contrast, the uptake of ABT-737 by the retinal organoids was more efficient than that of luminespib.

Together, these findings highlight ABT-737 and luminespib as promising, selective therapeutic candidates for Rb and demonstrate the utility of integrated retinal organoid and RPE models for preclinical drug screening and pharmacokinetic evaluation.

References

1. Dyer, M. A. Lessons from Retinoblastoma: Implications for Cancer, Development, Evolution, and Regenerative Medicine. Trends Mol Med 22, 863 (2016).
2. Kivelä, T. T. & Hadjistilianou, T. Neonatal retinoblastoma. Asia Pac J Oncol Nurs 4, 197–204 (2017).
3. Yang, C. P., Hung, I. J., Jaing, T. H., Shih, L. Y. & Chang, W. H. CANCER IN INFANTS: A Review of 82 Cases. Pediatr Hematol Oncol 22, 463–481 (2005).
4. Rabinowicz, R. et al. Cancer incidence and survival among infants in Israel, 1998-2007. Pediatr Hematol Oncol 30, 646–654 (2013).
5. MacCarthy, A. et al. Retinoblastoma: Treatment and survival in Great Britain 1963 to 2002. British Journal of Ophthalmology 93, 38–39 (2009).

Integrative small molecule drug screening, chemoinformatics and transcriptomics identify targetable vulnerabilities in MYC-amplified medulloblastoma

Authors and Affiliations
Louisa Taylor[1], Gemma Llargués-Sistac[1], Dean Thompson[1], Janice Law[1], Janet Lindsey[1], Connie McCoy[1], Ibrahim Kurdieh[1], Marie Claire Buttigieg[1], Alaide Marcavallo[2], Colin Kwok[2], Bethany Poole[1], Harvey Che[2], Laura K. Donovan[3], Rebecca M Hill[1], Louis Chesler[2], John Anderson[3] and Steven C Clifford[1]

1 Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Translational and Clinical Research Institute, Newcastle University, Newcastle-upon-Tyne, UK
2 Division of Clinical Studies, The Institute of Cancer Research, Sutton SM2 5NG, UK
3 UCL Institute of Child Health, London, UK

Scientific Abstract
MYC-amplified Group 3 medulloblastomas (MYC-MB$_{Group3}$) do not respond to conventional up-front therapies and represent one of the worst prognosis childhood brain tumours (<5% survival). MYC is not directly targetable using small molecule inhibitors. Thus, the systematic development of therapies which target complementary MYC-dependencies offers clear potential to rationally re-design effective therapeutic strategies.

We derived three MYC-regulable avatars from cellular models of MYC-MB$_{Group3}$ (D425; D283; HD-MB03). Transcriptomic analysis, alongside our primary tumour cohort (n=321), identified a common MYC-dependent signature enriched for DNA damage response (DDR) and proliferative processes. Parallel high-throughput drug screens (>500 compounds) revealed differential MYC-dependent sensitivities to small molecule inhibitors (80 molecules spanning 25 drug classes), consistent across our MYC-regulable avatars. Bespoke chemoinformatics of screening data, integrated with MYC-dependent transcriptomics, identified proceedable targets, which included the DDR-signalling proteins, CHEK1 and ATR. Lead compounds were advanced to medium-throughput combination drug screens in MYC-MBGroup3 cell lines, alongside standard-of-care agents (n=105). Validation of synergy and efficacy in advanced cell models, alongside toxicity counter-screens, identified five specific and effective drug combinations for progression to in vivo trials. In-line with transcriptomic analysis, all prioritised combinations included a DDR inhibitor. Extensive in vitro validation confirmed dysregulation of DDR pathways (elevated expression of CHEK1 and ATR, increased R-loop formation and γH2AX staining), representing a novel and targetable hallmark of MYC-MB$_{Group3}$. In vivo pharmacokinetic/pharmacodynamic trials and artificial intelligence-led toxicity modelling identified best-in class DNA damage repair inhibitors that are selective, MYC-dependent and central nervous system penetrant. On-going pre-clinical trials are now underway to assess top DNA damage repair inhibitor drug-drug combinations across in vivo MYC-MB$_{Group3}$ model systems (Myc-DNp53 allografts and patient derived xenografts).

In summary, we have utilised integrated human tumour and model-based screening approaches to identify and validate critical MYC co-dependencies, targetable using established small-molecules, to inhibit MYC-MB$_{Group3}$ tumour growth. Our findings reveal that inhibition of the DNA damage response, specifically via inhibition of CHEK1 and ATR, in combination with standard chemotherapeutics and/or other targeted agents is highly effective and warrants further clinical investigation. Importantly, we demonstrate the development of an effective pre-clinical screening pipeline that can be utilised to identify novel therapeutic strategies for clinical evaluation in medulloblastoma.

Temporal transcriptomic reprogramming following cranial radiotherapy in a medulloblastoma treatment model reveals targets for mitigating neurocognitive late effects

Authors and Affiliations
Simaran Johal[1*], Jemma Castle[1*], Samantha Jepson Gosling[1], Sasha Gartside[2], Steve Clifford[1], Teklu Egnuni[3], Susan C. Short[3], Debbie Hicks[1†], Daniel Williamson[1†]

1 Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK
2 Institute of Neuroscience, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom
3 Leeds Institute of Medical Research, Wellcome Trust Brenner Building, St James's University Hospital, Beckett St, Leeds, UK

* joint first authors † joint senior authors

Introduction
Current multimodal therapies -surgery, cranial radiotherapy (CRT), and chemotherapy- have significantly improved survival rates for children diagnosed with brain tumours, currently an estimated 80% of children survive this diagnosis. However, CRT exposure during critical periods of brain development comes with a cost; exposure of CRT to the developing brain increases the risk of neurocognitive deficits, particularly in processing speed, attention and working memory. High-dose CRT, used to treat medulloblastoma (MB), poses a significant risk, with >50% of survivors developing progressive neurocognitive deficits, including global intellectual impairment, which can substantially reduce quality of life. While CRT has been linked to late effects, the biological mechanisms driving these outcomes remain poorly understood. To address this gap, we developed an in vivo model system that recapitulates the radiotherapy dose, delivery and late-effect profile of childhood medulloblastoma, at an equivalent developmental stage[1]. In this project, we aim to map the biological mechanisms by which late effects manifest to reveal new targets for amelioration.

Methods
We administered CRT to C57BL/6J mice, mirroring human-equivalent dosages (36Gy over 10 fractions) at Post-Natal Day (PND) ~33, using the small animal radiation research platform (SARRP). To capture regional and temporal specificity in the murine brain's response to CRT we performed a longitudinal transcriptomic analysis using RNA-sequencing at six timepoints (1, 3, 7, 28, 90, 180 days post-CRT) across four different brain regions (cerebellum, hippocampus, cortex and forebrain) (total N=152, samples with and without CRT treatment). Differential expression was carried out using time and region-dependent models. Gene Set Enrichment Analysis (GSEA) was used to identify enrichment of hallmark and biological process pathways to ascribe likely functions of genes and pathways disrupted by CRT.

Results
CRT induced two distinct waves of transcriptomic reprogramming. The first wave, observed at 3 days post-CRT reflects acute injury and includes signatures of apoptosis, blood-brain barrier disruption, cellular stress, and inflammatory cytokine expression. The second wave, at 28 days post-CRT, is characterised by persistent microglial activation, suggesting a chronic pro-neuroinflammatory state. Interestingly, though there is evidence of recovery in neuronal precursor proliferation and neuroblast activity at this stage, pathways associated with neuronal dysfunction remain active long-term from 3 months post-CRT onwards. This indicates that while some regenerative processes are initiated, long-term functional recovery is incomplete, and chronic neuroinflammation persists. This temporal reprogramming in response to CRT was independent of brain region.

Conclusion
Our findings demonstrate temporally distinct transcriptomic responses to CRT, with the 28 days post-CRT timepoint representing the critical window during which neuroinflammatory states are sustained and likely to contribute to long-term neurocognitive impairment. The persistent activation of microglia and incomplete neuronal recovery highlight key regulatory nodes amenable to pharmacological modulation. These insights could pave the way for the development of neuroprotective agents or immune-modulating therapies that target chronic inflammation and support neuronal regeneration. We will further model this key temporal window by constructing a high-resolution single cell atlas to investigate vulnerable cell identities, states and trajectories that are sensitive to CRT and identify novel molecular targets for therapeutic intervention.

[1] Castle, J., Shaw, G., Weller, D., Fielder, E., Egnuni, T., Singh, M., Skinner, R., von Zglinicki, T., Clifford, S. C., Short, S. C., Miwa, S., & Hicks, D. (2024). In vivo modelling recapitulates radiotherapy delivery and late-effect profile for childhood medulloblastoma. Neuro-oncology advances, 6(1),

Iberdomide plus anti-GD2 as a potential combination treatment for Neuroblastoma

Authors and Affiliations
Jane Willoughby[1], Maryam Yazdanparast[1], Martin Taylor[1], Vici Pitic[1], Kerry Cox[1], Ben Wigley[1], Lisa Dunning[1], Cathy Buontempo[2], Salim Khakoo[3], Ali Roghanian[1], Michael Amatangelo[2], Juliet Gray[1]

1 Antibody and Vaccine Group, Centre for Cancer Immunology, University of Southampton
2 Translational Medicine, Bristol Myers Squibb,
3 School of Clinical and Experimental Sciences, University of Southampton

Scientific Abstract
Neuroblastoma is a paediatric cancer with a poor prognosis for those children who are diagnosed with high-risk disease despite an intense multi-modal treatment regime. Addition of anti-GD2 immunotherapy to standard treatment of care has improved outcomes but despite this, survival for those with high-risk disease is still only approximately 50%. Furthermore due to the expression of GD2 on peripheral nerves, neuropathic pain is a significant side effect of the treatment. Thus there is still an unmet clinical need for improved therapies. Iberdomide is a novel cereblon-modulating agent that induces potent degradation of the hematopoietic transcription factors Aiolos and Ikaros, which enhances T and NK cell anti-tumour responses and exerts direct anti-tumour effects in multiple myeloma. Since part of anti-GD2 activity involves NK cells, it was hypothesised that combining Iberdomide with anti-GD2 may synergise to improve outcomes in neuroblastoma patients.

Iberdomide was tested in whole PBMC cultures in vitro and the activation of T cells and their subsequent impact on NK cells was assessed both in terms of proliferation and cytokine production. The project was then expanded into an in vivo model using humanised NSG and NSG-IL-15 Tg mice. Iberdomide was also compared against an existing clinically approved compound, lenalidomide, to investigate potential to slow tumour growth in these models either as a monotherapy or in combination with anti-GD2.

Total PBMCs cultured with anti-CD3 and Iberdomide resulted in a significant increase in NK cells compared to DMSO control and non-significant increase in IL-2 production. Expansion into a humanised model using the NSG strain of mice demonstrated limited efficacy of either Iberdomide or anti-GD2, however there was an indication that the combination was better than monotherapy alone. Subsequently, NSG-IL-15 Tg mice were humanised in order to obtain increased numbers of NK cells. In this model the Iberdomide alone was able to induce a reduction in tumour growth.

In conclusion, Iberdomide may enhance NK mediated anti-neuroblastoma responses. Although further understanding of the mechanisms involved would provide the necessary insight to determine favourable combination therapies.

Inducible degradation of a B7H3 targeting chimeric antigen receptor by an IMiD drug sensitive degron tag

Authors and Affiliations
Sophie Munnings-Tomes[1], Marta Barisa[1], Rajeev Rai[1], Thomas Jackson[1], Habib Bouguenina[2], Yann-Vai Le Bihan[2], Ian Collins[2], John Anderson[1]

1 Cancer Section, Developmental Biology and Cancer Programme, UCL Institute of Child Health, 20c Guildford Road, London, WC1N 1DZ
2 Centre for Cancer Drug Discovery, the Institute of Cancer Research, 15 Cotswold Road, Sutton, London SM2 5NG, UK.

Introduction
A common determinant of CAR-T failure in solid cancers is T cell exhaustion associated with sustained signalling. Reversible downregulation of CAR expression during manufacture or following infusion holds promise to allow for CAR-T recovery. One method of selective degradation makes use of immunomodulatory drugs (IMiDs). These bring zinc finger degron motifs and the E3 ubiquitin ligase complex together, resulting in ubiquitination and proteasomal degradation of degrons. By addition of degron tags to our CAR constructs we aimed to modulate expression for improved efficacy of therapy.

Method
An optimised novel degron tag sensitive to IMiD drug downregulation (designated iTAG2) was obtained by mutational screening of known zinc finger binding degron motifs. Mutations were designed to prevent nuclear localisation of the degron tag due to zinc finger's intrinsic affinity for DNA. iTAG2 was added to the c terminus of our B7H3 binding CAR, TE9-28z. IMiD drug addition was analysed for its ability to reduce CAR expression in vitro and in vivo and the presence of iTAG2 on the CAR evaluated for killing and cytokine production capacity post co-culture with target cell lines. Re-challenge cytotoxic assays, with intermittent pomalidomide addition toggling CAR expression or degradation tested the effect of resting CARs on their long term functionality against tumour cells. In order to assess periodic degradation of CARs during treatment stage and the immunomodulatory effects of IMiDs in immunocompetent models, mouse TRAC-CARs containing iTAG2 were produced using an AAV vector.

Results
iTAG2 tagged TE9-28Z CAR showed reversible degradation at nanomolar concentrations of IMiDs. iTAG2 CARs had superior cell surface retention and antigen specific cytokine production in vitro compared published degron tags. Use of IMiDs switched off CAR expression preventing tonic signalling. In vivo degradation of iTAG2 CARs, containing luciferase under degron control, could be seen additionally with p.o. administration of pomalidomide with subsequent re-expression. Intermittent administration of pomalidomide, during rechallenge assays with target antigen positive cell lines, improved CAR effector function.
AAV transduction methods delivering our iTAG2 CAR construct into the murine TRAC locus resulted in high transduction efficiency that was maintained during the period of manufacture. iTAG2 CARs generated using this method with cereblon transgenic mouse splenocytes, showed sensitivity to IMiDs.

Conclusion
We have developed a B7H3 CAR that can be reversibly switched on and off by the IMiD drugs. This design may be optimal for clinical combination trials in which pulses of IMiD drug simultaneously control tumour growth and modulate tumour microenvironment, whilst allowing CAR-T cell rest and recovery.

Identifying targetable driver genes to circumvent medulloblastoma relapse

Authors and Affiliations
Dr Melanie Beckett, Joe Edwards, Dr Dean Thompson, Dr Alistair Poll, Dr Stacey Richardson, Janice Law, Dr Claire Keeling, Bethany Poole, Prof Steven C Clifford and Dr Rebecca M Hill

Introduction
Medulloblastoma (MB) is the most common malignant brain tumour found in children. Medulloblastoma is clinically and molecularly heterogenous and can be subdivided into four principal molecular groups; Wingless (MBWNT), sonic hedgehog (MBSHH), Group 3 (MBGrp3) and Group 4 (MBGrp4)1. Relapse (rMB) after multimodal treatment (surgery, radiotherapy, and chemotherapy) occurs in 30-40% of patients and is typically fatal2. Our recent multi-omic characterisation (methylation array, RNAseq, whole exome sequencing) of >120 rMBs and their diagnostic counterparts revealed putative drivers of relapse3. Functional validation and investigation of these candidates has strong potential to underpin the development of more personalised and specific therapies for rMB.

Methods
Genes (n>150) from our 'omic discovery set with acquired/enriched alterations in rMB were assessed as rMB drivers in functional genomic screens by RNAi. These included known cell cycle regulators, epigenetic regulators, and genes of unknown function. Doxycycline regulable shRNAs (n=5/gene) were introduced into MB cell-based models (n=3) and radiotherapy applied (1-3Gy, weekly). Changes in hairpin abundance, compared to baseline and controls, were used to prioritise genes with roles in tumour fitness/survival; further filtering considered availability of targeting compounds and clinical tractability. Candidate validation approaches included generation of knockdown/knockout (shRNA/CRISPR) models followed by model characterisation (growth, colony formation, protein expression), and single agent and combination drug testing.

Results
Prioritised genes include cell cycle regulator CDK6 and nuclear exporter XPO1. CDK6 amplifications are both acquired and maintained at relapse in MBGrp3 and MBGrp4. Importantly the use of a CDK4/CDK6 inhibitor Ribociclib has been trialled in a small number of rMB patients as part of SJDAWN (NCT03434262) – a phase I study evaluating doublet therapies for paediatric and young adults with recurrent brain tumours. Provisional reports of some patient benefit, coupled with our discoveries, suggest inhibition of CDK6 has potential as a targetable driver of rMB in selected patients. XPO1 mutations identified in SHH patients are maintained at relapse. Selinexor, an oral inhibitor of XPO1 is used clinically in the treatment of refractory multiple myeloma. We show that Selinexor enhances effects of radiotherapy in in vitro MB models, concordant with its significance under radiotherapeutic pressure in our RNAi screens. Ongoing work will assess Selinexor treatment in in vivo models of high-risk and rMB.

Conclusion
In summary, we show that focused screening approaches can identify and prioritise candidate rMB drivers, functionally relevant to disease biology, for pre-clinical assessment with a view to potential clinical exploitation.

1. Taylor, M. D. et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol 123, 465–472 (2012).
2. Hill, R. M. et al. Time, pattern, and outcome of medulloblastoma relapse and their association with tumour biology at diagnosis and therapy: a multicentre cohort study. The Lancet Child & Adolescent Health 4, 865–874 (2020).
3. Richardson, S. et al. Emergence and maintenance of actionable genetic drivers at medulloblastoma relapse. Neuro-Oncology 24, 153–165 (2022).

Phospho-LCK predicts dasatinib sensitivity in paediatric T-ALL

Authors and Affiliations
Alistair Poll
Newcastle University

Background
Children with T-cell acute lymphoblastic leukaemia (T-ALL) who relapse have very poor outcomes, with few treatment options available once resistance to first-line therapy occurs. We have previously shown that the tyrosine kinase inhibitor dasatinib can overcome dexamethasone resistance in a subset of patients. The combination is being explored in two clinical settings, but current approaches either require labour-intensive and logistically challenging centralised drug response profiling, restricting use to small trial cohorts, or apply the combination broadly in ways that risk overtreating patients and underpowering studies. This highlights the need for a scalable, clinically implementable method to identify which patients are likely to benefit. We hypothesised that this could be achieved through flowcytometric profiling of activating phosphorylation sites within the pre T-cell receptor signalling pathway, a known target of dasatinib.

Methods
A cohort of 28 patient-derived xenograft (PDX) models of T-ALL were stained with antibodies against p394Y-LCK, p319Y-ZAP70, p142Y-CD3ζ and analysed by flow cytometry. Marker intensities were normalised against positive and negative controls. Dasatinib sensitivity was assessed via two independent drug screening pipelines. Regression models quantified the correlation of each marker with dasatinib IC50 and the ability to discriminate resistant from sensitive patients at tow sensitivity thresholds: a hypersensitivity threshold (IC50 < 10 nM), reflecting strong preclinical activity, and a literature standard threshold (IC50 < 100 nM), commonly used to distinguish sensitive from resistant cases.

Results
pLCK showed the was the strongest correlation and was strongest single predictor of response. It achieved complete discrimination of hypersensitive cases (IC50 < 10 nM, ROC AUC = 1, PR AUC = 1), with pZAP70 performing only marginally less well. At the literature standard threshold (IC50 < 100 nM), both pLCK and pZAP70 remained excellent predictors in isolation (AUC ≥ 0.89). The benefit of including multiple markers in identifying sensitive patients at the higher threshold was explored by multivariate models. This did not improve the ability to discriminate sensitive from resistant samples, with pLCK in isolation shown to be the most parsimonious model of dasatinib sensitivity state by Akaike Information Criterion (AIC). pLCK and pZAP70 were highly correlated (Spearman ρ ≈ 0.91) and showed multicollinearity (Variance Inflation Factor > 15), consistent with their shared biological function, indicating further their redundancy. We have demonstrated the feasibility of including of pLCK within diagnostic flow cytometry protocols using spiked-in samples.

Conclusions
Phospho-LCK is a robust single-marker predictor of dasatinib response in T-ALL, capable of identifying both hypersensitive and sensitive cases. By providing a rapid, scalable and accurate alternative to centralised in-vitro testing, this approach could improve patient selection, expanding the context in which this therapy could be used whilst avoiding overtreatment. A multicentre collaboration across Europe is already underway to expand the cohort of PDX samples, alongside implementing the protocol in diagnostic laboratories t support translation of this marker into routine care.

NAMPT inhibition enhances sensitivity of glioblastoma cells to radiation

Authors and Affiliations
Kamila Rosiak-Stec
University of Glasgow

Introduction
The prognosis for patients with glioblastoma (GBM), the most prevalent and aggressive brain tumor in adults, remains extremely poor. Standard care involves maximal safe surgical resection followed by radiotherapy and temozolomide chemotherapy, but radioresistance remains a major obstacle. Cancer cells exhibit profound metabolic rewiring, including reliance on the nicotinamide adenine dinucleotide (NAD^+) salvage pathway. Nicotinamide phosphoribosyltransferase (NAMPT), the pathway's rate-limiting enzyme, is frequently overexpressed in GBM and other cancers, and high NAMPT expression correlates with poor survival. This in vitro study aimed to investigate the interaction between radiation and FK866 (Daporinad), a highly specific NAMPT inhibitor.

Method
In vitro, FK866 was assessed for effects on proliferation, clonogenic survival, cell cycle, gene expression, and DNA damage induction using genetically diverse, patient-derived glioblastoma stem-like cells (GSCs). In vivo, a maximum tolerated dose (MTD) study was conducted in CD1 nude mice (n=5/group), treated intraperitoneally with FK866 (15 or 30 mg/kg), alone or with 2 Gy irradiation on days 3, 5, 7, 10, 12, and 14.

Results
Treatment with FK866 significantly reduced total cell numbers across all ten GSC lines tested after a five-day exposure, with IC50 values ranging from 0.25 nM to 8.8 nM. Notably, this single agent activity was consistent irrespective of genetic subtype, O6-methylguanine-DNA methyltransferase (MGMT) methylation status, or baseline NAMPT expression and activity levels. No activity was observed in normal human astrocytes. On-target specificity of FK866 was confirmed by rescue experiments, wherein supplementation with NAD or nicotinamide mononucleotide reversed cytotoxic effects, validating NAMPT inhibition and NAD depletion as the primary mechanisms. Cytotoxic effects correlated with progressive alterations in gene expression profiles up to 72 hours after continuous FK866 exposure. However, removal of drug after 72 hours permitted recovery and proliferation of a subset of cells, indicating the need for combination approaches to achieve durable tumour control.
We therefore evaluated FK866 in combination with ionising radiation and demonstrated potent radiosensitising effects at low nanomolar concentrations in GBM cell culture models when added two hours pre-radiation.
Radiosensitisation was confirmed by clinically relevant clonogenic survival (2D) and spheroid growth delay assays (3D). Mechanistically, FK866 induced DNA replication stress, increased DNA double strand break formation after radiation and exacerbated radiation induced G2/M accumulation and mitotic block.

Concomitantly, FK866 suppressed RAD51 expression at the RNA level and in a dose-dependent manner at the protein level, consistent with impaired homologous recombination repair.

Finally, FK866 administered at 15 or 30 mg/kg, alone or in combination with brain irradiation (6 x 2 Gy), was well tolerated by non-tumour bearing CD1 nude mice, with no weight loss and no neurological or systemic symptoms or signs.

Conclusion
Targeting NAMPT with FK866 has therapeutic potential in glioblastoma both as a single agent and a potent radiosensitiser. The absence of overt toxicity supports its suitability for further investigation in forthcoming orthotopic efficacy studies.

Embracing Research and Impact

Birmingham | 24-25 November 2025

CHILDREN WITH CANCER UK

Poster abstracts

Exposure to metals and trace elements and risk of childhood leukaemia: An Italian case-control study

Authors and Affiliations
Tommaso Filippini[1,2], Marcella Malavolti[1], Teresa Urbano[1], Carlotta Malagoli[1], Giovanni Palazzi[3], Maria Caterina Putti[4], Giovanna Russo[5], Margherita Ferrante[6], Bernhard Michalke[7], Marco Vinceti[1,8]

(1) CREAGEN Research Center of Environmental, Genetic and Nutritional Epidemiology and Center for Neurosciences and Neurotechnology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; (2) Department of Epidemiology, School of Public Health, University of California Berkeley, Berkeley, CA, USA; (3) Division of Paediatric Oncology-Haematology, Modena Policlinico-University Hospital, Modena, Italy; (4) Paediatric Haematology Oncology Unit, Department of Woman's and Child's Health, Padova University Hospital, Padova, Italy; (5) Paediatric Haematology/ Oncology Unit, University of Catania, Catania, Italy; (6) Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Italy; (7) Research Unit Analytical BioGeoChemistry, German Research Center for Environmental Health, Helmholtz Center Munich, Neuherberg, Germany; (8) Department of Epidemiology, Boston University School of Public Health, Boston, USA

Abstract
Background and aims: Childhood leukaemia is the most common form of cancer in children. Although advances in therapy have significantly improved prognosis, the aetiology remains largely unknown. Recent evidence suggests that various heavy metals and metalloids may be involved in the onset of various types of cancer. The aim of this study was to investigate the association between the concentrations of certain trace metals and the risk of childhood leukaemia.

Methods
Between 2019 and 2023, we recruited children newly diagnosed with leukaemia and healthy controls from the Paediatric Haematology-Oncology Units of the University Hospitals of Modena, Padua, and Catania. Each child underwent blood sampling to quantify plasma levels of the following trace metals using plasma mass spectrometry: cadmium (Cd), iron (Fe), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn). We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models to correlate leukaemia risk with different tertiles of plasma metals, using the lowest tertile as a reference. Analyses were adjusted for age, sex, and second-hand smoking by quantifying urinary cotinine.

Results
The median age of the population was 6 years (interquartile range 3–10 years). Cu, Mn, and Se concentrations were higher in children with leukaemia than in controls, while Cd and Zn concentrations were slightly higher in controls than in children with the disease. Fe concentrations did not differ between the two groups. We found a higher risk of leukaemia for all metals examined, except for Zn. The highest risk was found for Cu, with an OR of 36.94 (95% CI 3.77–362.40). A dose-response relationship was found for all metals except for Mn, for which ORs of 5.27 (95% CI 1.21-22.91) and 2.72 (95% CI 0.67-11.05) were found for the second and third tertiles, respectively. For Se, an association with leukaemia risk was found only in the third tertile, with an OR of 1.33 (95% CI 0.57-3.10).

Conclusions
Our results indicate a positive association between blood levels of Cd, Cu, Fe, Mn, and Se and increased risk of childhood leukaemia, with Cu showing the strongest relation. These data suggest a potential role for trace metals in the pathogenesis of childhood leukaemia, highlighting the importance to lower exposure in children.

Polycyclic aromatic hydrocarbons and leukaemia risk: an Italian case-control study

Authors and Affiliations
Marcella Malavolti [(1)], Teresa Urbano[(1)], Tommaso Filippini[(1)(2)], Carlotta Malagoli[(1)], Giovanni Palazzi[(3)], Maria Caterina Putti[(4)], Giovanna Russo[(5)], Maria Fiore[(6)], Margherita Ferrante[(6)], Silvia Fustinoni[(7, 8)], Elisa Polledri[(7)], Marco Vinceti[(1)(9)] on behalf of the CwC2019 study group.

(1) CREAGEN Research Center of Environmental, Genetic and Nutritional Epidemiology and Center for Neurosciences and Neurotechnology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; (2) Department of Epidemiology, School of Public Health, University of California Berkeley, Berkeley, CA, USA; (3) Division of Pediatric Oncology-Hematology, Modena Policlinico-University Hospital, Modena, Italy; (4) Pediatric Hematology Oncology Unit, Department of Woman's and Child's Health, Padova University Hospital, Padova, Italy; (5) Pediatric Hematology/Oncology Unit, University of Catania, Catania, Italy; (6) Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Italy; (7) Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy; (8)Toxicology lab, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; (9) Department of Epidemiology, Boston University School of Public Health, Boston, USA

Abstract
Background and aims: Childhood leukaemia is the most common form of cancer in children. Although advances in therapy have significantly improved prognosis, the aetiology remains largely unknown. Recent evidence suggests that various heavy metals and metalloids may be involved in the onset of various types of cancer. The aim of this study was to investigate the association between the concentrations of certain trace metals and the risk of childhood leukaemia.

Methods
Between 2019 and 2023, we recruited children newly diagnosed with leukaemia and healthy controls from the Paediatric Haematology-Oncology Units of the University Hospitals of Modena, Padua, and Catania. Each child underwent blood sampling to quantify plasma levels of the following trace metals using plasma mass spectrometry: cadmium (Cd), iron (Fe), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn). We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models to correlate leukaemia risk with different tertiles of plasma metals, using the lowest tertile as a reference. Analyses were adjusted for age, sex, and second-hand smoking by quantifying urinary cotinine.

Results
The median age of the population was 6 years (interquartile range 3–10 years). Cu, Mn, and Se concentrations were higher in children with leukaemia than in controls, while Cd and Zn concentrations were slightly higher in controls than in children with the disease. Fe concentrations did not differ between the two groups. We found a higher risk of leukaemia for all metals examined, except for Zn. The highest risk was found for Cu, with an OR of 36.94 (95% CI 3.77–362.40). A dose-response relationship was found for all metals except for Mn, for which ORs of 5.27 (95% CI 1.21-22.91) and 2.72 (95% CI 0.67-11.05) were found for the second and third tertiles, respectively. For Se, an association with leukaemia risk was found only in the third tertile, with an OR of 1.33 (95% CI 0.57-3.10).

Conclusions
Our results indicate a positive association between blood levels of Cd, Cu, Fe, Mn, and Se and increased risk of childhood leukaemia, with Cu showing the strongest relation. These data suggest a potential role for trace metals in the pathogenesis of childhood leukaemia, highlighting the importance to lower exposure in children.

Identifying circulating microRNA biomarkers to facilitate minimally-invasive Wilms tumour diagnosis and monitoring

Authors and Affiliations
Jessica L. Balls[1], Dawn Ward[1], Georgia E. Martin[1], Zachary G. L. Scurlock[1], Tammie Waplington[1], Michael Boemo[1], Reem Al-Saadi[2], Kathy Pritchard-Jones[2], Nicholas Coleman[1*], Cinzia G. Scarpini[1*], Matthew J. Murray[1*]
*Joint senior authors

1 Department of Pathology, University of Cambridge, UK
2 University College London, UK

Background/Aims
Wilms Tumour (WT) is a rare paediatric cancer diagnosed in ~80 children annually in the UK. Current European treatment protocols allow for the initiation of chemotherapy based solely on typical clinical and imaging findings without histological confirmation. On occasion, at interval nephrectomy, the diagnosis is of a non-WT diagnosis: i.e., a more aggressive renal malignancy or a benign lesion. Accordingly, the identification of additional, minimally-invasive diagnostic methods is an urgent clinical need. This study aimed to determine whether a panel of circulating microRNAs (miRNAs) can accurately diagnose WT.

Methods
Following global miRNA (n=754) quantification using Taqman Cards A&B, two complementary methods were used to identify potential candidates for a WT diagnostic panel. The first, using the Applied Biosystems Relative Quantification App, identified differentially expressed circulating (serum) miRNAs in patients with WT (n=38) versus an age-matched non-cancer control group (n=15) and refined the list based on adjusted p-value and log2FC. The second, a Machine Learning (ML)-based approach, employed random forest classifiers, and incorporated additional Taqman Card data (n=38; from patients with malignant germ-cell-tumours) to prevent overfitting of the model and add discriminatory power. SHapley Additive exPlanations (SHAP) values were used to rank the candidate list, with log2FC used as additional stringency. Additionally, publicly available serum RNA sequencing data for WT patients (n=27) and healthy controls (n=10) was analysed to add further confidence to identified miRNAs (Mohamed et al, Front Mol Biosci, 2024; PMID:39473825).

Results
The ML-based random forest approach and Applied Biosystems relative quantification analysis identified 82 and 75 candidate biomarker miRNAs, respectively, with substantial overlap (n=61 miRNAs; 81% of the total possible). Eight of these 61 were also common to the independent RNA sequencing analysis, supporting their biological significance/ relevance.

Conclusion
This preliminary study identifies potential circulating miRNA candidates for a WT diagnostic panel. Further confirmatory and validation work on the candidate biomarker list is underway to determine the optimal panel for clinical testing for this heterogeneous disease.

Unravelling the Complexity of the Rhabdomyosarcoma Tumour Microenvironment

Authors and Affiliations
Rosa Gomes Alves Martins[1], Alex Look[1], Janet Shipley[2], Edward James[3], Tim J. Underwood[1], Aymen Al-Shamkhani[3], Matthew D. Blunt[4], Zoë S. Walters[1]

1 Innovation for Translation Research Group, School of Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.
2 Divisions of Molecular Pathology and Cancer Therapeutics, The Institute of Cancer Research, London, UK.
3 Antibody Vaccine Group, School of Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.
4 Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.

Introduction
Rhabdomyosarcoma (RMS) is a rare soft-tissue sarcoma mainly affecting children and adolescents. It is characterised by genetic lesions, with the presence of a fusion protein (PAX3/7-FOXO1), denoted fusion positive (FP-RMS) or mutations in MYOD1, denoted spindle cell/sclerosing (SS-RMS), which both confer poor outcomes, alternatively the absence of the fusion protein is denoted as fusion negative (FN-RMS). Although current treatments have increased overall survival rates, these can lead to irreversible side effects and are largely ineffective in relapsed or advanced patients, with 5-year survival <30%. Consequently, developing innovative treatment regimens, such as immunotherapies, is urgently required.

B7-H3, an immune checkpoint and tumour promoting molecule, has gained interest as a promising target antigen for novel immunotherapies as RMS tumours express high levels of B7-H3 with limited expression in normal tissues. Additionally, B7-H3 antibodies, e.g., Enoblituzumab, can potentiate the inherent ability of natural killer (NK) immune cells to kill cancer cells, in turn potentiating the activity of immunotherapies in RMS. However, to date, Enoblituzumab has reportedly no clinical effectiveness in RMS patients.

Methods
To understand why Enoblituzumab is ineffective in RMS, we negatively isolated NK cells from primary healthy peripheral human blood mononuclear cells (PBMCs) and co-cultured these with either established cell lines, patient-derived xenograft cells, or patient-derived tumouroids, representing FP- and FN-RMS subtypes, in 2D or as 3D spheroids in ultra-low attachment plates with or without Enoblituzumab. NK and RMS cells were either in direct contact or co-cultured indirectly by using a permeable support system (Transwell) or a 3D microfluidic device separating endothelial cells plus PBMCs from RMS cells with an extracellular matrix.

Results
In both 2D or 3D co-cultures, we found increased NK cell-mediated cytotoxicity in the presence of Enoblituzumab, independent of fusion status. With the Transwell system, we showed that though RMS spheroids recruited NK cells, these NK cells could not fully infiltrate the tumour with Enoblituzumab-mediated killing occurring only at the edge of the tumour spheroids. Conversely, when applying a more complex microfluidic model, RMS cells did not have the ability to attract PBMCs and in the presence of the chemoattractant CXCL12, dampened PBMC migration.

Conclusion
Thus, it is imperative to fully understand the relationship between the tumour microenvironment, immune infiltration, and immunotherapy response before trialling possible novel therapies. This research, and application of this novel 3D microfluidic device, will aid in unravelling the complexity of these interactions to ultimately find ways to improve immunotherapy responses in children with this deadly disease.

Longitudinal MR Spectroscopy to provide added value in response assessment for Paediatric Glioma

Authors and Affiliations
Katherine.Crombie[1,2], Daniel Griffiths-King[3], Tim Mulvany[3], Lara Worthington[2,4], Sara Burling[2], Andrew Peet[2], Jan Novak[3], John Apps[1,2]

1 University of Birmingham
2 Birmingham Children's Hospital
3 Aston University
4 Radiation Physics and Protection Service, University Hospitals Birmingham

Introduction
Central nervous system (CNS) tumours, of which gliomas are the most common, are the leading cause of cancer-related death in children. In cases where complete surgical resection is not possible, chemotherapy and radiotherapy may lead to complete or partial (>50%) response, however often only achieve "stable disease" as defined by the RAPNO Criteria (<25% change in size, stability of non-enhancing FLAIR/T2W lesions, and absence of new lesions in a clinically stable patient).

Importantly, the "stable disease" category is heterogeneous: some patients exhibit therapeutic response and tumours entering quiescent or senescent states, while others harbour slow but undetectable progression that becomes evident only over time. Distinguishing these groups earlier could support treatment planning optimise follow-up imaging strategies and support the development of novel therapeutic approaches (e.g. senolytics).

Magnetic resonance spectroscopy (MRS) has shown potential to provide complementary information in diagnosis, tumour subtyping, prognosis, and treatment monitoring. Its ability to detect biochemical changes may therefore add value in refining response assessment.

Methods
This study analysed data from The CCLG Imaging of Tumours Study, a multi-centre, single-arm, observational study (REC 04/MRE04/4; IRAS 32486) sponsored by the University of Birmingham.

Patients with glioma treated at Birmingham Children's Hospital and enrolled in the study who had longitudinal MRS data were identified (n=202). Spectroscopy data were processed using **TARQUIN (Totally Automatic Robust Quantitation in NMR) software, version 4.3.10.**

Metabolite concentrations were assessed longitudinally in individual patients and correlated with clinical notes, radiology reports, and multidisciplinary team (MDT) documentation. Linear regression modelling was applied to identify significant metabolite changes while accounting for multiple clinical and technical variables.

Results
Preliminary findings suggest that metabolite alterations may reflect subtle changes in disease state not apparent on conventional imaging. Statistically significant changes were identified in specific metabolites, including the **Myo-inositol/ Choline ratio and Citrate**. These shifts were detectable from baseline and correlated with clinical context.

Conclusion
These early results indicate that MRS may contribute to distinguishing true stability from early progression in paediatric glioma. When combined with other advanced imaging modalities, spectroscopy could provide a more robust biomarker of disease behaviour and better inform clinical decision-making. The metabolites identified are consistent with published literature on indicators of tumour progression, supporting the biological plausibility of these findings.

This work forms part of a larger project integrating MRS with additional imaging techniques to improve the accuracy of treatment response assessment in paediatric glioma.

Targeting TGFβ pathway enhances anti-B7H3 CAR-T cell therapy in Group 3 Medulloblastoma

Authors and Affiliations
Dr Sonia Morlando
University College London

Introduction
Group 3 medulloblastoma (G3 MB) is among the most aggressive paediatric brain tumours, with limited treatment options and poor prognosis in high-risk cases. Chimeric Antigen Receptor (CAR) T cell therapy targeting B7H3, an immune checkpoint molecule overexpressed in medulloblastoma, has emerged as a promising therapeutic approach in both paediatric and adult brain tumours. However, the highly immunosuppressive tumour microenvironment (TME), particularly mediated by Transforming Growth Factor-Beta (TGFβ), poses a significant barrier to CAR-T cell efficacy and persistence. We hypothesised that pharmacological inhibition of TGFβ signalling via blockade of TGFβ receptor I (TGFBRI) could preserve CAR-T cell functionality and promote sustained anti-tumour responses against G3 MB.

Method
To evaluate this hypothesis, we first screened G3 MB cell lines and mouse models for activation of the TGFβ pathway. Phosphorylated SMAD3 (pSMAD3) via immunohistochemistry (IHC) was used as a readout to confirm active TGFβ signalling within the tumour context. For CAR-T cell generation, peripheral blood mononuclear cells (PBMCs) were freshly isolated from healthy donors, stimulated with anti-CD3/CD28 antibodies, and transduced with a retroviral vector encoding either an anti-B7H3 CAR alone or an anti-B7H3 CAR co-expressing a truncated form of TGFBRII (DNR). Expanded CAR-T cells were then used in in vitro co-culture assays with G3 MB cell lines in the presence or absence of exogenous TGFβ (10ng/ml) to assess TGFβ-mediated immunosuppression. Pharmacological inhibition of TGFβ signalling was tested using two TGFBRI (ALK5) inhibitors, Vactosertib (TEW197) and LY3200882.

Results
Firstly, we evaluated TGFβ pathway activation across multiple models of medulloblastoma, including patient tumour samples and G3 MB established in both immunocompetent and immunodeficient mouse models. Our results confirmed high levels of phosphorylated SMAD3, indicating robust pathway activation within tumour tissues. Notably, in patient samples (n=71 (SHH), n=63 (Group 3), n=71 (Group 4), n=15 (WNT)), levels of phosphorylated SMAD3 were significantly higher in G3 tumours compared to other medulloblastoma subgroups.

After establishing this, we investigated the effects of exogenous TGFβ on anti-B7H3 CAR T cells using in vitro co-culture with MB cell lines. Our results demonstrated that TGFβ markedly impaired CAR T-cell proliferation, phenotype and cytokine release and, these suppressive effects were significantly reversed by co-expression of a dominant-negative form of TGFBRII (DNR) in the CAR construct.
We next explored combinatorial strategies using anti-B7H3 CAR T cells and two TGFBRI inhibitors, Vactosertib and LY3200882, to evaluate their ability to counteract TGFβ-mediated dysfunction. Both inhibitors preserved CAR T-cell proliferation and, importantly, effectively rescued effector function in a TGFβ-rich environment. Strikingly, these protective effects were sustained in re-challenge assays with repeated exposure to both tumour cells and exogenous TGFβ, where cytokine release and proliferation were otherwise significantly impaired. Current experiments are now evaluating the efficacy of these combinations in in vivo models.

Conclusion
Collectively, our findings demonstrate that both genetic (DNR) and pharmacological (TGFBRI inhibitors) strategies restore anti-B7H3 CAR T-cell function in TGFβ-rich environments. These results provide strong preclinical rationale for the development of combination approaches to overcome TME-mediated immunosuppression in Group 3 medulloblastoma.

Circulating microRNAs to assist the diagnosis and management of CNS malignant germ cell tumours

Authors and Affiliations
Cinzia G. Scarpini[1], Dawn Ward[1], Silvia Caprari[1], Shivani Bailey[2], Nicholas Coleman[1,3], Matthew J. Murray[1,4].

1 Department of Pathology, Tennis Court Road, University of Cambridge, Cambridge, CB2 1QP, UK
2 Department of Paediatric Haematology and Oncology, Birmingham Children's Hospital, Birmingham, UK
3 Department of Histopathology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK
4 Department of Paediatric Haematology and Oncology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK

Background
Circulating (serum) miR-371a-3p quantification has shown substantial clinical utility over the existing conventional serum tumour markers alpha-fetoprotein (AFP) and human chorionic gonadotrophin (HCG) for testicular malignant germ cell tumour (GCT) diagnosis and follow-up [1]. Using matched cerebrospinal fluid (CSF) and serum samples, this assay has also shown early promise for suspected central nervous system (CNS) malignant GCTs [2-4]. Here, we sought to determine assay performance characteristics in a larger independent series of patients presenting with CNS lesions, including suspected malignant GCTs.

Methods
Samples were sent frozen from Paediatric Oncology Principal Treatment Centres (UK/Europe); minimal serum/CSF volume requested 200µl. Samples underwent miRNA isolation and pre-amplified qRT-PCR using established protocols including quality-control checks [5]. Sensitivity, specificity, and area-under-the-curve (AUC) for the assay was assessed for these cohorts using previously reported raw miR-371a-3p Cq thresholds, with positive (raw-Cq<28), indeterminate (≥28-35), and negative (≥35) results reported, as described [5].

Results
To date, 70 CSF and 57 serum samples from 62 patients with intracranial lesions have been analysed, typically from presentation/diagnosis (n=55/62 patients), but also during treatment/relapse (n=7/62). Of these, 46/62 patients had not previously been published, had definitive diagnosis/treatment, and/or sufficient follow-up data to be included in the current analysis. CSF miR-371a-3p results were positive (raw-Cq<28) in 18/19 malignant GCT cases, indeterminate in 1/19 malignant GCT case, and negative in 27/27 remaining control cases (other e.g., suprasellar/pineal lesions/tumours). Resulting assay sensitivity 94.7%, specificity 100%, with AUC 0.947 for intracranial malignant GCT diagnosis, with 95%-confidence-intervals=0.872-1.000. Serum miR-371a-3p levels positive in 2/19 malignant GCT cases (both CSF positive) and negative in all controls.

Conclusion
The CSF miR-371a-3p assay run by the Cambridge, UK lab using highly sensitive pre-amplified qRT-PCR testing shows excellent performance characteristics for CNS malignant GCT detection. Such compelling evidence will facilitate clinical adoption for this indication, in addition to testicular cases.

References
[1] Piao J, Lafin L, Scarpini CG, Nuno M, Syring I, Dieckmann KP, Belge G, Ellinger J, Amatruda JF, Bagrodia A, Krailo MD, Coleman N, Frazier AL, Murray MJ. A multi-institutional pooled analysis demonstrates the utility of circulating miR-371a-3p alone for testicular malignant germ cell tumour diagnosis. Clinical Genitourinary Cancer, 2021 Dec;19(6):469-479. doi: 10.1016/j.clgc.2021.08.006. Epub 2021 Sep 15.

[2] Murray MJ, Bell E, Raby K, Rijlaarsdam M, Gillis A, Looijenga LH, Brown H, Destenaves B, Nicholson JC, Coleman N. A pipeline to quantify serum and cerebrospinal fluid microRNAs for diagnosis and detection of relapse in paediatric malignant germ cell tumours. British Journal of Cancer, 2016;114:151-62.

[3] Murray MJ, Ajithkumar TV, Harris F, Williams RM, Jalloh I, Cross J, Ronghe M, Ward DM, Scarpini CG, Nicholson JC and Nicholas Coleman, on behalf of the CCLG. Clinical utility of circulating miR-371a-3p for the management of patients with intracranial malignant germ cell tumors. Neuro-Oncology Advances, 2020 Apr 13;2(1):vdaa048. doi: 10.1093/noajnl/vdaa048. eCollection 2020 Jan-Dec.

[4] Schönberger S, Mohseni MM, Ellinger J, Tran GVQ, Becker M, Claviez A, Classen C-F, Hermes B, Driever PH, Jorch N, Lauten M, Mehlitz M, Schäfer N, Scheer Preiss J, Schneider DT, Troeger A, Calaminus G, Dilloo D. MicroRNA profiling of miR 371~373 and miR 302/367 clusters in serum and cerebrospinal fluid identify patients with intracranial germ cell tumors

[5] Lafin JT, Scarpini CG, Amini A, Konneh B, Howard JM, Gerald T, Nuno M, Piao J, Savelyeva A, Wang Z, Jeffrey Gagan, Jia L, Lewis CM, Murray S, Sawa YC, Margulis V, Woldu SL, Strand DW, Coleman N, Amatruda JF, Frazier AL, Murray MJ, Bagrodia A. Refining the serum miR-371a-3p test for viable germ cell tumor detection: identification and definition of an indeterminate range. Scientific Reports, 2023, June 29;13(1):10558. doi: 10.1038/s41598-023-37271-1.

Insights across indications from the Paediatric Bespoke Therapeutic Development Workshop initiative

Authors and Affiliations
Itziar Areso, Joseph S Baxter, Claudia A Montiel-Equihua, Itziar Areso, Jan J Molenaar, Sheena Patel, Siwan Oldham, Andrew D J Pearson, David Jenkinson.

LifeArc, Lynton House, 7-12 Tavistock Square, London WC1H 9LT

Introduction
The Paediatric Bespoke Therapeutic Development Workshop (PBTDW) initiative is a collaborative multi-stakeholder effort aimed at mapping unmet needs across childhood cancer indications. Each workshop convenes a global panel of experts, including researchers, patient advocates, and representatives from charitable organizations, for a virtual discussion focused on emerging therapeutic targets, combination drug strategies, as well as other clinical challenges and opportunities.

Method
To support prioritisation of targets for novel therapeutic development, detailed diligence is prepared and shared prior to the meeting, considering biological rationale, strength of target validation, tractability, and existing commercial development of each target.

Results
To date, seven workshops have been conducted, addressing rhabdoid tumours, medulloblastoma, rhabdomyosarcoma, osteosarcoma, acute myeloid leukaemia, desmoplastic small round cell tumours and high-grade glioma. An eighth workshop, focused on Ewing sarcoma, is scheduled for early November.

Conclusion
Despite the biological and clinical diversity of these indications, several recurring themes have emerged. Most targets require some level of further validation. For a target where the ideal therapeutic would modulate target activity, such as small molecule inhibitors, it is critical to show a strong functional and mechanistic association with the tumour type. For antigen-directed therapies independent of functional modulation, such as cell therapies or antibody-drug conjugates, expression studies should show sufficiently high and homogeneous expression (ideally at the protein level) to stimulate on-tumour activity. Many prominent targets are involved in early stages of childhood development and require rigorous evaluation for potential toxicity and safety concerns. However, the limited availability of developmental gene expression and functional datasets in paediatric populations remains a significant barrier to progress. Notably, targets once deemed “undruggable”, such as transcription factors and fusion genes, are increasingly viewed favourably, with ongoing efforts to develop targeted degraders and cell-based therapies.

From a clinical perspective, paediatric oncology shares many of the challenges inherent to rare diseases. Small patient populations require innovative trial designs and international collaborative efforts to coordinate and enable effective recruitment. Access to promising therapies remains constrained, often due to industry challenges around return on investment and the complexities of extending adult indications to paediatric populations, despite existing regulatory incentives. The Paediatric Bespoke Therapeutic Development Workshops aim to identify approaches with promise and accelerate therapeutics to clinical development.

The tumour microenvironment of primary and metastatic Medulloblastoma in a novel MYC-driven genetically engineered mouse model

Authors and Affiliations
Sumana Shrestha[1,2], Ruby Wang[2], Chantelle Bowers[2], Carlos Nery[2], John Anderson[2], Louis Chesler[1] and Laura Donovan[2]

1 Division of Clinical Studies, Institute of Cancer Research, London, UK.
2 University College London Great Ormond Street Institute of Child Health and Zayed Centre for Research in Rare Diseases of Childhood, London, UK.

Introduction

Medulloblastoma (MB) is the most common malignant brain tumour in children. The Group 3 subtype is the most aggressive, characterised by high rates of leptomeningeal dissemination to the spine at diagnosis, which is a major driver of mortality. Despite this, current therapeutic strategies are directed at the primary cerebellar tumour and do not specifically target these metastatic lesions. While the tumour microenvironment (TME) of primary Group 3 MB has been investigated, the cellular composition of the spinal metastatic niche remains poorly understood, representing a critical knowledge gap. To address this, we developed a novel, genetically engineered mouse model (GEMM) that recapitulates MYC-amplified Group 3 MB to perform the first detailed comparative analysis of the TME in primary versus metastatic sites.

Method

We used a novel GEMM that spontaneously develops MYC-amplified, Group 3-like medulloblastoma giving rise to spinal leptomeningeal metastases. Cerebellar primary tumours and matched spinal metastatic lesions were harvested at endpoint. We performed a comprehensive characterisation of the TME using multiplex immunohistochemistry and flow cytometry. Key cell populations were identified and quantified using a panel of markers, including NeuN for mature neurons, GFAP for astrocytes, Iba1 for microglia/macrophages, and CD45 for pan-leukocytes, alongside markers for stemness and other immune subsets.

Results

We identified key similarities in the immune landscape in both the primary and metastatic lesions. Both areas showed a significantly altered TME compared to non-tumour-bearing regions in wild-type controls. This was characterised by a significant reduction in the proportion of Iba1+ myeloid cells and a notable decrease in infiltrating CD45+ leukocytes, findings validated by flow cytometry. This shared feature strongly indicates the presence of a potent, actively maintained immunosuppressive microenvironment common to both the primary and metastatic sites. Our analysis also showed interesting differences in the non-malignant cellular composition between the two sites. Spinal metastases were significantly enriched with well-preserved, mature NeuN+ neurons and GFAP+ reactive astrocytes, cell types that were virtually absent from the core of the primary cerebellar tumours. This suggests a distinct interaction between metastatic cells and the resident cells of the spinal cord.

Conclusion

This study provides the first in-depth characterisation of the spinal metastatic microenvironment in Group 3 medulloblastoma using a clinically relevant, MYC-driven mouse model. Our findings showed that while metastatic lesions are uniquely integrated with mature neural cells, they share a immunosuppressive microenvironment with the primary tumour. This shared immune-depleted state represents a key therapeutic vulnerability. Targeting this immunosuppression could therefore be a powerful strategy to treat not only the primary tumour but also the metastatic disease that is the ultimate cause of treatment failure. This work provides critical insights for the development of novel immunotherapies aimed at reactivating an anti-tumour response across all disease sites in patients with Group 3 medulloblastoma.

In-silico characterisation of the medulloblastoma tumour immune microenvironment (TIME) at high resolution

Authors and Affiliations
Dean Thompson, John Anderson, Steven Clifford, Laura Donovan, Rebecca Hill, Louis Chesler

Newcastle University

Introduction

Medulloblastoma (MB) is the most common malignant brain tumour of childhood and remains a leading cause of cancer-related death and morbidity [1]. MB is molecularly heterogenous, comprising four groups – WNT, SHH, Group 3 and Group 4 – each characterised by distinct genetic and chromosomal abnormalities which influence prognostic outcome [2]. Beyond molecular variation, the tumour immune microenvironment (TIME) has also been shown to be relatively suppressive and group-specific, although existing studies have been largely limited to low-throughput in vitro or low-resolution in silico approaches [3]. As immune infiltration is increasingly recognised to influence both patient outcome and immunotherapeutic response in multiple cancers [4], it is imperative that the immune ecology of MB is characterised both at scale and at high resolution. To address this need, we developed an MB-specific high-resolution deconvolution signature based on gene expression data capable of estimating the proportions of immune and non-immune cell types. Application of this signature to a large RNA-sequencing cohort of over 1,200 tumours provides the most comprehensive description of the MB TIME landscape to date in silico, spanning both groups and subgroups.

Method

A MB-specific deconvolution signature capable of estimating malignant, neuronal and immune cell types was constructed from transcriptomic reference datasets [5-7]. Cell-type markers were derived using CIBERSORTx [5], with proportion estimates validated in silico using artificially generated pseudo-bulk mixtures and the cytolytic score index (CYT) [8]. The signature was validated and applied to a large RNA-seq cohort of primary MB samples (n=1,262) to characterise the TIME and evaluate differences across molecular groups and subgroups. TIME profiles were then subjected to k-means clustering to identify tumour subsets defined by immune infiltrate.
Associations between TIME clusters and clinico-molecular features were tested using chi-squared statistics.

Results

A human expression-based deconvolution signature comprising 919 genes and representing 26 cell types was developed. Validation against synthetic pseudo-bulk mixtures demonstrated excellent performance, with predicted cell fractions showing near-perfect correlation with ground truth ($R=0.998$, $p,0.001$). Predicted T-lymphocyte proportions were positively correlated with CYT score ($R=0.53$, $p<0.001$). Application of the signature to primary medulloblastoma tumours revealed significant differences in immune cell infiltrate across molecular group/subgroups, with variation observed in 24/26 cell types. Unsupervised consensus clustering of MB TIME profiles identified three robust immune clusters, distinguished by overall immune infiltration and cell type composition. These clusters were significantly associated with multiple clinico-molecular features, including molecular group ($p<0.005$) and MYC amplification ($p<0.05$).

Conclusion

We present the development of a high-resolution deconvolution signature capable of accurately characterising TIME in medulloblastoma. Significant differences in immune cell infiltrates were observed across molecular groups and subgroups, and unsupervised clustering revealed a subset of tumours with markedly elevated immune infiltration. The presence of this group challenges the prevailing view of medulloblastoma as uniformly immune ‘cold’ and suggests that certain tumours may be amenable to immunotherapeutic intervention.

Moreover, TIME clusters were significantly associated with key clinico-molecular features, indicating a link between immune infiltrate and disease outcome. Together, these findings provide a foundation for future studies exploring the biological and therapeutic relevance of the immune microenvironment in medulloblastoma.

Reshaping tumour immunity in MYC-driven medulloblastoma

Authors and Affiliations
Ben Draper[1], Dean Thompson[2], Zhen You[3], Alberto Delaidelli[4], Bethany Remeniuk[5], Rebecca Hill[2], Tom Jacques[1,6], Claudia Faria[7], Olivier Ayrault[8], Poul Sorenson[4], John Anderson[1,6], Louis Chesler[9], Steve Clifford[2], Frank Huang[10], Laura Donovan[1]

1 UCL Great Ormond Street Institute of Child Health, London, United Kingdom.
2 Newcastle University cancer Centre, Newcastle, United Kingdom.
3 Cincinnati Children's Hospital Medical centre, Cincinnati, USA.
4 BC Cancer research Centre, Vancouver, Canada.
5 Akoya, Marlborough, USA.
6 Great Ormond Street Hospital for Children, London, United Kingdom.
7 Hospital Santa Maria, Lisbon, Portugal.
8 Institut Curie, Paris, France.
9 Institute of Cancer Research, Sutton, United Kingdom.
10 Mayo Clinic College of Medicine and Science, Minnesota, United Kingdom

Scientific Abstract
One of the most significant unmet clinical challenges in paediatric oncology is the development of novel therapeutic strategies for recurrent medulloblastoma (R-MB). MYC-driven MBs are defined as classically cold tumours with a low incidence of infiltrating immune cells, resulting in a therapeutic challenge. The identification of cell-cell interactions between tumour and immune cells may provide insight into critical intercellular communications and manipulations occurring within the tumour immune microenvironment (TME). We hypothesised that the key cell-cell interactions in MYC-driven primary and recurrent MB may reveal dominant immune-suppressive mechanisms and uncover targetable therapeutic vulnerabilities.

Paired primary-recurrent bulk RNA-sequencing data, confirmed myeloid cells as the most infiltrating immune cell type in group3-MB and group4-MB. Comprehensive spatial phenotypic and cell-cell communication analyses corroborated this discovery, validating an increased incidence of macrophages in the matched-recurrent tumours. Subsequently, we used innovative algorithms for 10X MB single-cell data to predict interactions between tumour-cell ligands and immune-cell receptors within the TME; macrophages emerged as the core immune-cells involved in interactions throughout the TMEs, with the most significant ligand-receptor interaction and inflammatory response between MIF and CD74.

In-depth immunohistochemistry analyses of primary and recurrent group3 and group4 tumours, and exhaustive tissue microarrays demonstrated expression of both CD74 and MIF, with limited expression of CD74 within the brain. To investigate the therapeutic potential of CD74, we developed recurrent, immune competent MYC-driven medulloblastoma mouse models. Comprehensive deconvolution analysis confirmed the TME integrity of our models to mirror that of the human disease. Locoregional delivery and repeat dosing of a bioactive-CD74 peptide demonstrated complete tumour clearance in our immune-competent mouse models of primary and recurrent MB, demonstrating the significant therapeutic potential of targeting the CD74-MIF axis in MYC-driven primary and recurrent MB.

Key cellular interactions and therapeutic vulnerabilities within the tumour microenvironment of MYC-driven medulloblastoma (MB) have been identified, highlighting the CD74-MIF axis as a target for next-generation immunotherapies.

Our findings establish diagnostic and relapsed MB as profoundly immune-compromised malignancies. Targeting the MIF-CD74 axis, either alone or in combination with other immunotherapeutic approaches, may offer a promising strategy to enhance anti-tumor immune responses. The interaction between MIF and CD74 serves as an exemplar of broader targeting strategies within such disease mechanisms. The proof of concept successfully validates the effectiveness of this pipeline, demonstrating its potential for wider application in therapeutic interventions.

Targeting MYB in paediatric acute myeloid leukaemia

Authors and Affiliations
Dr Olivia Ciampa

UCL Great Ormond Street Institute of Child Health
30 Guilford Street, London, WC1N 1EH

Introduction
Acute myeloid leukaemia (AML) is a highly aggressive malignancy and remains one of the deadliest childhood cancers. Treatment relies heavily on highly toxic chemotherapeutic regimens, which cause severe acute and long-term side effects. More importantly, in relapsed AML, intensifying chemotherapy often becomes redundant and fails to improve outcomes. This underscores the urgent need for novel, non-toxic therapies that exploit disease specific vulnerabilities. The transcription factor MYB has emerged as a compelling therapeutic target, as many AML subtypes exhibit increased dependency on MYB activity compared to normal haematopoietic cells. Our lab has previously identified the anti-helminth drug mebendazole as a potential MYB-directed therapeutic capable of inducing rapid MYB ablation and exerting potent, specific anti-AML therapeutic efficacy. This study aims to define core pathways and genetic vulnerabilities in AML that govern MYB dependence and mebendazole's therapeutic response.

Method
To further characterise the molecular mechanisms underlying AML cells' responses to MYB inhibition by mebendazole, we performed genome-wide CRISPR/Cas9 dropout screens. These screens were designed to identify genes and pathways that influence AML sensitivity or resistance to MYB targeting. Specifically, we sought to determine which genetic alterations would impact the effectiveness of mebendazole and whether any pathways were implicated in mediating therapeutic resistance in AML cells. Candidate genes identified from the screen were subsequently validated using targeted CRISPR/Cas9 knockout approaches. Additionally, we conducted further CRISPR screens to identify genes whose inhibition could potentially enhance the anti-AML efficacy of mebendazole.

Results
The genome-wide CRISPR/Cas9 screens revealed a set of novel genes and biological pathways that influence AML survival when MYB is inhibited by mebendazole. Among these, we identified anti-apoptotic survival mechanisms which may mediate therapeutic resistance. Additionally, a subset of genes were identified whose inhibition sensitised AML cells to mebendazole, enhancing its therapeutic potential.

Conclusion
This study aims to define core pathways and genetic vulnerabilities in AML that govern MYB dependence and mebendazole's therapeutic response. By characterising mechanisms of resistance and genetic vulnerabilities that can be exploited to enhance treatment efficacy we aim to develop a rational design of targeted therapies that exploit these dependencies. Future efforts will focus on developing low-toxicity combination strategies that integrate MYB-directed therapies to improve therapeutic outcomes while reducing reliance on traditional chemotherapy. These findings bring us closer to developing more effective, less toxic treatment options for paediatrics with AML who are most vulnerable to the long-term consequences of chemotherapy.

Inotuzumab Ozogamicin-resistant B-ALL cells reside within a protective bone marrow niche

Authors and Affiliations
Penny Sparkes

Imperial College London

Scientific Abstract
Inotuzumab ozogamicin (IO) is a CD22-targeted antibody conjugated to the DNA-damaging toxin calicheamicin used in the treatment of relapsed/refractory B-cell acute lymphoblastic leukaemia (B-ALL). IO is highly effective at inducing remission in B-ALL, however patients inevitably relapse, and mechanisms of IO resistance are poorly understood. Previous studies suggest chemo-resistant B-ALL cells are not genetically distinct from the treatment sensitive bulk population and reside within in a protective 'niche' with nestin-positive mesenchymal stromal cells (MSC) (Duan, 2014; Ebinger 2016; Turati 2021). We have demonstrated that leukaemia cells resistant to DNA-damaging chemotherapy are protected in a bone marrow niche containing nestin-positive mesenchymal stromal cells (MCS) via mitochondrial transfer and antioxidant protection (Burt, 2019).

We sought to determine if IO-resistant cells, like chemo-resistant B-ALL cells, survive within a bone marrow niche in human xenograft models of B-ALL. The human B-ALL cell lines SEM (KMT2A-AF4) and REH (ETV6-RUNX1) were transduced with tdTomato and injected into NOD-scid IL2Rgammanull (NSG) nestin-GFP reporter mice. This allowed us to visualise in real time in live anaesthetised mice, the interactions between B-ALL cells and nestin-positive MSC in the bone marrow before and after treatment with IO using intravital microscopy (IVM). On sufficient engraftment, mice were treated with 3 doses of IO or vehicle control (PBS). IVM of the calvarium was performed 1 day prior to the first dose and 24 hours after each dose and 3D images and time lapse videos acquired. After imaging, mice were sacrificed and femurs collected for immunofluorescence imaging to validate the IVM calvarium findings. Niche formation was assessed by 1) proximity of B-ALL cells to nestin-positive MSC and 2) changes in motility or behaviour of B-ALL cells in treated versus untreated mice.

We observed that both SEM and REH cells were found close to and in contact with nestin-positive MSC in vivo. This was despite markedly different responses in disease burden to IO in the REH and SEM xenograft models (SEM untreated 81.1% vs IO-treated 73.0%; REH untreated 19.6% vs IO-treated 0.13%). The minimum distance of SEM B-ALL cells to their nearest nestin-positive MSC neighbour was lower in IO-treated mice versus PBS controls in both the calvarium (all time points, $p<0.0001$) and femur bone marrow (after doses 2 and 3, $p<0.0001$), suggesting IO-resistant cells associate with MSC. Analysis of SEM cell motility showed that IO-treated B-ALL cells moved more slowly ($p=0.0012$) and shorter distances compared to PBS controls ($p=0.0024$) consistent with niche formation. Preliminary examination of REH is consistent with SEM results but analysis is ongoing.

Our findings suggest IO-resistance B-ALL cells are protected within a bone marrow 'niche' with nestin-positive MSC. This appears to be independent of the degree of response to IO or the B-ALL genetic sub-type and similar to DNA-damaging chemotherapy. This suggests a conserved protective mechanism in the leukaemic bone marrow to genotoxic and oxidative stress induced by both DNA damaging chemotherapy and IO, a targeted antibody drug conjugate. We are currently validating these results in patient derived xenograft models and exploring mechanisms to inhibit niche formation to overcome IO-resistance.

Iberdomide enhances drug-switchable CAR-T cell function but hinders scale-up manufacturing in GMP-compatible platforms

Authors and Affiliations
Ksenia Zornikova[*1], Marilena Nicolaidou[*1], Sonia Morlando[1], Annie Etuk[1], Marta Barisa[1], John Anderson[1]
*equal contribution

UCL GOS Institute of Child Health, London, United Kingdom

Scientific Abstract
Weak ligand-independent tonic signalling in CAR-T cells is a marker of efficiency and plays an important role in cell homeostasis. However, excessive autonomous signalling can lead to overstimulation and exhaustion. Switchable CAR-T cell technologies enable control of CAR expression through drug-inducible degrons, such as iTAG2, which reduce tonic signalling during both manufacturing and post-infusion. Iberdomide, a member of the thalidomide-derivative immunomodulatory drug (IMiD) family, acts as a molecular glue that induces proteasomal degradation via recruitment of the E3 ubiquitin ligase cereblon.

We developed a GMP protocol for phase I clinical trials in paediatric brain and extracranial tumours. Various serum-free protocols were tested to produce anti-B7H3 TE9-28z-iTAG2 CAR-T cells in the presence or absence of Iberdomide, using two GMP-compatible large-scale manufacturing platforms - the CliniMACS Prodigy and the G-Rex gas-permeable bioreactor- as well as conventional tissue culture plates. During manufacture, expansion and transduction efficiency were assessed, while cell functionality and cytotoxicity were evaluated post-manufacture.

CAR-T cells treated with Iberdomide during manufacturing in conventional tissue culture plates with GMP-compatible reagents demonstrated enhanced functionality both in vitro and in vivo. Consequently, we translated Iberdomide-based manufacturing into large-scale platforms. However, Iberdomide-treated cells exhibited reduced expansion compared to untreated cells in both the CliniMACS Prodigy and the G-Rex.

Transduction efficiency was higher in the Prodigy compared to the G-Rex and to cells produced in a traditional tissue culture plates. In both large-scale manufacturing platforms, every-other-other treatment with 100 nM Iberdomide was insufficient to downregulate CAR expression, requiring modifications to the original protocol established in conventional tissue culture plates. Despite this incomplete CAR downregulation, CAR-T cells treated with Iberdomide exhibited higher proportion of non-exhausted $CD4^+/PD\text{-}1^-/Tim\text{-}3^-$ cells compared to untreated controls, whereas this effect was not observed in $CD8^+$ cells. Furthermore, Iberdomide-treated CAR-T cells demonstrated enhanced cytotoxicity and cytokine release against target cells derived from a B7H3-expressing medulloblastoma cell line.

These findings indicate substantial therapeutic potential for IMiD-treated CAR-T cells. However, scaling up manufacturing from traditional 2D tissue culture systems to 3D bioreactor platforms appears to face significant challenges. Optimisation of current protocols will be necessary to enable large-scale production of drug-switchable CAR-T cells and to support their translation into clinical trial applications.

Temporal transcriptomic reprogramming following cranial radiotherapy in a medulloblastoma treatment model reveals targets for mitigating neurocognitive late effects

Authors and Affiliations
Simaran Johal[1*], Jemma Castle[1*], Samantha Jepson Gosling[1], Sasha Gartside[2], Steve Clifford[1], Teklu Egnuni[3], Susan C. Short[3], Debbie Hicks[1†], Daniel Williamson[1†]

1 Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK
2 Institute of Neuroscience, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom
3 Leeds Institute of Medical Research, Wellcome Trust Brenner Building, St James's University Hospital, Beckett St, Leeds, UK

* joint first authors
† joint senior authors

Introduction

Current multimodal therapies - surgery, cranial radiotherapy (CRT), and chemotherapy - have significantly improved survival rates for children diagnosed with brain tumours, currently an estimated 80% of children survive this diagnosis. However, CRT exposure during critical periods of brain development comes with a cost; exposure of CRT to the developing brain increases the risk of neurocognitive deficits, particularly in processing speed, attention and working memory. High-dose CRT, used to treat medulloblastoma (MB), poses a significant risk, with >50% of survivors developing progressive neurocognitive deficits, including global intellectual impairment, which can substantially reduce quality of life. While CRT has been linked to late effects, the biological mechanisms driving these outcomes remain poorly understood. To address this gap, we developed an in vivo model system that recapitulates the radiotherapy dose, delivery and late-effect profile of childhood medulloblastoma, at an equivalent developmental stage[1]. In this project, we aim to map the biological mechanisms by which late effects manifest to reveal new targets for amelioration.

Methods

We administered CRT to C57BL/6J mice, mirroring human-equivalent dosages (36Gy over 10 fractions) at Post-Natal Day (PND) ~33, using the small animal radiation research platform (SARRP). To capture regional and temporal specificity in the murine brain's response to CRT we performed a longitudinal transcriptomic analysis using RNA-sequencing at six timepoints (1,3,7,28, 90, 180 days post-CRT) across four different brain regions (cerebellum, hippocampus, cortex and forebrain) (total N=152, samples with and without CRT treatment). Differential expression was carried out using time and region-dependent models. Gene Set Enrichment Analysis (GSEA) was used to identify enrichment of hallmark and biological process pathways to ascribe likely functions of genes and pathways disrupted by CRT.

Results

CRT induced two distinct waves of transcriptomic reprogramming. The first wave, observed at 3 days post-CRT reflects acute injury and includes signatures of apoptosis, blood-brain barrier disruption, cellular stress, and inflammatory cytokine expression. The second wave, at 28 days post-CRT, is characterised by persistent microglial activation, suggesting a chronic pro-neuroinflammatory state, reduced glutamatergic and GABAergic synaptic transmission and reduced myelin assembly. Interestingly, though there is evidence of recovery in neuronal precursor proliferation and neuroblast activity at this stage, pathways associated with neuronal dysfunction remain active. This indicates that while some regenerative processes are initiated, functional recovery is incomplete, and chronic neuroinflammation persists. This temporal reprogramming in response to CRT was independent of brain region.

Conclusion

Our findings demonstrate temporally distinct transcriptomic responses to CRT, with the 28 days post-CRT timepoint representing the critical window during which neuroinflammatory states are sustained and likely to contribute to long-term neurocognitive impairment. The persistent activation of microglia and incomplete neuronal recovery observed at 28 days post-CRT may highlight key regulatory nodes amenable to pharmacological modulation. These insights could pave the way for the development of neuroprotective agents or immune-modulating therapies that target chronic inflammation and support neuronal regeneration. We will further model this key temporal window by constructing a high-resolution single cell atlas to investigate vulnerable cell identities, states and trajectories that are sensitive to CRT and identify novel molecular targets for therapeutic intervention.

Modelling ETMR: MYCN overexpression in human neuroepithelial stem cells drives tumour formation

Authors and Affiliations
Sumana Shrestha[1,2], Colin Kwok[1], Diego Chillon Pino[2], Yura Grabovska[3], Barbara Martins da Costa[1], Kevin Greenslade[1], Karen Barker[1], Claire Lynn[1], Yann Jamin[4], Ben Ho[5], Fupan Yao[5], Ulrich Shüller[6], Laura Donovan[2], Annie Huang[5] & Louis Chesler[1]

1 Division of Clinical Studies, Institute of Cancer Research, London, UK.
2 University College London Great Ormond Street Institute of Child Health and Zayed Centre for Research in Rare Diseases of Childhood, London, UK.
3 Molecular Pathology, Institute of Cancer Research, London, UK.
4 Radiotherapy and Imaging, Institute of Cancer Research, London, UK.
5 Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Canada.
6 Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Introduction

Embryonal Tumour with Multilayered Rosettes (ETMR) is a rare and exceptionally aggressive paediatric brain cancer, with five-year survival rates below 30%. A lack of clinically relevant preclinical models has severely hampered efforts to understand its biology and develop effective therapies. The transcription factor MYCN, essential for normal neurogenesis, is frequently overexpressed in ETMR and linked to its rapid progression. However, whether MYCN is sufficient to initiate this disease, and from which cell of origin, remains a critical unanswered question. Given the embryonal and neural characteristics of the disease, we hypothesised that overexpression of MYCN in human hindbrain-derived neuroepithelial stem (hbNES) cells, a physiologically relevant progenitor population, is sufficient to drive tumorigenesis and create a relevant model of ETMR.

Method

We generated stable hbNES cell lines with doxycycline-inducible overexpression of a stabilised MYCN mutant (MYCN-T58A) using a Tet-On system. These cells were injected intracranially into immunocompromised NSG mice. Tumour initiation and progression were monitored non-invasively using high-resolution MRI. Upon reaching endpoint, tumours were harvested for comprehensive analysis, including histopathology, immunohistochemistry for key ETMR markers, bulk RNA-sequencing, and global DNA methylation profiling using the EPIC array.

Results

MYCN-overexpressing hbNES cells resulted in the formation of aggressive, high-grade tumours with 100% penetrance, leading to a median survival of ~50 days. Tumours arose in multiple locations, including both supratentorial and infratentorial regions, mirroring the clinical presentation of ETMR. Histologically, the xenograft tumours were highly similar to patient ETMRs, displaying characteristic features such as primitive neuroepithelial cells, high mitotic activity, and classic multilayered rosettes. Molecularly, the tumours showed significant fidelity to the human disease. Global DNA methylation profiling demonstrated that the hbNES-derived tumours robustly clustered with clinical ETMR samples, clearly separating from other paediatric brain tumour entities. Furthermore, gene expression analysis confirmed the activation of hallmark ETMR transcriptional programs.

Conclusion

This study shows for the first time that MYCN overexpression is sufficient to transform human neuroepithelial stem cells into tumours that robustly recapitulate the key histological and molecular features of ETMR. We have successfully established a reproducible, and clinically relevant human-derived model for a disease that has historically been incredibly difficult to study. This inducible system provides a new opportunity to dissect the temporal molecular events of MYCN-driven tumour initiation and progression. This model will serve as an invaluable platform for future preclinical studies aimed at identifying novel therapeutic vulnerabilities and accelerating the development of more effective treatments for children with ETMR.

Charactering the Tumour Microenvironment of PAWS-GIST through Spatial Transcriptomics and Single-Nucleus RNA Sequencing

Authors and Affiliations
Jack Harrington, Debbie Glencross, Zehor Belkhatir, Luke Hill, Dean Bryant, Zoë Walters

University of Southampton

Introduction

Paediatric, adolescent, wild-type and syndromic gastrointestinal stromal tumours (PAWS-GISTs) are a rare and poorly understood subset of GISTs that account for the majority of cases in children and adolescents. These tumours lack the common KIT or PDGFRA mutations that drive most adult cases and are frequently associated with succinate dehydrogenase (SDH) deficiency. Consequently, these tumours are resistant to the standard treatment tyrosine kinase inhibitors, leaving patients with limited treatment options and a poor prognosis [1]. Due to the limited access to material and lack of suitable preclinical models, PAWS-GISTs remain underexplored with a limited understanding on the tumour's biology. This project aims to provide a better understanding of PAWS-GISTs to ultimately identify more effective treatment strategies and improve outcomes for patients.

Method

In collaboration with the National GIST Biobank, this project aims to characterise the cellular and spatial landscape of PAWS-GISTs using a combination of spatial transcriptomics (10x Visium) [2] and single nucleus RNA sequencing (snPATHO-seq) [3] of matched samples. This allows for detailed spatial and transcriptional characterisation of matched tumour regions from the same PAWS-GIST patient and allows for the identification of cell types and tumour microenvironment interactions.

In tandem, primary cell cultures have been derived from fresh PAWS-GIST samples, in addition to isolating matched peripheral blood mononuclear cells (PBMCs) and cancer associated fibroblasts (CAFs). The establishment of these models provide a platform for functional exploration of TME interactions such as co-culture models and the discovery and testing of new therapeutics.

Results

Preliminary data reveal a diverse and spatially structured microenvironment, including areas of proliferative cancer cells and heterogeneous fibroblast and immune cell populations. These findings highlight the biological complexity of PAWS-GISTs and could provide novel therapeutic targets with further exploration and sample numbers.

Primary cultures from fresh tumour material have been successfully established and are currently being expanded. Matched CAF and PBMC populations provide the opportunity to model tumour, stroma and immune interactions in vitro, enabling functional validation of candidate pathways identified from spatial and single-cell analysis.

Conclusion

This study represents the first spatial characterisation of PAWS-GIST and lays important groundwork for understanding the biology of this rare and clinically challenging tumour type. By combining spatial transcriptomics, single-nucleus RNA-seq and matched functional models, we aim to better characterise the tumour microenvironment. Ultimately, this work will support the development of more targeted treatments for PAWS-GIST and help create a resource that will be valuable to the wider GIST and rare tumour research community.

References

1. Mavroeidis L, Kalofonou F, Casey R, et al. Identifying and managing rare subtypes of gastrointestinal stromal tumors. Expert Rev Gastroenterol Hepatol 2025;19(5):549-61.
2. Dong Y, Saglietti C, Bayard Q, et al. Transcriptome analysis of archived tumors by Visium, GeoMx DSP, and Chromium reveals patient heterogeneity. Nat Commun 2025;16(1):4400.
3. Wang T, Roach MJ, Harvey K, et al. snPATHO-seq, a versatile FFPE single-nucleus RNA sequencing method to unlock pathology archives. Commun Biol 2024;7(1):1340.

Serine/glycine biosynthesis is a novel targetable therapeutic vulnerability in MYC-amplified medulloblastoma

Authors and Affiliations
Bethany Poole[1*], Magretta Adiamah[1*], Janet C Lindsey[1], Alaide Morcavallo[2], Helen J Blair[1], Mankaran Singh[1], Samantha Jepson Gosling[1], Melanie Beckett[1], Janice Law[1], Dean Thompson[1], Shanel Swartz[1], Sarah Kohe[3], Tong Zhang[4], Oliver Maddocks[4], Andrew Peet[3], Louis Chesler[2], Ian Hickson[1], Ross Maxwell[1], Rebecca M Hill[1*], Steven C Clifford[1*]

1 Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Newcastle University, Newcastle upon Tyne, UK
2 Division of Clinical Studies, Institute of Cancer Research (ICR), London and Royal Marsden NHS Trust, Sutton, UK
3 Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK
4 Institute of Cancer Sciences, University of Glasgow, Glasgow, UK

Introduction
Medulloblastoma is the most common high-grade paediatric brain tumour. International consensus recognises four principal molecular groups: wingless (MBWNT), sonic hedgehog (MBSHH), Group 3 (MBGRP3) and Group 4 (MBGRP4). MBGRP3 accounts for approximately 25% of cases and is enriched for amplification of the proto-oncogene MYC. MYC-amplified MBGRP3 (MYC-MBGRP3) tumours are highly aggressive, refractory to current multi-modal therapy, and are almost universally fatal (<10% survival). Identifying novel therapeutic strategies for these patients therefore represents one of greatest clinical unmet needs in paediatric oncology. Since direct targeting of MYC remains elusive, attention has focused on targeting more therapeutically amenable MYC co-dependencies. MYC-MBGRP3 tumours undergo substantial metabolic rewiring; we have previously demonstrated that these tumours exhibit a dependence on the de novo serine/glycine synthesis pathway (SSP), catalysed by the rate-limiting enzyme PHGDH. Given the metabolic constraints imposed by the low levels of exogenous serine/glycine in the brain and surrounding cerebrospinal fluid, targeting PHGDH potentially represents a novel, synthetically lethal, therapeutic strategy for MYC-MBGRP3 tumours.

Method
Pharmacological inhibition of PHGDH using NCT-503 was assessed in MYC-MBGRP3 cell lines, alongside two independent subcutaneous xenograft (D425) and spontaneous transgenic (GTML) mouse models. Genetic loss-of-function studies were performed using three independent PHGDH gene knockout (PHGDH-KO) MYC-MBGRP3 cell lines by CRISPR. Phenotypic characterisation of PHGDH-KO cell lines (cell proliferation, colony formation, SSP expression, reactive oxygen species (ROS) production) was undertaken in vitro under serine/glycine-replete and serine/glycine-limiting conditions. Bulk RNA sequencing (RNAseq) was performed on PHGDH-KO MYC-MBGRP3 cells to investigate changes in gene expression and metabolic pathway activation under serine/glycine-replete and serine/glycine-limiting conditions. PHGDH-KO MYC-MBGRP3 cells were injected subcutaneously and orthotopically into immunodeficient mice to understand the role of PHGDH and the surrounding tumour microenvironment on MYC-MBGRP3 tumour progression.

Results
NCT-503 treatment demonstrated on-target inhibition of PHGDH and antiproliferative efficacy in MYC-MBGRP3 cell lines. In vivo, NCT-503 slowed tumour progression and significantly prolonged survival across both MYC-MBGRP3 mouse models ($p=0.024$). Under serine/glycine-limiting conditions in vitro, PHGDH-KO impeded cell proliferation, reduced colony formation, and increased oxidative stress across all three MYC-MBGRP3 cell line models. Mechanistically, inhibition of serine/glycine metabolism induced a global metabolic stress response consistent with reduced glutathione metabolism, nucleotide synthesis, and mTOR pathway activation following RNAseq analysis. Interestingly, PHGDH-KO also downregulated MYC expression and MYC-associated transcriptional

continued

programmes. Critically, over 70% of orthotopic PHGDH-KO tumours failed to engraft in mice (29% (4/14) vs. 100% (14/14) engraftment of MYC-amplified PHGDH expressing controls; $p<0.0001$). In contrast, 100% (3/3) of subcutaneous PHGDH-KO tumours engrafted and displayed comparable growth dynamics to MYC-amplified PHGDH expressing controls (100%, 3/3).

Conclusion

Inhibition of serine/glycine metabolism represents a novel indirect MYC-targeting strategy in MYC-MBGRP3. We have uncovered a potential role for PHGDH in MYC-MBGRP3 tumour formation that appears to be selective to the brain tumour microenvironment. These findings provide the foundations for further exploration of the role of PHGDH in MYC-MBGRP3 tumour maintenance to validate PHGDH as a therapeutic target. The development of more potent PHGDH inhibitors is now essential to advance clinical translation of this strategy for this poor-prognosis disease group.

Modelling Metastatic Medulloblastoma for Therapeutic Discovery

Authors and Affiliations
Chantelle E Bowers[1], Diego Pino[1], Sumana Shrestha[2], Carlos Lorenzo G Nery, Melanie Schoof, Dean Thompson[3], Karen Barker[2], Ulrich Schuller, John Anderson[1], Steve Clifford[3], Louis Chesler[2], Laura K Donovan[1]

1 Great Ormond Street Institute of Child Health, University College London, UK
2 The Institute of Cancer Research, Sutton, UK
3 Wolfson Childhood Cancer Research Centre, Newcastle University, Newcastle upon Tyne, UK
4 Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction
Medulloblastoma is the most common malignant paediatric brain tumour. Group 3 tumours, characterized by c-Myc amplification, have the poorest prognosis, with metastases present at diagnosis in approximately 30% of patients, and the leading cause of cancer-related deaths. Progress in understanding the biology of these metastases and their local tumour immune microenvironment has been limited by the lack of representative preclinical models, slowing the development of targeted therapies. To address this gap, we have generated an immunocompetent genetically engineered mouse model (GEMM) of metastatic Group 3 medulloblastoma, driven by human c-Myc amplification under the Blbp promoter. Hence, providing a platform to investigate the molecular mechanisms underlying leptomeningeal dissemination and vulnerabilities suitable for therapeutic interventions.

Materials & Methods
Comprehensive phenotypic characterisation of this model was initially performed, including survival analysis, histology, comprehensive flow cytometry and immunohistochemistry to validate its fidelity to the human disease. To dissect the molecular drivers of metastasis, we have produced multi-omic analyses, including bulk and single RNA-sequencing, methylation arrays, and spatial transcriptomics on both primary tumours and their corresponding metastatic lesions. Furthermore, we have developed a novel dissection technique that allows for the precise isolation of the meninges, enabling an unprecedented characterisation of the metastatic tumour cells and the leptomeningeal microenvironment.

Results
Our c-MYC model faithfully recapitulates key features of human Group 3 medulloblastoma, including the development of highly aggressive primary tumours and spontaneous, robust leptomeningeal metastasis, with tumour histology consistent with the classic, large-cell/anaplastic features seen in patients. Comparative analysis revealed that the leptomeningeal metastases are transcriptionally distinct from their matched primary tumours, occupying a unique molecular state within an immunologically active microenvironment, comprising mostly of immunosuppressive myeloid cells
Further transcriptomic and epigenomic analyses are currently underway to identify distinct molecular signatures that differentiate metastatic clones from the primary tumour and to map the cellular architecture of the metastatic niche.

Conclusions
This highly relevant GEMM represents a powerful tool for studying the pathogenesis of Group 3 medulloblastoma. By integrating advanced molecular profiling with detailed spatial analysis, we aim to uncover the key mechanisms that enable tumour cells to colonise and thrive in the leptomeningeal space. Ultimately, this model will serve as a crucial platform for the pre-clinical evaluation of novel therapeutic strategies designed to target both the primary tumour and the metastatic component of this devastating disease, with the goal of improving outcomes for children with Group 3 medulloblastoma.

Development of novel MT1-MMP activated peptide prodrugs for targeted delivery in Osteosarcoma

Authors and Affiliations
Hannah LM Spencer, Alaa Abdullah, Jad Kattan, Steven D Shnyder, Paul M Loadman, Robert A Falconer.

Institute of Cancer Therapeutics, Faculty of Life Sciences, University of Bradford, Bradford, BD7 1DP

Introduction

Introduction: Osteosarcoma (OS) is the most common form of primary malignant bone tumour and exhibits its highest prevalence in children and adolescents. The survival rate for those who develop OS has not improved significantly over the past 30 years primarily due to the lack of novel therapeutics1.

Methotrexate (MTX) is widely used in the treatment of OS, despite the many adverse side effects associated with its administration. One approach to this problem is to selectively target the drug to the tumour, using tumour-expressed enzymes to activate a prodrug. One such family of enzymes are the matrix metalloproteinases (MMPs), that have roles in cancer progression and metastasis2. MT1-MMP is overexpressed and functionally active in osteosarcoma tissue, and plays a key role in tumour development3. This project focuses on the development of MTX prodrugs that utilise this proteolytic activity of MT1-MMP allowing for selective delivery of MTX.

The overall aim of this work is to synthesise novel MMP activated methotrexate prodrugs with enhanced efficacy and lower toxicity in vivo, thereby resulting in fewer side effects for patients.

Method

Initially the project focused on the chemical synthesis and purification of a series of MTX-conjugated peptide conjugates. Following assessment of activation of the prodrug in tumour tissues ex vivo, the metabolic stability of all the compounds synthesised is screened in normal tissues on the bench (liver, kidney). Following lead identification a full body mouse pharmacokinetics (PK) study was performed.

Results

The rates of metabolism and stability of MTX-conjugated prodrugs has been determined. 4 compounds were identified which displayed enhanced normal tissue stability and successful release of methotrexate ex vivo. These were assessed in a preliminary PK study. Compound 14 was taken forward for complete evaluation. HT1080 tumour bearing mice were treated with compound 14 or an equivalent dose of MTX. We observed successful release of MTX from 14 in tumour tissue with a notable reduction in the Cmax of MTX in plasma (10-fold) and liver (14.5 fold) samples, when compared to MTX alone.

Conclusion

Following the successful design and synthesis of a series of novel MTX-conjugated prodrugs, the rates of the ex vivo stability of these conjugates were investigated. Lead compound 14 demonstrated differential tumour release in vivo, and is currently the subject of a lead optimisation programme.

References

1. Spencer HLM, Shnyder SD, Loadman PM, Falconer RA. The role of MT1-MMP in the progression and metastasis of osteosarcoma. J Cancer Metastasis Treat 2022;8:2
2. 2. Visse R, Nagase H, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovascular Research 2006;69:562-73.
3. Gong Y, Chippada-Venkata UD, Oh WK. Roles of matrix metalloproteinases and their natural inhibitors in prostate cancer progression. Cancers 2014;6:1298-327.

Developing a 3D model of the bone marrow niche to investigate the influence of folate on childhood leukaemia-initiating events

Authors and Affiliations
Dr Jessica Saville, Jill McKay, Kate Porter, Kay Padget, Lisa Russell, Deepali Pal

Northumbria University

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common cancer in children. Although aetiology is unclear, various genetic aberrations, such as chromosomal translocations, are suggested to be initiating events in ALL development and have been retrospectively detected at birth, suggesting these events occur in utero. Further factors, such as epigenetic modifications, are also likely required for disease progression. Epidemiological evidence suggests maternal folate status is likely to influence risk of childhood leukaemia. Folate is known to influence DNA damage, repair and methylation status. Current guidelines recommend pregnant women consume synthetic folic acid (FA) supplements during the first trimester, however, biologically active folate, 5-methytetrahydrofolate (5mTHF), is becoming more popular in pregnancy targeted supplements. Our aim is to investigate the influence of FA and 5mTHF on leukaemia-initiators using organoid models to replicate the bone marrow niche.

Method

Mesenchymal stem cells (MSCs) were used to create 2D and 3D organoids supporting growth of GM12878 B cells in media containing physiologically relevant concentrations of FA or 5mTHF. Cell growth was evaluated through trypan blue exclusion. Intracellular and media folate was measured using LC-MS. Reverse-transcription PCR is used to monitor cell markers and identify leukaemia-initiating events.

Results

When grown in co-culture with MSCs, B cell growth is increased compared to growing alone. A further increase in B cell growth was observed when grown in the presence of 3D spheroids compared to 2D monolayers of MSCs. When grown in folate deficient conditions a reduction in B cell growth compared to standard media is observed for both FA and 5mTHF supplementation over 9 days. A reduction in intracellular folate was observed as early as 2 days following folate depletion.

Conclusion

We have optimised a 3D model of folate in B cells, which will allow us to investigate the induction of leukaemia-initiators. Such knowledge may be useful to influence public health policy for folate guidance during pregnancy.

Folate depletion alters DNA methylation in tissues relevant for childhood leukaemia

Authors and Affiliations
Dr Jessica Saville, Jill McKay, Akram Ghantous Kay Padget, Lisa Russell, Farah Nassar

Northumbria University

Introduction
Epidemiological evidence suggests maternal folate status influences childhood leukaemia risk; however, the underlying mechanisms are unclear. Genetic aberrations, are considered leukaemia-initiating events, however, alone they are not sufficient for disease, with additional 'hits' required. Epigenetic alteration, i.e. DNA methylation, is one mechanism by which maternal folate is likely to contribute to disease. We investigated the relationship between folate depletion and DNA methylation in murine tissues relating to childhood leukaemia.

Method
Female mice were allocated low or normal folate diets for 4 weeks before mating and onwards. Offspring were sampled at 17.5 days gestation and weaning. DNA was isolated from fetal livers and bone marrow of weaned mice. Tissues correspond to specific sites of haematopoiesis throughout the lifecycle relevant to leukaemic blasts. Epigenome-wide DNA methylation was profiled using Infinium® MouseMethylation BeadChips. R/Bioconductor packages identified differentially methylated points (DMP) and regions (DMRs). Pathway analysis was conducted by DAVID.

Results
There were 598 DMPs (382 hypermethylated/216 hypomethylated) and 363 DMRs with significantly (FDR) altered methylation in response to folate depletion in the fetal liver. In bone marrow, 1206 DMPs (334 hypermethylated/852 hypomethylated) and 565 DMRs had significantly altered methylation in response to folate depletion. Only 3 DMPs and 13 DMRs were altered in both tissue types, suggesting highly tissue specific responses. Pathway analysis suggested 15 and 44 pathways could be altered in response to folate depletion in liver and bone marrow respectively, with 8 overlapping between tissues.

Conclusion
Whilst loci specific altered methylation appears to be different between tissues, the impact on biological pathways is similar.

Evaluating the combinatory anti-tumour efficacy of radiotherapy with CAR-T cell therapy against paediatric neuroblastoma

Authors and Affiliations
Gaya Nair, Courtney Himsworth, Thomas Jackson, Louis Chesler & John Anderson

Zayed Centre for Research into Rare Disease in Children

Introduction
Neuroblastoma (NB) is the most common extracranial solid tumour in children. Despite intensive multimodal therapies, outcomes for high-risk NB remain among the poorest of all paediatric cancers. Targeted approaches such as immunotherapy offer new opportunities; for example, clinical trial data show that the monoclonal antibody dinutuximab, which targets the tumour associated antigen GD2, improves overall survival rates. Chimeric Antigen Receptor (CAR) T cell therapy is another promising immunotherapy, harnessing T cells to exert a direct cytotoxic effect against tumours. For effective CAR-T cell engraftment, patients first undergo lymphodepletion, a process shown to be essential for therapeutic efficacy. This is commonly achieved with Fludarabine and Cyclophosphamide, though radiotherapy may also be used. Preliminary data in immunodeficient NB mouse models suggests that radiotherapy-based pre-conditioning enhances the anti-tumour response of CAR-T cell therapy. Building on this, we aim to evaluate lymphodepletion, focal radiotherapy pre-conditioning, and anti-GD2 CAR-T cells, individually and in combination, all in the context of immunocompetent NB mouse models.

Methods
Spleens were harvested from C57B/6 mice from which T cells were isolated and activated with anti-CD3 and anti-CD28. T cells were cultured in RPMI media supplemented with IL-2 for 48hrs, after which retrovirus and homology directed repair were used to generate CAR-T cells, assessed via flow cytometry. CAR-T cells were cryopreserved and thawed to assess viability. CAR-T cells were plated in 24-hour co-cultures in vitro against a GD2 positive tumour line, and cytotoxicity was assessed via flow cytometry. C57Bl/6 mice were total body irradiated (TBI) and lymphodepletion was evaluated by assessing the immune profile of peripheral blood via flow cytometry.

Results
Anti-GD2 CAR-T cells demonstrated cytotoxicity, specifically against antigen-positive cell lines. Following cryopreservation, CAR-T cells maintained adequate viability upon thawing. In C57Bl/6 mice, analysis of peripheral blood after TBI revealed reductions in both myeloid and lymphocyte compartments that persisted for up to 10 days. By 28 days post-irradiation, circulating leukocytes are fully recovered.

Conclusions
We have demonstrated that our anti-GD2 CAR-T cells effectively kill target cells in vitro. Importantly, we have optimised a freeze–thaw process that preserves viability, enabling the production of large CAR-T cell batches for subsequent in vivo studies. To study interactions between the tumour immune microenvironment and treatment components, we have developed immunocompetent NB mouse models. In this setting, we have evaluated lymphodepletion using total body irradiation, showing that a 3 Gy dose is sufficient to ablate leukocytes for up to 4 weeks whilst not impacting tumour growth. We are now positioned to assess lymphodepletion and focal radiotherapy pre-conditioning in combination with CAR-T therapy in vivo, in immunocompetent NB models.

Evaluating the combinatory anti-tumour efficacy of radiotherapy with CAR-T cell therapy against paediatric neuroblastoma

Authors and Affiliations
Gaya Nair, Courtney Himsworth, Thomas Jackson, Louis Chesler & John Anderson

Zayed Centre for Research into Rare Disease in Children

Introduction
Neuroblastoma (NB) is the most common extracranial solid tumour in children. Despite intensive multimodal therapies, outcomes for high-risk NB remain among the poorest of all paediatric cancers. Targeted approaches such as immunotherapy offer new opportunities; for example, clinical trial data show that the monoclonal antibody dinutuximab, which targets the tumour associated antigen GD2, improves overall survival rates. Chimeric Antigen Receptor (CAR) T cell therapy is another promising immunotherapy, harnessing T cells to exert a direct cytotoxic effect against tumours. For effective CAR-T cell engraftment, patients first undergo lymphodepletion, a process shown to be essential for therapeutic efficacy. This is commonly achieved with Fludarabine and Cyclophosphamide, though radiotherapy may also be used. Preliminary data in immunodeficient NB mouse models suggests that radiotherapy-based pre-conditioning enhances the anti-tumour response of CAR-T cell therapy. Building on this, we aim to evaluate lymphodepletion, focal radiotherapy pre-conditioning, and anti-GD2 CAR-T cells, individually and in combination, all in the context of immunocompetent NB mouse models.

Methods
Spleens were harvested from C57B/6 mice from which T cells were isolated and activated with anti-CD3 and anti-CD28. T cells were cultured in RPMI media supplemented with IL-2 for 48hrs, after which retrovirus and homology directed repair were used to generate CAR-T cells, assessed via flow cytometry. CAR-T cells were cryopreserved and thawed to assess viability. CAR-T cells were plated in 24-hour co-cultures in vitro against a GD2 positive tumour line, and cytotoxicity was assessed via flow cytometry. C57Bl/6 mice were total body irradiated (TBI) and lymphodepletion was evaluated by assessing the immune profile of peripheral blood via flow cytometry.

Results
Anti-GD2 CAR-T cells demonstrated cytotoxicity, specifically against antigen-positive cell lines. Following cryopreservation, CAR-T cells maintained adequate viability upon thawing. In C57Bl/6 mice, analysis of peripheral blood after TBI revealed reductions in both myeloid and lymphocyte compartments that persisted for up to 10 days. By 28 days post-irradiation, circulating leukocytes are fully recovered.

Conclusions
We have demonstrated that our anti-GD2 CAR-T cells effectively kill target cells in vitro. Importantly, we have optimised a freeze–thaw process that preserves viability, enabling the production of large CAR-T cell batches for subsequent in vivo studies. To study interactions between the tumour immune microenvironment and treatment components, we have developed immunocompetent NB mouse models. In this setting, we have evaluated lymphodepletion using total body irradiation, showing that a 3 Gy dose is sufficient to ablate leukocytes for up to 4 weeks whilst not impacting tumour growth. We are now positioned to assess lymphodepletion and focal radiotherapy pre-conditioning in combination with CAR-T therapy in vivo, in immunocompetent NB models.

VIVO Biobank – Supporting academic research into children and young people with cancer

Authors and Affiliations
Eszter Tuboly[1], Anne Thomson[2], Alex Smith[2], John Moppett[3], Chris Bacon[1], Anthony Moorman[1], Bethan Lloyd-Lewis[4],Rachel Howarth[1], Dona Saji[1], Robyn Watson[1] & Deb Tweddle[1]

1 Translational & Clinical Research Institute, Newcastle University, Newcastle upon Tyne
2 University of York, York,
3 Royal Bristol Hospitals Trust, Bristol,
4 University of Bristol, Bristol.

Introduction
VIVO Biobank is the first national biomedical research resource dedicated to storing a wide range of samples and associated data from the full spectrum of cancers in children and young people (CYP). It is a collaboration between the Universities of Newcastle, Bristol and York and is funded by Cancer Research UK and Blood Cancer UK.

Methods
Sample biobanking is open to all centres treating CYP who are registered with VIVO. Therefore, the biobank is population-based and holds samples covering the full range of cancers found in the CYP population.

Results
VIVO Biobank provides the opportunity for academic researchers to explore novel hypotheses and establish research collaborations.

Case Study 1 – Discovery of a novel biological process in childhood leukaemia
Research using VIVO Biobank samples has identified a new biological phenomenon in the cancer cells of patients with leukaemia. The study showed that leftover fragments of DNA (called excised signal circles - ESCs) generated as a by-product from normal antibody production are not, as previously thought, lost from the cancer cells. Using novel technologies, the authors proved that these ESCs persist in cancer cells and can increase in number. , Importantly, the study found that patients who relapsed tended to have more copies of ESCs than patients who did not relapse; raising the possibility that they may contribute to relapse. This discovery could provide an early warning for patients who are at more likely to relapse.
VIVO Biobank was also able to facilitate collaboration with other researchers who had worked on the same samples enabling sharing of published genomic data.
Nature (2025).
https://doi.org/10.1038/s41586-025-09372-6

Case Study 2 – Unlocking a New Treatment Target in Anaplastic Large Cell Lymphoma (ALCL)
ALCL is a rare type of childhood lymphoma which is treated with toxic multi-agent chemotherapy and has a 25-50% rate of relapse.
Using frozen tumour samples and constitutional DNA samples provided by VIVO Biobank, researchers discovered a mutation in the NOTCH1 gene (T349P) that appears to make tumours more aggressive. The study showed that blocking NOTCH1 activity, especially in combination with Anaplastic Lymphoma Kinase (ALK) inhibitors, can effectively kill cancer cells, even those resistant to existing treatments. This finding could offer hope for more effective and less toxic treatments for patients with ALCL
Haematologica: (2021)
https://doi.org/10.3324/haematol.2019.238766

Conclusion
VIVO Biobank supports a wide range of international research representative of all CYP with cancer. This together with collaborations which encourage data sharing, has facilitated novel research with the potential to improve treatments for CYP with cancer.

Acknowledgements
We are grateful to patients and families for donating samples, to clinical research staff at centres for consenting patients and sending samples and to CRUK and Blood Cancer UK for funding VIVO Biobank.

International variation in child health surveillance and healthcare practices: a mixed-methods study focused on childhood cancer diagnosis

Authors and Affiliations

[*]Angela Lopez-Cortes, MSc
UCL GOS Institute of Child Health, UCL Developmental Biology & Cancer Research Department, London, UK

[*]Aisha Al Mahdy, MSc

[**]Zsuzsanna Jakab, PhD
National Childhood Cancer Registry (NCCR), Hungarian Pediatric Oncology Network (HuPON). Department of Pediatrics, Semmelweis University, Budapest, Hungary.

Masako Shimato, MD

Laura Botta, PhD
Evaluative Epidemiology Unit, Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Fabio Didonè, MSc
Evaluative Epidemiology Unit, Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Adela Cañete Nieto, PhD
Hospital UiP La Fe, Paediatric Oncology and Hematology Unit, Valencia, Spain.

Emmanuel Desandes, MD
National Registry of Childhood Cancers, CRESS, UMRS 1153, INSERM, Paris-Cité University / National Registry of Childhood Solid Tumors, CHRU Nancy, Vandœuvre-lès-Nancy, France.

Lisa L. Hjalgrim, PhD
Department of Paediatrics and Adolescent Medicine, University of Copenhagen, Rigshospitalet, Copenhagen, Denmark.

Charles A. Stiller, MSc
National Disease Registration Service, Transformation Directorate, NHS England, UK.

Bernward Zeller, PhD
Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway.

Gemma Gatta, MD
Evaluative Epidemiology Unit, Department of Epidemiology and Data Science, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

[**§]Professor Kathy Pritchard-Jones, PhD
UCL GOS Institute of Child Health, UCL Developmental Biology & Cancer Research Department, London, UK.

The BENCHISTA Project Working Group.

*First author (co-shared authorship).

**Joint senior authors.

§Corresponding author.

Background

Childhood cancer survival and stage at diagnosis vary internationally. We aimed to explore how child health surveillance and healthcare practices vary between countries involved in the International Benchmarking of Childhood Cancer Survival by tumour Stage (BENCHISTA) project in relation to the timely diagnosis of childhood cancer.

Methods

We conducted a scoping review of five databases (MEDLINE, Embase, SCOPUS, Web of Science, and ProQuest Central) for articles published in English or Spanish between Jan 1, 2012, and July 31, 2024, describing child health surveillance and acute care pathways. We also searched PROSPERO for relevant protocols. Two reviewers independently screened abstracts, with a third resolving conflicts. In parallel, we sent a semi-structured questionnaire to one general practitioner and one general paediatrician per country in the 27 countries submitting data to the BENCHISTA project to collect standardised information on national child health practices and programmes, with findings validated against published national guidance.

continued

Findings
Of 2,788 articles screened, 31 met the inclusion criteria. We identified three central components influencing the diagnostic process: the healthcare-seeking behaviour of families and their interaction with frontline professionals; awareness of alarm symptoms by both caregivers and clinicians; and health system and training-related factors that affect timely referral and diagnosis. Twenty-four studies reported on diagnostic pathways, 15 provided insights into the alarm signs and symptoms, and 28 reported on other influencing factors. Key challenges included poor symptom recognition, variation in paediatric training requirements for frontline healthcare professionals, and lack of referral guidance. Questionnaire responses revealed variation in the number of routine child health checks with physical examination for children under 5 years (median 10; range 2–21), and variation in paediatric training, access/referral pathways and national diagnostic support tools.

Interpretation
Substantial international variation exists in routine child health surveillance and access to paediatric assessment for acute illness. These differences are mirrored in limited national guidance on alarm symptoms, including those for childhood cancer. Our findings may inform the interpretation of stage at diagnosis variation in the BENCHISTA project and support the development of strategies to enhance early cancer detection.

Funding
Children with Cancer UK (grant 20-329) and the Associazione Italiana per la Ricerca sul Cancro, Italy (grant IG 2020 - ID 24933).

Targeting High-Risk Paediatric Tumours with an Inducible Dimerization System Chimeric Antigen Receptor Controlled by IMiDs

Authors and Affiliations
Ferry, G.M.[1], Giota, E.[1], Rintoul, J.[1], Munnings-Tome, S.[1], Muller, H.[1], Kapllani-Mucaj, S.[1], and Anderson, J.[1]

Developmental Biology and Cancer Section,
University College London (UCL)
Great Ormond Street Institute of Child Health,
London, UK

Scientific Abstract
High-risk childhood solid cancers demand the development of novel efficacious therapies despite extensive frontline approaches currently used in standard of care treatments. Many paediatric brain and extracranial solid tumours express B7-H3, suggesting its potential utility as a target for chimeric antigen receptor (CAR) therapy. A major limitation to CAR-T cells targeting solid cancers is effector cell exhaustion and dysfunction mediated by ongoing antigenic stimulation. Potent CAR-T cell activation has also been associated with T cell hyperactivation toxicity, suggesting that CAR-T control systems could improve therapeutic efficacy. To obviate CAR-T cell exhaustion, our group has developed an OFF-switch system using a 60-amino acid peptide sequence (iTAG2) at the intracellular terminus of the CAR construct. We have demonstrated that a B7-H3 targeting CAR-iTAG2 is sensitive to reversible degradation using nanomolar concentrations of thalidomide-derivative immunomodulatory (IMiD) drugs. Using the iTAG2 degron in a dual-CAR system, we endeavoured to generate an inducible ON-switch version of the B7H3-CAR to complement the existing OFF-switch CAR being evaluated in clinical studies such that intermittent scheduling of IMiD drugs could allow more sustained effector cell activation and antitumour activity. We have made both mono- and bicistronic lentiviral vectors expressing the two CAR modules that contain the different configurations of the signalling and dimerising components of a canonical second-generation CAR specific for the B7-H3 target antigen. We initially screened the modules using the NFAT-reporter Jurkat cell line as a readout of effector cell function in the presence of IMiD drugs. Our first iterations showed that T cell activation is dependent on the presence of both B7-H3-expressing target cells and low nanomolar concentrations of IMiD drugs. Ongoing studies are optimising the module configuration and delivery via lentiviral vectors and homology-directed repaid (HDR) to further enhance expression in primary human T cells.

Development of tumour-targeted prodrugs of DNA damage repair inhibitors for neuroblastoma therapy

Authors and Affiliations
April J Baral, Goreti Ribeiro Morais, Francis Barnieh, Steven D Shnyder, Paul M Loadman, Robert A Falconer

Institute of Cancer Therapeutics, Faculty of Life Sciences, University of Bradford, Bradford BD7 1DP, U.K.

Introduction
Neuroblastoma is the most common extracranial solid cancer in children, with survival rates of less than 50% in high-risk patients despite intensive chemotherapy and radiotherapy. Most high-risk patients relapse or fail to achieve remission after completing initial therapy, while a few survivors are usually burdened with long-term toxicities arising from the intensive treatment received. Therefore, novel tumour-targeted therapies and combinations to reduce the burden of toxicity remain an urgent unmet need.

DNA damage response (DDR) proteins, including ATR and CHK1, have emerged as key therapeutic targets in cancer. The ATR/CHK1 pathway mitigates DNA damage caused by cytotoxic chemotherapy; therefore, inhibition of this pathway can increase chemotherapy efficacy. However, despite the clinical promise of ATR and CHK1 inhibitors, there are ongoing concerns over acute and long-term systemic toxicities2. A tumour-targeted inhibitor in the form of a peptide prodrug has the potential to minimise the exposure of normal tissues to drug toxicity and enhance the therapeutic window of these drugs. In this approach, an inactive form of a drug is attached to a specific peptide sequence that undergoes enzymatic cleavage to selectively release the active compound at the tumour site. We aim to utilise the increased expression and activity of CD13 (aminopeptidase N) in neuroblastoma and tumour vasculature, but not in normal vasculature, to develop tumour-targeted ATR and CHK1 inhibitors. CD13-activated prodrugs of AZD6738 (ATR inhibitor) and AZD7762 (CHK1 inhibitor) were designed to be selectively cleaved by the exo/endopeptidase activity of CD13, releasing the active drug within the tumour microenvironment.

Method
The CD13 selective prodrugs were synthesised using solid phase peptide synthesis and purified by semi-preparative HPLC. Using ex vivo metabolism assays and LC/MS methodology, CD13-specific cleavage of the prodrugs was assessed in normal tissues (liver and kidney), and neuroblastoma tumour homogenates, non-MYC (SH-SY5Y) and MYC-amplified (LAN-1). The synergistic effect of the prodrugs in combination with cytotoxic agents relevant to neuroblastoma therapy was also evaluated in CD13-expressing neuroblastoma cells in vitro using MTT assay.

Results
Ex vivo metabolic studies of CD13 prodrugs showed significant differential release of AZD6738 and AZD7762 in tumour homogenates compared to normal tissues, confirming tumour-selective activation. Synergism was also observed with WEE1 inhibition (adavosertib) and ALK inhibition (lorlatinib), in combination with the CD13-activated prodrugs of AZD6738 and AZD7762, supporting a rational combination strategy.

Conclusion
Our data demonstrate that CD13-activating ATR and CHK1 prodrugs minimise the cytotoxic effects of ATR or CHK1 inhibition in normal tissues by the selective release of cytotoxic agents in the neuroblastoma tumour. Combination of the CD13 prodrugs with clinically relevant inhibitors for WEE1 and ALK represents a rational approach to enhance the therapeutic index of ATR and CHK1 inhibitors, with the potential to maximise anti-tumour efficacy and reduce treatment-related toxicities in patients.

Investigating the potential of interfering with glutamine addiction to better treat MYC-driven medulloblastoma

Authors and Affiliations
Eales, K.L., Lyu, Y., and Tennant, D.A.

Department of Metabolism and Systems Science, School of Medical Sciences, College of Medicine and Health, University of Birmingham, UK. B15 2TT.

Introduction
In the UK, ~50-70 children are diagnosed each year with Medulloblastoma (MB), making it the most common malignant brain tumour in children and young adults. MB are biologically heterogeneous and initially classified into four groups (WNT, SHH, Group 3, Group 4) based on the tumour's genetic profile. While 5-year survival rates are significantly better for some groups (70-90%), Group 3 tumours have the poorest prognosis (30-60%). Group 3 MB are often driven by oncogenic MYC signalling, causing metabolic transformation and an 'addiction' to the amino acid glutamine, thereby perpetuating increased tumour growth and resistance to conventional therapies. We therefore hypothesise that blocking glutamine metabolism could represent a more efficacious means of targeting these highly resistant tumours, however we lack sufficient understanding of the metabolic basis of this MB group to advance from hypothesis to treatment.

The first step for incorporation of glutamine carbons into cellular metabolites is catalysed by the glutaminase (GLS) and glutaminase 2 (GLS2) enzymes. These are upstream of several metabolic pathways that provide building blocks for cellular proliferation and repair following chemo- and radiotherapy-induced damage. Our research therefore aims to investigate the contribution of these two enzymes and their downstream metabolic pathways to glutamine-mediated proliferation and therapy resistance of Group 3 MB cells, subsequently providing evidence for translational studies to take the next step in developing kinder and more effective therapies for these children.

Methods
Compartment specific localisation and expression of GLS/GLS2 was assessed through cellular fractionation and western blotting using Group 3 HD-MB03 cells compared with SHH group UW.221 cells. To investigate the contribution of GLS/GLS2, we created specific knockdowns of each of the GLS and GLS2 isoforms (KGA/GAC and LGA/GAB) in HDMB-03 cells using the pLKO.1-puro lentiviral system. Using stable isotope-enriched nutrients and gas and liquid chromatography mass spectrometry (GCMS/LCMS) technologies, we subsequently assessed how these isoforms function metabolically in this model. Furthermore, previous investigations into glutamine metabolism have used non-physiological conditions, and so we conducted these metabolic studies in physiologically relevant medium, enhancing translatability of results.

Results
We observed that there is differential expression of GLS and GLS2 between SHH and Group 3 MB cells. Additionally, we saw that hypoxia (0.3% and 1% O2), differentially influences GLS/GLS2 expression. Upon cell separation, we determined for the first time, presence of the GLS2 isoform LGA in the cell cytoplasm in Group 3 MB cells. Metabolic analysis identified a series of tricarboxylic acid cycle (TCA) metabolites and amino acids which were differentially altered in these isoform knockdown models.

Conclusion
These data represent a novel and exciting finding relating to GLS/GLS2 expression, cellular localisation and their differential contribution to the fate of glutamine in MYC-amplified Group 3 MB cells. Following these metabolic findings, we are now expanding this investigation using patient-derived Group 3 cells and investigating the contribution of isoform-specific glutamine metabolism to the acquired resistance to conventional therapies to provide evidence for translational studies to take the next step in developing new, kinder therapies for these children.

Clinico-molecular correlates of quality of survival and neurocognitive outcomes in medulloblastoma: A meta-analysis of the SIOP-UKCCSG-PNET3 and HIT-SIOP-PNET4 trials

Authors and Affiliations
Ellie Butler[1], Mathilde Chevignard[2], Kim S. Bull[3], James Holt[1], Bertie Harrington[1], Jemma Castle[1], Marie-Amelie Heng[4], Colin Kennedy[3], Francois Doz[4], Birgitta Lannering[5], Stefan Rutkowski[6], Maura Massimino[7], Simon Bailey[1], Steven C. Clifford[1], Debbie Hicks[1]

1 Newcastle University, Newcastle upon Tyne.
2 Saint Maurice Hospitals, Saint Maurice, France.
3 University of Southampton, Southampton, UK.
4 Institut Curie, Paris, France.
5 University of Gothenburg, Gothenburg, Sweden.
6 University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
7 Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Introduction

Medulloblastoma (MB) is the most common malignant paediatric brain tumour accounting for ~63% of childhood intracranial embryonal tumours1. Five-year overall survival rates for standard-risk patients are ~75% with cure rates >95% in the MBWNT subgroup[2,3]. However, standard-of-care treatment for MB comprising a combination of chemotherapy, radiotherapy, and surgical resection results in severe long-term neurocognitive, social, physical, emotional, and behavioural functioning deficits in survivors of childhood MB[2,4]. Subsequently, there has been an increased focus on identifying both therapeutic strategies for reducing toxicity, and risk factors associated with diminished quality of survival[4-11].

Method

We undertook an integrated analysis of clinico-demographic, biological, and molecular features in a cohort of patients treated on the SIOP-UKCCSG-PNET3 and HIT-SIOP-PNET4 clinical trials. Quality of survival (QoS) data (n=218) or neuropsychometric data (n=140) were assessed to ascertain key correlates of survivorship. Demographic and treatment factors, as well as molecular subgroup (MBWNT, MBSHH, MBgrp3, MBgrp4) were assessed against patient/proxy-reported outcome measures (PROMs); the Health Utilities Index (HUI3), Strength and Difficulties Questionnaire (SDQ), and the Pediatric Quality of Life Inventory (PedsQL), as well as neuropsychometric assessments using the Wechsler Intelligence Scale (WISC IV) scores. Additionally, 39 candidate SNPs with known modifying effects on neurocognitive outcomes were genotyped from HIT-SIOP-PNET4 patients only (n = 74) and assessed against the WISC IV scores.

Results

SIOP-UKCCSG-PNET3 patients receiving chemotherapy before craniospinal irradiation (CSI) had significantly lower PROMs scores when compared to those receiving CSI alone in the self-reported HUI3 ($p = 0.0117$), the proxy-reported PedsQL ($p = 0.0361$), and the proxy-reported SDQ ($p = 0.0024$), as well as to those treated on both arms of HIT-SIOP-PNET4 ($p < 0.012$). As expected, the MBSHH subgroup had improved PROMs scores compared to the MBWNT and MBgrp4 subgroups in the proxy-reported PedsQL assessment ($p < 0.0191$). Neither molecular group nor the clinico-demographic features tested were associated with neurocognitive outcomes. In contrast, 8 SNPs were significantly associated with ≥1 WISC domain, with 5/8 showing multiple associations.

Conclusion

This large, integrated analysis of two independent trials cohorts has revealed multiple factors predictive of medulloblastoma survivorship. Further assessment is required to determine their potential as a basis for modifications to disease management.

Understanding what helps and hinders survivors of childhood sarcoma to be physically active: qualitative research to inform intervention development. The BEACON - Sarcoma study

Authors and Affiliations

Elizabeth Pharoah[1,2], Linda Sharp[1,2], Quentin Campbell-Hewson[2,3,] Sherron Furtado[4], Craig Gerrand[4], Tasnim Arif[3], Morven C Brown[1,2]

1. Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne;
2. Centre for Cancer, Newcastle University, Newcastle upon Tyne;
3. Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne;
4. Royal National Orthopaedic Hospital NHS Trust, Stanmore.

Introduction

Despite the importance of physical activity (PA) to cardiovascular health, survivors of childhood, adolescent and young adult (CAYA) sarcoma are less active than non-cancer peers, and other CAYA cancer survivors. Moreover, PA support for survivors (and particularly for sarcoma survivors) is lacking.

We recently completed empirical, evidence-synthesis and co-design work to develop an early prototype of a theoretically-informed, multi-component intervention to improve PA levels of survivors of CAYA cancer in follow-up care (the BEACON study) with the purpose of reducing survivors' risk of cardiovascular morbidity and mortality.

However, it is likely that survivors of sarcoma face additional and specific challenges to PA. Therefore, this project will identify and understand barriers to, and enablers of, PA from the perspectives of CAYA sarcoma survivors; to identify survivors' preferences for PA support; and use this information to further inform design of the BEACON intervention.

Methods

Workpackage 1: we are currently carrying out in-depth interviews with 25-30 young people diagnosed ≤24 years with sarcoma in the pelvis or extremities; currently aged 10-24 years and 2-15 years post-treatment. Workpackage 2: will begin in spring 2026 and will involve co-design workshops with survivors and healthcare professionals (HCPs) to identify the design changes needed to refine the BEACON intervention to meet the preferences and needs of survivors of CAYA sarcoma.

Results

Our early prototype comprises a blended multi-component intervention delivering multiple behaviour change techniques both face-to-face and online: 1) provision of brief PA advice by a trusted HCP at clinic and referral to a trained PA coach; 2) online 'real-time' regular exercise and support sessions with PA coach; and 3) access to an interactive web-app.

Based on the results of the interviews with young people who have had a sarcoma, and the co-design activities with young people and a range of HCPs, we will refine the design of the BEACON intervention to ensure it is tailored to the needs and preferences of CAYA sarcoma survivors.

Preliminary analysis of the interviews with CAYA sarcoma survivors reveals key themes around survivors needing to gain confidence in engaging in PA post-surgery. Central to this are the need for individually tailored goals that reflect both age-specific and ability-specific requirements, and acquiring knowledge of movement adaptation strategies to accommodate their unique physical needs.

Conclusion

The project will aim to maximise intervention effectiveness by enabling tailoring to accommodate survivor- and tumour-specific impairments and barriers to PA. Next steps will be to gain funding so that we can work with stakeholders to operationalise the intervention and test it for feasibility and acceptability.

A preclinical model for evaluating interventions against radiotherapy-induced late-effects in childhood medulloblastoma

Authors and Affiliations
Jemma Castle[1], Gary Shaw[2], Edward Fielder[3], Teklu Egnuni[2], Dominic Weller[1], Magdalena Voldrichova[1], Mankaran Singh[1], Roderick Skinner[1], Thomas von Zglinicki[3], Steven C. Clifford[1], Susan C. Short[2], Satomi Miwa[3], Debbie Hicks[1].

1 Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK.
2 Leeds Institute of Medical Research, Wellcome Trust Brenner Building, St James's University Hospital, Beckett St, Leeds, UK.
3 Biosciences Institute, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, UK.

Background
Medulloblastoma (MB) is the most common malignant paediatric brain tumour, with 5-year survival rates over 75%. Survivors often suffer debilitating late-effects from their tumour and its treatment. Cranial radiotherapy (CRT) plus posterior fossa boost (PFB) is the mainstay of treatment for non-infants and has contributed to increasing survival rates. However, radiotherapeutic insult to the surrounding normal brain tissue has deleterious consequences and often causes cognitive deficits, endocrine impairment, secondary tumours, and reduced physical function, ultimately leading to poorer quality of life. Among the most devasting late-effect is the neurocognitive impairment; survivors often suffer reduced processing speed, attention and working memory. Currently, there are no interventions to treat/prevent the radiotherapy-induced late-effects in widespread clinical use, and a paucity of appropriate model systems hinders their development.

Methods
We have developed an in vivo model system that recapitulates the radiotherapy dose, delivery and late-effect profile of childhood medulloblastoma, at an equivalent developmental stage. To mimic clinical treatment regimens, age-equivalent (postnatal days 28-37) C57Bl/6J mice received targeted, CT image-guided CRT (human-equivalent 38 Gy, n=12) or CRT with PFB (human-equivalent 49 Gy, n=12), via the small animal radiation research platform (SARRP). Mice were longitudinally assessed for over a year (frailty; frailty index, physical function; rotarod and grip strength, and neurocognition; Y-maze and Barnes maze). Late-effects were compared to a sham-irradiated group (n=12). To achieve sophisticated, close-to-human, assessment of distinct neurocognitive domains, we employed gold-standard operant touchscreen chamber technology to assess learning and cognitive flexibility (Pairwise Visual Discrimination; PVD, CRT; n=11 and sham; n=12).

Results
CRT was well tolerated, independent of PFB receipt, and no mice suffered severe acute toxicity. Irradiated mice exhibited increased frailty (frailty index; p=0.0002) and reduced physical functioning; time to fall and grip strength were significantly lower (rotarod; p=0.026 and grip strength; p=0.006, respectively). Neurocognitive deficits were also apparent; irradiated mice displayed significantly worse working memory (Y-maze; p=0.009) and long-term memory (Barnes maze; p=0.029). However, cognitive flexibility and learning were maintained following CRT (PVD; p>0.05). Receipt of PFB did not induce a more severe late-effect profile.

Conclusion
Our in vivo model of childhood MB radiotherapy recapitulates the profile of late-effects experienced by MB survivors, with mice exhibiting significant neurocognitive deficits, reduced physical function and increased frailty following CRT. CRT induced deficits in working memory and long-term memory, however, MB survivors are also frequently debilitated by reduced processing speed and attention, and further assessment of these neurocognitive domains is required. Next, we will further utilise our operant touchscreen chamber platform to benchmark the extent of neurocognitive deficits associated with attention and processing speed. Together, this model offers a robust platform for preclinical testing of therapeutic strategies aimed at mitigating radiotherapy-induced late effects in MB survivors.

Increasing the physical activity levels and quality of life of Paediatric Cancer Patients and their family: A Family-Based Approach

Authors and Affiliations
Dr. Elizabeth Pharoah, Dr. Clare Stevinson, Dr. Silvia Costa, and Dr. Elizabeth Stamp

Loughborough University

Introduction/Background
During childhood cancer treatment, a child and their family can experience many pressures that can detrimentally impact their physical activity levels and quality of life. However, physical activity interventions supporting families through childhood cancer treatment are rare. To address this, this research aimed to use a pragmatic approach to design a theory-driven physical activity intervention. A programme of formative research was undertaken to examine the relevant family-based physical activity literature and explore the experiences of families during treatment to meet their needs.

Methods
The research included:

1) eighteen interviews with parents of children who had received a cancer diagnosis explored their experiences, and barriers and facilitators for family members' physical activity;

2) three individual-family focus groups with childhood cancer survivors and their family members (n=13) explored their experiences of family activities during treatment; and

3) a systematic review which examined the efficacy, feasibility, and acceptability of 'family-based' physical activity interventions for childhood cancer patients and survivors. The findings from the systematic review and the thematic analysis of the qualitative data were used to inform the development of an intervention using the Behaviour Change Wheel.

Results
The Active Living for Families intervention (ALFIE) was developed using the Behaviour Change Wheel to target physical activity for childhood cancer patients and their family during treatment. This family-based, multi-component intervention to improve physical activity levels and quality of life, targets seven intervention functions and 17 behaviour change techniques (including information about health consequences, demonstration and instruction on how to perform the behaviour, restructuring the physical and social environments, and social support). In a proposed feasibility study, outcome and process evaluations of the intervention would examine the feasibility of the intervention.

Conclusions
This research took a pragmatic approach (the first-hand experiences of family members, evidence from a systematic review and a behaviour change framework) to develop a family-based physical activity intervention. Further research through a feasibility study, outcome and process evaluations of the intervention would be conducted to inform modifications required to improve the support delivered and to meet the needs of the whole family during treatment.

VIVO Biobank – Supporting Translational Research linked to innovative clinical trials

Authors and Affiliations
Anne Thomson[2], Eszter Tuboly[1], Alex Smith[2], John Moppett[3], Chris Bacon[1], Anthony Moorman[1], Bethan Lloyd-Lewis[4],Rachel Howarth[1], Dona Saji[1], Robyn Watson[1] & Deb Tweddle[1]

1 Translational & Clinical Research Institute, Newcastle University, Newcastle upon Tyne,
2 University of York, York,
3 Royal Bristol Hospitals Trust, Bristol,
4 University of Bristol, Bristol.

Introduction
VIVO Biobank is the first national biomedical research resource dedicated to storing a wide range of samples and associated data from the full spectrum of cancers in children and young people (CYP). It is a collaboration between the Universities of Newcastle, Bristol and York and is funded by Cancer Research UK and Blood Cancer UK.

Methods
VIVO Biobank is open to all CYP with cancer in the UK, treated at centres registered with VIVO. VIVO Biobank also supports sample collection for many current clinical trials and surplus samples may be made available for non-trial research. Samples and data from legacy clinical trials, providing a rich source of research samples with well-annotated clinical and outcome data, are also available.

Results
Research using clinical trial samples collected by VIVO Biobank has been instrumental in changing patient treatment.

Case study 1 – UKALL2003 trial
UKALL2003 was a UK-wide clinical trial for children with acute lymphoblastic leukaemia and the first to systematically bank bone marrow via VIVO Biobank. Leveraging these samples, a series of inter-related projects identified, characterised and evaluated several genetic biomarkers including iAMP21, UKALL copy number alteration (CNA) profile, EBF1::PDGFRB. In addition, research using samples from UKALL2003 led to the redefinition of refractory disease and the concept of genotype-specific MRD thresholds. These research findings led to the development of a novel integrated risk classification algorithm which has been adopted by the international ALLTogether01.

Nature 508:98 (2014), J.Clin Oncol. 31:2289 (2013), Blood 124(9): 1434 (2014), Blood 127(18): 2214 (2016), J Clin Oncol 35(6): 660 (2017). J Clin Oncol 36(1): 34 (2018), Blood Adv 3(2): 148 (2019), Br J Haematol 191(5): 844 (2020), Blood. 137(13):1835 (2021)

Case study 2 – High Risk Neuroblastoma 1 (HRNBL1 trial)
Investigation into ALK genetic modifications in high-risk neuroblastoma patients recruited onto the SIOPEN HR-NBL1 trial revealed that particular alterations, such as clonal mutations and amplifications, are associated with worse survival outcomes. This was a large international European study and VIVO Biobank provided samples for UK patients. As a result of this research patients with ALK alterations will be randomised to receive an ALK inhibitor (Lorlatinib) in addition to standard treatment in an upcoming amendment to the SIOPEN HR-NBL2 trial

J.Clin Oncol. (2021) 39(30), 3377;. Clin. Cancer Res.(2023) 29(7):1317

Conclusion
Both of these international research projects highlight the importance of VIVO Biobank in supporting centralised sample collection for clinical trials facilitating access to samples to support research that has changed treatment for CYP with cancer in the UK and around the world.

Acknowledgements
We are grateful to patients and families for donating samples, to clinical research staff at centres for consenting patients and sending samples and to CRUK and Blood Cancer UK for funding VIVO Biobank.

Validation of novel CDK12/Cyclin K inhibitor CT7439 in high risk MYCN amplified neuroblastomas

Authors and Affiliations
Dr Nikita Lad, Chloe Harris, Ian Sudbery, Helen Bryant

University of Sheffield, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX

Introduction
Neuroblastoma is a rare paediatric cancer, mainly affecting children under the age of 5. Amplification of the gene MYCN is associated with high-risk cases of neuroblastoma, with a poor 5-year survival rate of 50%. MYCN amplification increases DNA replication, which therefore increases the rate of DNA damage and leads to a larger reliance on DNA damage repair (DDR) pathways. CDK12, which interacts with Cyclin K when activated, is known to be involved with transcription elongation and is associated with the DDR, making it a target of interest. CT7439 is a novel molecular glue inhibitor of CDK12, which leads to the degradation of Cyclin K, therefore disrupting transcription dynamics. This study aimed to investigate the effects of CT7439 in neuroblastoma, particularly its effects in MYCN amplified cells.

Method
A range of MYCN amplified and non-amplified neuroblastoma cell lines were used to explore the effects of CT7439. This included the tet-inducible SHEP21N MYCN expression cell model. CT7439 was assessed as a monotherapy to determine its effects on survival via clonogenic survival assays, DNA repair capacity via immunofluorescent staining, mRNA expression via RNAseq, and protein expression via western blotting. Combination therapies were also assessed by clonogenic assay using various chemotherapy drugs and inhibitors.

Results
Target and functional validation of CT7439 was confirmed with a reduction in CDK12 and Cyclin K. CT7439 decreased cell survival in all neuroblastoma cell lines, with MYCN amplification leading to increased sensitivity compared to the non-amplified cells. In the SHEP21 MYCN expression model, an increase of damage marker yH2AX was seen over 48hrs suggesting accumulation of DNA damage particularly in the MYCN expressing cells. RNAseq analysis and western blotting revealed a reduction in the DDR, particularly proteins involved with homologous recombination, including BRCA1. Again, this was more pronounced in the MYCN expressing cells compared to the non-expressing cells.

Conclusion
These findings demonstrate the efficacy of CT7439 in neuroblastoma cell models, particularly the MYCN amplified models, and suggest this comes about via a down regulation of the DNA damage response pathway on which we have previously shown MYCN amplified cells are more dependent. This suggests CDK12 as a novel therapeutic target to improve the treatment of high-risk neuroblastoma.

Minimally invasive bioelectronic device for voltage-controlled intratumoural chemotherapeutic delivery

Authors and Affiliations
Immi Van der Auweraert, Gary Shaw, Joshua Killilea, Estelle Cuttaz, Heiko Wurdak, Ryan Mathew, Rylie Green

Imperial College London

Background
Paediatric high-grade gliomas remain one of the leading causes of cancer-related mortality in children in the UK. Current standard of care, namely tumour resection, radiotherapy, and adjuvant chemotherapy, sees limited efficacy with a 5-year survival of less than 20%[1]. Complete resection is often difficult due to the infiltrative nature of these tumours and their frequent location in functionally critical brain regions. In addition, the choice of chemotherapeutic drugs is limited due to the blood-brain barrier preventing sufficient drug concentrations reaching the tumour site. Novel drug delivery strategies, including bioelectronic approaches, are emerging as promising technologies to address some of these limitations. A recently developed, minimally invasive bioelectronic device capable of intratumoural drug delivery upon external voltage application has shown successful drug release and efficacy in both 2D and 3D in vitro models. However, drug distribution within the tumour upon release from the device has not yet been assessed.

Aim
The aim of this study is to assess drug distribution after voltage-driven release from the device in terminal studies in glioma-bearing mice.

Methods
The device consists of a probe compatible with standard neurosurgical frames. The chemotherapeutic drug doxorubicin is housed in a conductive elastomer matrix (made of poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) and polyurethane) and coated onto the tip of the device interfacing within the tumour. For this study, the device was first adapted for rodent studies. A device was stereotactically inserted into the tumour site of terminal SB-28 glioma-bearing mice (n=3 for each group). Drug release was triggered by applying a +2V stimulus for 30 min, with controls receiving no stimulus to assess passive release. Brains were subsequently resected, cryosectioned, and imaged by confocal microscopy exploiting the fluorescent properties of doxorubicin to assess drug distribution.

Results
Devices were successfully manufactured in a format compatible with rodent studies. Drug release quantification studies in PBS demonstrated increased release after voltage stimulus compared to passive diffusion. In terminal studies, voltage-driven release of doxorubicin produced a circular dispersion of roughly 0.7-1mm in diameter, whereas passive release without stimulation was minimal and largely confined to the insertion site.

Conclusion
These findings validate the feasibility of intratumoural voltage-driven chemotherapy release in glioma-bearing mice. These results highlight the potential of this bioelectronic device to achieve controlled, localised chemotherapy delivery within tumours. Future work will focus on device optimisation, refinement of voltage paradigms, and validation in additional paediatric glioma models, alongside in vivo survival studies to assess therapeutic efficacy. Ultimately, this technology offers a promising strategy to reduce systemic toxicity and improve treatment outcomes for children with high-grade gliomas.

Paediatric Cancer Survivors: Do current UK Guidlines meet the physical and psychosocial needs of patients in the Medium and Long-Term?

Authors and Affiliations
Jemima Korvin

Barts and the London Medical School

Purpose
Modern day therapies are resulting in a five-year survival rate of 84% for paediatric cancer patients. In the UK, 45,000 survived a childhood cancer diagnosis, two-thirds of whom will experience treatment-induced physical and psychosocial late effects. The objectives of this review were to identify current guidelines for paediatric cancer survivorship care and appraise whether UK guidelines meet the needs of paediatric cancer survivors.

Methods
Ovid Medline and Embase were systematically searched. Studies into late effects, follow-up care programmes, survivorship care, and the transition from paediatric to adult centred care were included. Data was synthesised into tables, and the Critical Appraisal Skills Programme (CASP) checklists were used to assess the quality and reliability of studies. 14 studies were an appropriate quality for inclusion.

Results
Important themes emerging demonstrated the need for improvement in guidelines at all stages of care. These themes were: improved reproductive care, more supported transition into adult care, need for widened access into follow-up care, improved screening programmes, and minimisation of late effects.

Conclusion
UK guidelines do not meet the physical nor psychosocial needs of paediatric cancer survivors throughout the cancer care continuum, making guidelines ethically inappropriate and unfit for purpose. An individualised survivorship care pathway should be implemented, focusing on patient autonomy. Recommendations are based on studies from other countries whose guidelines, policies, and programmes better meet survivors' needs. Future work into piloting implementations in the UK, and performing a wider review is imperative.

Miss Korvin's time on the work was supervised by Dr Alex Collinson, University of St Andrews.

Supporting young cancer survivors who smoke and vape: The PRISM study

Authors and Affiliations
Morven C Brown[1], Vera Araújo-Soares[2], Roderick Skinner[1,3], Jamie Brown[4], Adam W Glaser[5], Helena Hanratty[6], Martin McCabe[7], Ana-Ecaterina Amariutei[8], Sabrina Mauri[8], Linda Sharp[1]

1. Newcastle University Centre for Cancer, Newcastle upon Tyne;
2. Medical Faculty Mannheim, Heidelberg University, Heidelberg;
3. Great North Children's Hospital, Newcastle upon Tyne;
4. Department of Behavioural Science and Health, University College London, London;
5. Leeds Teaching Hospitals NHS Trust, Leeds; 6. Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield
7. Division of Cancer Sciences, The University of Manchester, Manchester;
8. Patient and public representatives for the study.

Introduction
Childhood, adolescent and young adult (CAYA) cancer survivors are vulnerable to adverse late-effects. Whilst it is known that tobacco smoking is an important preventable cause of ill-health and early death, the long-term effects of vaping are not yet established. Formative research exploring smoking and vaping in CAYA cancer survivors is lacking.

To address this, we recently completed a programme of empirical work (the PRISM study) which explored 1) published smoking cessations interventions for CAYA cancer survivors; 2) the views and experiences of CAYA cancer survivors who smoke and/or vape; and 3) the views and current practices of healthcare professionals regarding smoking and vaping discussions with CAYA cancer survivors in their care.

Methods
Our programme of formative research included:

1) a scoping review of published smoking cessation interventions in CAYA cancer survivors;
2) theoretically-informed interviews with both CAYA cancer survivors who smoke and/or vape;
3) theoretically-informed interviews with healthcare professionals who care for CAYA cancer survivors.

Results
We found that few smoking cessation interventions have been developed, tested and published for CAYA cancer survivors. In the CAYA cancer clinical context, multiple barriers exist which impede the potential for disclosure, discussion and support provisions for smoking and vaping behaviours. These include factors which hinder patient's willingness to disclose their smoking status (e.g., presence of parents, feelings of embarrassment, worries of confidentiality). Healthcare professionals also reported barriers to initiating conversations around smoking such as factors which prevented ascertaining patients true smoking status, a lack of engagement from known smokers and vapers regarding offers of cessation support, and ultimately a view that they as healthcare professionals had limited influence over their patients choice of behaviours. Both healthcare professionals and survivors acknowledged the limited information on the long-term effects of vaping, and even more limited knowledge around vaping cessation.

Conclusion
Several challenges to addressing smoking and vaping behaviours within cancer care exist from both the healthcare provider and the survivor perspective. Further considerations are needed to enable open and honest discussions between survivors and their clinicians, and how to best support young cancer survivors who smoke or vape.

We fund research

Our investment in research is advancing our understanding of childhood cancer. We are developing our knowledge about why children develop cancer and we are gaining new insights into the genetic and environmental causes of cancer to see if prevention is possible. We hope to improve the diagnosis, treatment and long-term care of children with cancer.

Thank you

Children with Cancer UK
Third Floor
21-27 Lamb's Conduit Street
London
WC1N 3NL

0800 222 9000
info@childrenwithcancer.org.uk
www.childrenwithcancer.org.uk

Registered Charity Number: 298405
Children with Cancer UK is a company limited by guarantee.
Registered in England No. 4960054

@childrenwithcanceruk
@CwC_UK
@childrenwithcanceruk