Childhood Cancer 2019
9th - 10th September 2019
Newcastle upon Tyne

Exploiting genomic medicine throughout the patient journey
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Dear Colleagues,

On behalf of Children with Cancer UK, we would like to welcome you to Childhood Cancer 2019

We are delighted to be back here in Newcastle. In 2017 we held our first major conference outside London at the same venue. It was a highly successful conference.

This year we address “Exploiting genomic medicine throughout the patient journey”. There is now real hope that the use of precision medicine will improve survival and quality of survival from cancer at all ages. Children with Cancer UK is playing its part too, having already granted many millions of pounds to support both research and infrastructure that will help ensure that all children and young people with cancer in the UK have their tumour DNA sequenced in a clinically relevant time frame.

This meeting aims not only to highlight state-of-the-art in precision paediatric cancer medicine and research, but also the current challenges particularly those related to trial design, funding, ethics and access for children in the UK to newer, kinder and more effective, therapies and technologies.

We hope that in holding this conference we provide a useful forum for Education and Networking. It is also an opportunity for you to learn more about Children with Cancer UK and for us to refine our understanding of how our annual research budget (c. £5 million at present) can be best channelled to increase survival and quality of life.

We wish to particularly thank Professor Steve Clifford, Professor Anthony Mooman and members of the organising committee for their enthusiastic support. We also wish to thank our session chairs and plenary speakers who have so generously given us their time in delivering the programme. We would also like to thank those of you who have shared your current research with us by free communication. Lastly, thank you for attending and taking part - we look forward to meeting you and learning more about your interests and research and how together we can further improve the outlook for all children, teenagers and young adults suffering from cancer.

For Children with Cancer UK
Nick Goulden, Medical Research Director
Denis L Henshaw, Scientific Director
Alasdair Philips, Trustee
Joseph Bryan, Research Grants Manager
“Thank you to Children with Cancer UK and Newcastle University for hosting this year’s International Childhood Cancer conference. I am pleased to welcome experts from around the world to our great city. It is fantastic to see this important event being held in Newcastle. Our city has a long history of innovation and scientific research and I am proud to see educational and medical institutions coming together to share expertise in the global fight against childhood cancer. As Shadow Minister for Science and Innovation I believe we need to do more to support the huge potential for better treatments and longer, more productive lives that scientific research offers.

While childhood cancer diagnosis and survival rates are steadily improving, there is still more work to be done. Every day, 12 families in the UK get the devastating news that their child has cancer - over 4,500 children and young people are diagnosed with cancer every year in the UK.

The ground-breaking research taking place here in Newcastle at the Wolfson Childhood Cancer Centre is just one example of the many projects that are working to develop kinder treatments and improve outcomes for childhood cancer patients and their families. The work taking place here and in other institutions along with the support of Children with Cancer UK, will hopefully lead to more children ringing that end of treatment bell.”

Chi Onwurah MP is the Labour MP for Newcastle upon Tyne Central and is also Shadow Minister for Industrial Strategy Science & Innovation. Chi is a chartered engineer who has worked around the world in hardware & software development, product management, network roll out & regulation.
About Children with Cancer UK

Children with Cancer UK is the leading national charity devoted to the causes, treatment and prevention of cancer in children and young people.

We raise and invest money for vital specialist research to save the life of every child with cancer and keep their family together.

Our work is helping to drive up survival rates and develop kinder and more effective treatments so that more children with cancer can ring the end of treatment bell.

We fund research

Every day, 12 families in the UK receive the devastating news that their child has cancer. Huge progress has been made over the last 30 years, but now we need kinder and safer treatments so that children can survive cancer without a lifetime of health problems.

We have helped fund over 200 research projects, including two of the most successful childhood cancer clinical trials in the UK - UKALL2003 and UKALL2011. The development of a Minimal Residual Disease Test is now part of standard NHS treatment for all young acute lymphoblastic leukaemia patients. We are now also making Precision Medicine a reality for treating young cancer patients with solid tumours.

The incidence of cancer in children and young people is continuing to rise. Our 2019 international conference highlights what we already know about using genomic medicine to combat childhood cancer and how this can be exploited to deliver next generation therapies to improve the patient journey.

We are currently funding 60 active research projects at centres of excellence around the UK. In 2018, we made 7 new research awards, totalling over £1.7M.

We help families

We fund and provide a wide range of support services specifically designed to help children and their families cope during treatment.

We believe that it’s vitally important for families to stay together during their child’s cancer treatment, so we help to fund the building of free patient and family homes near hospitals around the UK. Through partner charities we also fund financial assistance to families during treatment to relieve the added financial burden a cancer diagnosis can bring.

We know how important it is for families to spend quality time together away from the hospital ward, so we also deliver unforgettable events like circus days and trips to the seaside - creating memories that will never be lost.
Day One: Monday 9th September 2019

08:30  Registration open. Refreshments available.
09:00  Poster set-up and viewing
09:30  Welcome and Introduction  
       Mark Brider, Acting CEO, Children with Cancer UK
09:40  Chi Onwurah MP, Newcastle upon Tyne Central Shadow Minister for Industrial Strategy Science & Innovation
09:50  Professor Steve Clifford, Professor of Molecular Paediatric Oncology; Northern Institute for Cancer Research Newcastle University
10:00  Session 1  Clinical Delivery of Genomic Medicine
10:00  Paediatric Solid Tumours - Professor Tom Jacques, UCL Institute of Child Health and Consultant in Neuropathology, Great Ormond Street Hospital for Children
10:25  Paediatric and TYA Sarcomas - Professor Adrienne Flanagan, Professor of Musculoskeletal Pathology UCL Cancer Institute and Royal National Orthopaedic Hospital
10:50  Haematological Malignancies - Professor Anthony Moorman, Professor of Genetic Epidemiology, Northern Institute for Cancer Research, Newcastle University
11:15 - 11:45  Refreshments and comfort break. Poster presentations are available to view.
11:45  INSTINCT - Professor Steve Clifford, Professor of Molecular Paediatric Oncology, Northern Institute for Cancer Research, Newcastle University
12:10  SM Paeds - Professor Darren Hargrave, Professor of Paediatric Neuro-oncology. ICH Development UCL GOS Institute of Child Health.
12:35  Phase I/II Networks -Dr Lynley Marshall, Consultant in Paediatric & Adolescent Drug Development ICR & Royal Marsden Hospital
13:00 - 14:00  Lunch
14:00  Session 2  Translating Tumour Biology into Next Generation Therapies
14:00  Brain Tumours - Professor Chris Jones, Glioma Team Lead, Institute of Cancer Research, London
14:50  Lymphoma - Dr Simon Bomken, Consultant Paediatric Oncologist at the Great North Children’s Hospital in Newcastle, Wolfson Childhood Cancer Research Centre, Newcastle University.
15:15 - 15:45  Refreshments and Comfort Break. Poster presentations available to view
15:45  Session 3  Immunotherapy and the Tumour Microenvironment
15:45  Immunotherapy of Solid Tumours - Dr Karin Straathof, Institute of Child Health London
16:10  Immunotherapy of Acute Leukaemia - Dr Sara Ghorashian, Institute of Child Health London
16:35  Tumour-Promoting Effects of Stem Cell Senescence in Pituitary Tumours - Professor J. P. Martinez-Barbera, Professor of Development Biology and Cancer, UCL Institute of Child Health, London.
17:00  Session 4  Poster Session with pre-dinner reception
       A call for Poster Abstracts of original research, for presentation at the meeting, focusing on the use of genomic medicine throughout the patient journey has been issued. Posters for all accepted abstracts will be displayed in this session.
18:00 - 19:15  Drinks reception in Upper Atrium of hotel.
19:30  Children with Cancer UK Conference Dinner
       The Conference Dinner is an integral part of this conference and is included in the price. It is at the same venue and will provide a good opportunity for networking with other researchers and clinicians.
Day Two: Tuesday 10th September 2019

08:30  Registration open. Refreshments available.
09:00  Session 5   Survivorship
       Clinical prediction of late effects in childhood cancer - Dr Rod Skinner, Consultant Paediatric Haematologist, Great North Children's Hospital, Newcastle.
       Molecular survivorship: future opportunities in understanding and managing late-effects - Dr Debbie Hicks, University Research Fellow, Paediatric Brain Tumour Group, Northern Institute for Cancer Research, Newcastle University.
       Neuropsychological Intervention Studies - Dr Sarah Verity, Clinical Psychologist in Paediatric Neuro-oncology, Great North Children's Hospital, Newcastle.
10:15 - 10:45  Refreshments and comfort break. Poster presentations are available to view.
10:45  Session 6   Early Diagnosis, Screening and Monitoring
       Myelodysplastic syndromes: what lies beneath - Dr Anupama Rao, Consultant in Paediatric Haematology, Great Ormond Street Hospital for Children.
11:10  NGS detection of MRD in ALL - Dr John Moppett, Bristol Children’s Hospital
12:00  Session 7  Causation and Epidemiology
       Highlights of Childhood Cancer 2018 - Alasdair Philips, Trustee Children with Cancer UK
       2018/19 Children with Cancer UK Causation Grant Round - Professor Denis L Henshaw, Scientific Director Children with Cancer UK
13:00  Lunch
14:00  Session 8   Low and Middle Income Countries - The Future
       Developing protocols in resource challenged countries - Professor Simon Bailey, Consultant, Paediatric Neuro-Oncology, Newcastle
       Lessons from the genetics of sporadic & endemic B-NHL - Dr Vikki Rand, Bloodwise Bennet Senior Fellow at Newcastle University
14:30  Session 9   Oral Presentation of Abstracts
       Ten poster abstracts will be selected for “elevator style” 3-minute presentation. The best presentation will be awarded a £1,000 travel bursary
15:15 - 15:45  Refreshments and Comfort Break
15:45  Session 10  Keynote Plenary Lecture
       Evolution of Cancer Cytogenetics, Professor Christine Harrison, Northern Institute for Cancer Research, Newcastle University
16:30  Meeting Closes
Day One
Monday 9th September 2019

Session 1
Clinical Delivery of Genomic Medicine

Session 2
Translating Tumour Biology into Next Generation Therapies

Session 3
Immunotherapy and the Tumour Microenvironment

Session 4
Poster Session
Day One: Invited Speakers

10:00

Professor Tom Jacques

Paediatric Solid Tumours

Childhood tumours are the commonest cause of death in children and the survivors are at risk of long-term disability. Therefore, it is crucial to stratify patients to the best treatment to maximise survival while minimising complications and late effects. Genomic medicine is at the heart of stratifying children with cancer for treatment. This is partly because it offers the opportunity to find targetable mutations for new treatments, but more so because it allows more accurate diagnosis that will ensure that children receive the best conventional treatment. Furthermore, some children will have an inherited predisposition, and this will not only affect theirs and their family’s genetic counselling but may affect their immediate treatment. Against this opportunity for genomics in children, there are considerable challenges for practical implementation including the rarity of the tumours, the multiple centres responsible for diagnosis, the many different molecular tumour subtypes, the need for rapid turnaround times and the need to integrate with clinical trials. There are several major initiatives in the UK that are central to solving these challenges including: the reorganisation of genomic medicine by NHS England with access to whole genome sequencing for all children with solid tumours; the Stratified Medicine for Paediatrics Study offering multi-omic analysis for relapsed and refractory tumours; and the routine incorporation of methylation profiling for childhood brain tumours. I will discuss the interactions of these initiatives and present the UK experience over a 2-year period of clinical methylation profiling.

Professor Tom Jacques

Professor of Paediatric Neuropathology at the UCL Great Ormond Street Institute of Child Health

Tom Jacques is Editor in Chief of Neuropathology and Applied Neurobiology and is the national lead for the Paediatric Tumour Genomics England Clinical Interpretation Partnership. He received the Cavanagh prize of the British Neuropathological Society. He was previously the chair of the scientific steering committee and chief investigator of the CCLG national children’s tumour bank and a former chair of the clinical practices committee of the British Neuropathological Society.
Diagnosing sarcoma subtypes has become more reproducible with the translation of basic research into clinical practice, and this has been demonstrated by the use of whole genome sequencing provided by Genomics England. In certain cases, the findings also allow patients to be better informed about their prognoses, a good example being the clinical outcomes in children with fusion positive congenital/infantile spindle cell rhabdomyosarcoma compared with rhabdomyosarcomas characterised with MYOD1 $L122R$ mutations. However, in many tumour types there remains little progress in utilising the genetic findings to influence response to therapy and clinical outcomes as exemplified in osteosarcoma.

The 100,000 Genomes Project and other DNA sequencing projects have highlighted that sarcomas have strong heritable genetic components. Although this has been recognised for some time, the frequency with which such testing is now being undertaken brings to the fore the challenges of interpreting rare genetic germline alterations in patients with sarcoma. The clinical implications of such findings are potentially important, and research is required to identify sarcoma pre-disposition genes and their variants and to determine the pathogenicity of such rare variants in sarcoma.

The Genomics England 100,000 Genomes Project has encouraged the oncology and pathology community to focus their efforts on delivering tissue which is suitable for advanced molecular pathology analysis, such as whole genome sequencing. To understand the pathogenesis of disease and the impact of chemo and radiotherapy, treatment-naive samples should be analysed as this allows the generation of an evidence base which can be exploited for the purposes of increasing the number of clinical trials for patients with sarcoma. The new NHSE initiative which allows all sarcomas to undergo whole genome sequencing provides significant opportunities to conduct research on these rare diseases and to improve treatment options.

Professor Adrienne M Flanagan

Professor of Musculoskeletal Pathology UCL Cancer Institute and Royal National Orthopaedic Hospital

Adrienne is the President of Path Soc. and the Head of Academic Pathology at UCL, and the Clinical Lead for the London Sarcoma Service, and the Royal National Orthopaedic Hospital. Adrienne is the Sarcoma Genomic England Clinical Interpretation Partnership (GECIP) Lead and for the last 15 years she has driven bone cancer research in the UK and delivered the molecular classification for many of these tumours. In recognition of her contribution to cancer research she has been awarded the Goudie Medal, the William Gerald Award from Memorial Sloan Kettering and an OBE.
Childhood leukaemia, like all cancers, is characterized by the sequential acquisition of genetic aberrations which drive the initiation and maintenance of the leukemic clone. Broadly speaking, genetic abnormalities can be considered as primary or secondary events. Primary abnormalities are responsible for the initiation of a pre-leukemic clone which converts, upon the acquisition of additional secondary or cooperating genetic changes, into overt disease. In acute lymphoblastic leukaemia (ALL), primary abnormalities are often chromosomal translocations which can result in chimeric fusion genes (e.g. BCR-ABL1, ETV6-RUNX1, KMT2A-AFF1 and EBF1-PDGFRA) or oncogene activation (e.g. IGH-CRLF2, STIL-TAL1, BCL11B-TLX3, TRA-DTLX1). Gross aneuploidy, gain or loss of multiple whole chromosomes, giving rise to karyotypes with high hyperdiploidy (51-65 chromosomes), near-haploidy (<30 chromosomes) or low hypodiploidy (30-39 chromosomes) is another prevalent primary event in ALL. In contrast, secondary abnormalities are usually copy number alterations (CNA) (frequently micro-deletions, e.g. IKZF1, CDKN2A/B and ETV6 deletions) and point mutations (e.g. TP53, NRAS and NOTCH1 mutations). Primary abnormalities are, by definition, present in all the cells comprising the leukemic clone and define the key features of the leukaemia. In contrast, secondary abnormalities are present only in a subset of the leukemic cells and give rise to a complex branching sub-clonal architecture. There is a strong correlation between the primary chromosomal abnormality and the spectrum of secondary or cooperating mutations observed in that sub-type. For example, up to 80% of patients with BCR-ABL1 fusions harbour IKZF1 deletions compared to fewer than 15% of patients overall. The spectrum of genetic drivers is rapidly expanding with the application of genome wide technologies (e.g. SNP arrays and whole exome/genome sequencing) to ever-increasing numbers of samples. One of the key challenges ahead is to identify the key prognostic and predictive biomarkers that can be utilized to improve the outcome of childhood with high risk ALL or reduce the intensity of therapy required to achieve lasting remissions. Comprehensive genetic testing of patients suspected of having ALL is standard of care in the UK. The risk stratification algorithm for the new paediatric ALL trial requires information on 19 different genetic abnormalities. Traditional screening will require a minimum of three different methodologies to detect this spectrum of genetic abnormalities. Whole genome sequencing offers the opportunity to detect all these abnormalities using a single test and, importantly, identify clinically actionable variants that may evade detection by traditional methods.

Childhood leukaemia is part of the haematological malignancies Genomics England Clinical Interpretation Partnership (GeCIP) and a few centres around the UK are now accessing clinical WGS on a regular basis for newly diagnosed patients. In the spring of 2019, we instigated a retrospective WGS study of paediatric ALL with both logistical and scientific aims. The logistic aims were (1) to optimize clinical data and sample collection, clinical reporting data validation and interpretation; (2) to improve understanding of the implications of genomic findings and improve the accuracy and reliability of information fed back to clinicians. The scientific aim was to improve our understanding of the genetic basis of disease. In particular, to characterize the spectrum of mutations associated with a slow response to induction therapy. We accessed paired tumour-germline samples from 88 patients treated on UKALL2003 who had a MRD ≥1% at the end of induction. All patients had undergone standard cytogenetic testing at initial diagnosis and some had undergone additional screening using FISH, MLPA and SNP array. Overall, they represented nearly the full spectrum of clinically actionable abnormalities observed in ALL. Samples were submitted to GEL for WGS using the standard pipeline via the North East & North Cumbria NHS Genomic Medicine Centre. I give update on the progress of this project at the Children with Cancer UK conference.

Stratified/precision medicine is a reality in paediatric ALL and many patients are already benefiting from our increased understanding of the genetic basis of this disease. Further genomic characterization of ALL patients using whole genome sequencing will undoubtedly identify novel and better therapeutic targets.
Professor Anthony Moorman
Professor of Genetic Epidemiology, Northern Institute for Cancer Research, Newcastle University

Anthony Moorman has worked in the field of leukaemia genetics for more than 25 years and, along with the other members of the Leukaemia Research Cytogenetics Group (LRCG), has been involved in the discovery, characterisation and clinical assessment of numerous genetic biomarkers in acute lymphoblastic and myeloid leukaemia. More recently, Anthony’s research has focussed on integrating risk factors to assist the design of novel risk stratification algorithms.
Day One: Invited Speakers

11:45

Professor Steve Clifford

INSTINCT

‘High-risk’ paediatric brain tumours (HR-PB Ts) are the leading cause of childhood cancer death. Biological discoveries, and their translation into effective therapies, will be essential to future clinical advances.

The INSTINCT research programme, co-funded by The Brain Tumour Charity, Children with Cancer UK and Great Ormond street Children’s Charity, integrates leading HR-PBT research programmes at The Institute of Child Health, Newcastle University and The Institute of Cancer Research to create a concerted clinical/research network, with the primary aim of delivering improved therapies for children with HR-PB Ts.

INSTINCT is built upon a series of ‘Strategic Initiatives’ which bring together combined strengths across the institutions, in: tumour collection, neuropathology, basic science, experimental modelling, biomarker and drug development, clinical investigations and Phase I/II/III clinical trials, to deliver the essential common infrastructure necessary to undertake state-of-the art biological investigations and translate findings into clinical advances.

These combined strengths are, in turn, applied to support translational research programmes in major HR-PBT types; high-risk medulloblastoma, high-grade and diffuse-intrinsic pontine gliomas, atypical teratoid/rhabdoid tumours and other rare tumour types.

INSTINCT combines the expertise and resources of three of the largest paediatric oncology research/treatment centres in Europe, and builds on established track records and collaborations. Moreover, the leading roles of the teams in international research and clinical trials co-ordinating groups, give the best possible opportunity to translate biological findings into clinical practice.

Professor Steve Clifford

Professor of Molecular Paediatric Oncology; Northern Institute for Cancer Research Newcastle University

Steve Clifford is Director of the Northern Institute of Cancer Research (NICR) at Newcastle University. He graduated with First Class Honours in Applied Biology (Cardiff University) and a PhD in Cancer Molecular Biology (Newcastle University) in the early ’90s. Following post-docs at Cambridge and Oxford Universities, he was appointed to a tenured Lectureship at Newcastle University in 2000, and as Professor of Molecular Paediatric Oncology in 2009. Prof. Clifford directs the NICR’s paediatric brain tumour research programme (25 scientists/clinicians), with major interests in understanding the biological basis of embryonal brain tumour development (principally medulloblastoma, ATRT and other embryonal tumours), and translating these findings into improved clinical treatments. He plays leading roles in national (CCLG, NCRI) and international (SIOP-Europe) research networks and clinical trials in medulloblastoma, and directs the UK national reference centre for medulloblastoma molecular diagnostics and pathology review. His research is supported by five-year programme grants from Cancer Research UK (Biomarker-driven therapies for medulloblastoma, £1.8M), The Brain Tumour Charity/Children with Cancer UK/Great Ormond Street Children’s Charity (INSTINCT: The ICR-Newcastle University-UCL high-risk childhood brain tumour network, £3.9M) and NECCR (Infrastructure funding for paediatric oncology research in Newcastle, £1.2M), and he has published over 100 peer-reviewed papers. In teaching, he leads under-graduate modules in Cancer at Newcastle University, supervises (>20 to date) and regularly examines MD and PhD candidates. Prof. Clifford sits on advisory panels for Cancer Research UK, the French National Cancer Institute (INCa), Children with Cancer UK and The Neuroblastoma Society, and also helps run an outreach programme for children’s cancer care in Malawi, Africa.
Day One: Invited Speakers

12:10

Professor Darren Hargrave

**SM Paeds**

SMPaeds is a UK research study testing tumour (somatic) and normal (germline) DNA and RNA for genetic and gene-expression changes in children, teenagers and young adults with relapsed/refractory cancer. The results of the tests performed will identify patients who may be eligible for new targeted anti-cancer therapies and will aid research that will help us to more precisely diagnose cancer and understand why some patients do not respond to standard treatments. All children, teenagers and young adults with solid paediatric tumours (including brain tumours and lymphoma) whose disease has come back (relapsed) or not responded to treatment (refractory) will be eligible to take part. In addition, the patient must have had a recent biopsy/operation to obtain tumour tissue on which molecular tests can be performed. The results of the testing will be relayed back to the patient’s doctor via an expert group of doctors who will make recommendations on any available treatments. Patients and/or their parents will be asked in advance to consider what information they wish to receive in relation to any abnormal genetic results either in the tumour or their normal (germline) genetic code. In addition, the data collected will be used and shared for the purposes of clinical research.

**Trial Design**

SMPaeds is a molecular profiling platform that will generate genomic, transcriptomic, and epigenetic (methylation) data in children, teenagers and young adults with relapsed/refractory cancer solid and brain cancers with four principle aims:

1. to detect and clinically report the presence of genomic alterations in patients who require molecular confirmation of targets for registration onto precision medicine clinical trials.

2. to increase the accuracy of tumour diagnosis through the inclusion of genomic data.

3. to contribute to the assembly of a comprehensive evidence-database on the prevalence of genomic alterations and a means to prioritise their importance.

4. to identify genomic alterations that increase in frequency between time of diagnosis and relapse.

Professor Darren Hargrave

*Professor of Paediatric Neuro-oncology. ICH Development UCL GOS Institute of Child Health*

Darren Hargrave specialises in paediatric neuro-oncology and the development of new anti-cancer drugs for children and adolescents. He trained in the UK and was a neuro-oncology clinical fellow at the Hospital for Sick Children in Toronto. Upon returning to the UK he was a Consultant Paediatric Oncologist at the Royal Marsden for 10 years before moving to Great Ormond Street Children’s Hospital in 2011 where he leads the experimental therapeutics programme and was appointed as the GOSH Children’s Charity Clinical Professor in Paediatric Neuro-oncology at the UCL Great Ormond Street Institute of Child Health in December 2017.

He is the Chair of the SIOP-E Brain Tumour Group where he previously Chaired the High-Grade Glioma working group. He served as the Chair of the Children’s Novel Agents Subgroup of the UK NCRI for 4 years and he sits on the ITCC Clinical Trials Committee and is a Chief Investigator of over 15 completed, on-going and planned clinical trials in paediatric cancer. He is a member of the Guy’s Complex NF1 clinic and has an interest in NF related tumours. His research interests include: the biology of childhood brain tumours, the use of innovative imaging techniques in childhood cancer and drug development of targeted therapies in childhood and adolescent oncology. He is the Co-chair of the UK Stratified Medicine Paediatrics (SMPaeds) molecular/ precision medicine platform and a member of the NHSE Task and Finish Group for the implementation of whole genome sequencing in childhood cancer.
Day One: Invited Speakers

12:35

Dr Lynley Marshall

Phase I/II Networks – Better Together: Accelerating Clinical Trials of Molecularly Targeted Agents in Children and Young People

Childhood cancer survival outcomes have improved continuously decade upon decade, due to enhanced diagnostic techniques, advances in multimodal intensive treatment strategies, better supportive care and (in no small part) national and international collaborative clinical trials edging survival curves upwards via serial gains. Nonetheless, the reality is that the positive statistics are skewed by some of the more common but better prognosis malignancies. For children and young people with high risk cancers at the outset, or certain relapsed/refractory diseases, outcomes range between suboptimal and dismally poor, and novel approaches are desperately needed. The explosion of more readily and affordably available molecular tumour profiling techniques over recent years has started to unlock the biological secrets underlying many adult cancer types, resulting in the development of vast numbers of new molecularly targeted agents which can be used in a more personalised, biologically selective way within endless potential treatment combinations. These advances are now increasingly being harnessed to further improve our knowledge of and treatment options for childhood and adolescent cancers, and ultimately patient outcomes. Whilst this is best done within the context of robust evidence gathered through adequately powered biomarker-driven clinical trials, the relative rarity of individual cancer types and even rarer molecular subpopulations, but a plethora of new agents even within the same class of drugs, present a numbers challenge. It is clearer than ever that the best chance of accelerating the most promising agents from preclinical testing, through well-designed biomarker-rich early clinical trials, and into more frontline regimens as efficiently as possible is via truly collaborative national and international efforts. This talk will thus focus on the current national and international landscape of clinical trials consortia, the important complementary roles of biologists, specialist early phase trial networks and tumour-specific/target-specific later phase trial experts in advancing the best drugs forward via efficient and novel trial designs, and the crucial importance of multi-stakeholder working, including with academics, clinicians, pharmaceutical companies, regulatory authorities and parent/patient representatives towards the common goals of providing equitable access to novel therapies and driving forward progress in drug development for children and young people with cancer. Specific phase I/II networks such as the Paediatric Experimental Cancer Medicine Centre (ECMC) network, Innovative Therapies for Children with Cancer (ITCC) consortium and others, and initiatives such as the ACCELERATE platform will be discussed, and specific molecularly targeted agent/personalised medicine clinical trials exemplified.

References

1. ECMC: https://www.ecmchenetwork.org.uk › paediatric-network
2. ITCC: http://www.itcc-consortium.org/
3. ACCELERATE: https://www.accelerate-platform.org/

Dr Lynley Marshall
Consultant in Paediatric & Adolescent Drug Development ICR & Royal Marsden Hospital

Dr Lynley Marshall (MB BCh DCH MRCPCH PhD) is the Oak Foundation Consultant/Lead for Paediatric and Adolescent Oncology Drug Development, Paediatric Clinical Research Lead and Paediatric Experimental Cancer Medicines Centre (ECMC) Lead at The Royal Marsden Hospital and Honorary Faculty member at The Institute of Cancer Research, London, UK where she previously obtained her PhD in the field of novel agents for paediatric high grade glioma. Prior to her appointment into her current role she served
a year as National Expert on Secondment (Paediatric Oncology) at The European Medicines Agency, working with the Agency’s Paediatric Committee in the field of Paediatric Oncology Drug Development. She is current Chair of the NCRI Children’s Cancer and Leukaemia Clinical Studies Group Novel Agents Subgroup, and a member of the Innovative Therapy for Children with Cancer (ITCC) European Early Phase Trial Consortium’s Clinical Trial Committee. She is also a member of the Executive Committee of ACCELERATE, an international multi-stakeholder platform working hard to drive forward access to novel anti-cancer therapies for children and young people with cancer, and a member of their ‘Fostering Age Inclusive Research’ (FAIR) trials working group. Her passion is facilitating access to promising new agents for children and young people with a high unmet medical need through high quality clinical trials, in the setting of providing excellent and holistic clinical care for young patients and their families. She has experience as UK Chief Investigator on more than 15 international pharma or academic sponsored early phase studies, including first in child studies of molecularly targeted agents and immunotherapy agents, and as local Principle Investigator on multiple other phase I/II trials. She is UK Chief Investigator for the ITCC E-SMART innovative multi-arm combination basket phase I/II study.
Day One: Invited Speakers

14:00

Professor Chris Jones

Brain Tumours

High grade gliomas are very different when they arise in children compared to adults. They occur in different locations, with very different biological drivers, and a long history of clinical trials based upon extrapolation from adult data have been doomed to failure. Moreover, they do not represent a single entity – there are in fact a bewildering array of very distinct subtypes within the umbrella term ‘high grade glioma’ in children, again occurring in different parts of the brain, at different ages, and within distinct biology. Although overall having a dismal survival, we are now beginning to make progress such that not all ‘high grade glioma’ are incurable – there are becoming several examples of subgroups for which we now have seemingly effective treatments which are beginning to show clinical responses for the first time. There are however subtypes for which this is not the case, and new therapies are desperately needed – I will give examples of how new models of drug development can take highly specific targets only found in childhood glioma subtypes and still produce drugs which can get to clinic, and how an extraordinary level of collaboration in the field can run international trials in these very rare patient populations.

Professor Chris Jones
Glioma Team Lead, Institute of Cancer Research, London

Chris Jones grew up in Perth, West Australia before moving to the UK, and did his undergraduate (Toxicology and Pharmacology) and PhD (Molecular Biology) studies at what is now UCL. He spent a year with Novartis in Switzerland, and first joined the ICR in Chelsea (Breast Cancer) in 2001, before starting his own lab in Sutton (the old Section of Paediatric Oncology) in 2003. Since around 2006 his lab has been focussed on the study of high grade glioma in children. He has been Chair of the SIOP Europe HGG/DIPG Biology Group and Biology Lead on the HERBY and BIOMEDE clinical trials. They carried out the first and largest molecular characterisation of these tumours, have turned a lot of their efforts into developing novel subgroup-specific models for mechanistic and preclinical analyses, and continue to explore their evolutionary dynamics and subclonal diversity and interactions.
Neuroblastoma

Neuroblastoma, a tumour arising from the sympathetic nervous system is the commonest extra-cranial malignancy of childhood. The clinical spectrum of disease is variable: Infants with widespread metastatic disease can have a spontaneous resolution, whereas approximately two thirds of children with neuroblastoma have high-risk disease at the time of presentation, associated with a poor outcome, despite intensive therapy. Recently, the molecular drivers of aggressive neuroblastoma have been identified, with the common underlying mechanism relating to altered telomere maintenance. Three distinct molecular subgroups exist: MYCN amplified and TERT rearranged, both associated with an increase in telomerase activity, and ATRX mutated, associated with Alternative Lengthening of Telomeres (ALT). Furthermore, it has been shown that when an altered telomere maintenance mechanism is present in association with other key somatic mutations (most commonly activating ALK mutations), then the outcome is uniformly dismal.

Pre-clinical studies in the Chesler laboratory are focused on effective targeting of the underlying drivers of aggressive disease. Recent preclinical data on strategies to target MYCN, ATRX and ALK will be presented, including how this work will be directly translated to clinical trials for children with neuroblastoma in the near future.

Dr Sally George

Sally George is an academic paediatric oncologist, currently working at The Royal Marsden Hospital (RMH) and The Institute of Cancer Research (ICR). She successfully conceived, wrote and developed her own PhD project, based on her clinical experience, focusing on identifying novel therapies for children with ATRX mutant neuroblastoma – associated with a distinct clinical phenotype and chemo-resistance. She is also involved in a number of translational research projects, including development of the clinical sequencing pilot programme for children with cancer which has directly lead to SMPaeds – the stratified medicine initiative for childhood cancers. She is also leading sequencing/translational projects associated with the SIOPEN high-risk neuroblastoma clinical trial and the BEACON clinical trial for children with relapsed/refractory neuroblastoma. She has recently been appointed to a fellowship position at the Francis Crick Institute to commence in 2020 to continue her work on ATRX mutant neuroblastoma.
Day One: Invited Speakers

14:50

Dr Simon Bomken

Lymphoma

The diverse malignancies which comprise non-Hodgkin lymphoma make up a notable proportion of the paediatric oncologists workload, with highly varied management and clinical course. Individually, however, these different diseases are rare and developing our understanding of their biology is challenging. These challenges are mirrored by the obstacles to developing and delivering early phase clinical studies for this group of patients.

Focusing on mature B cell lymphomas, I will discuss the current state of our understanding of the driving molecular events in the most common NHL subgroups, Burkitt and diffuse large B cell lymphoma. I will describe how the UK B-NHL cohort is being studied to provide critical insights into novel biomarkers and potential therapeutic targets to inform the development of molecularly driven clinical trials. Finally I will consider how, in place of relapsed patient samples, in vivo modelling can be used to study the development of therapy resistance, generating a detailed understanding of intratumoural heterogeneity and clonal evolution.

Dr Simon Bomken

Consultant Paediatric Oncologist at the Great North Children’s Hospital in Newcastle, Wolfson Childhood Cancer Research Centre, Newcastle University

Simon is an Honorary Consultant Paediatric Oncologist at the Great North Children’s Hospital in Newcastle, and a Clinical Fellow at the Wolfson Childhood Cancer Research Centre, Newcastle University. Clinically, he is responsible for the care of children with lymphomas and Langerhans cell histiocytosis (LCH) with a particular clinical interest in the development of lymphoproliferation in the setting of immune dysregulation.

The lymphoma research group at the Wolfson Centre focuses on developing our understanding of mature B cell lymphomas. They have ongoing projects working with cohorts from both the UK and Malawi. The aim of the group is to identify prognostic biomarkers to improve patient stratification and novel therapeutic targets to support the next generation of early phase drug development. Within those aims Simon is responsible for leading the functional and patient-derived xenograft (PDX) studies. He was recently awarded an MRC Clinician Scientist Fellowship to support his use of Burkitt lymphoma PDX models to study intratumour heterogeneity and evolution under therapeutic pressure. Understanding how Burkitt lymphoma evolves from an exquisitely chemosensitive disease to one with multidrug resistance and very poor outcome is critical to developing effective salvage strategies. In turn, the offer of effective salvage will allow us to implement genomically driven risk stratification in the setting of front-line clinical trials.

Simon is the current chair of the CCLG non-Hodgkin lymphoma special interest group and member of the NCRI paediatric NHL subgroup.

https://www.ncl.ac.uk/nicr/staff/profile/snbomken.html#background
Day One: Invited Speakers

15:45

Dr Karin Straathof

**Immunotherapy of Solid Tumours**

Treatment of high-risk neuroblastoma remains highly challenging. The current treatment regimen consists of a combination of chemotherapy, surgery, radiotherapy and more recently immunotherapy. This highly intensive regimen is associated with significant side effects and still less than half of patients will be long-term survivors highlighting a clear need for different treatment approaches. Neuroblastoma uniformly expresses disialoganglioside GD2, while its expression on normal tissue is highly restricted, which makes GD2 a suitable target antigen for immunotherapy. Indeed GD2-specific monoclonal antibodies as maintenance treatment have been shown to prolong the period during which the disease is controlled. Disease recurrence however remains a significant problem.

Redirecting specificity of patient T-cells to GD2 has the potential to induce durable anti-tumour immunity. One approach to achieve this uses a chimeric antigen receptor (CAR) which combines the specificity of an antibody with potent and persistent effector functions of a T-cell. A CAR effectively grafts the desired specificity onto the patient’s own T-cells. Ground-breaking clinical responses achieved in B-ALL demonstrate the potential of CAR T-cell therapy. Results to date of our clinical study of GD2-CAR T-cell therapy in patients with relapsed/refractory neuroblastoma (NCT02761915) shows that anti-tumour activity can be induced in this childhood solid tumour. Crucially, anti-tumour activity was achieved without neurotoxicity. Nevertheless, responses so far have been short-lived. This is likely due to immune inhibitory mechanisms employed by the tumour. Current work focuses on next generation CAR T-cell approaches whereby CAR T-cells are equipped with ‘functional modules’ which support their persistent function within a hostile tumour microenvironment.

An alternative approach to re-direct T-cell specificity to induce anti-tumour immunity are bi-specific T-cell engagers (BiTEs). BiTEs consist of a T-cell binding domain and tumour-antigen binding domain and act as engagers of a tumour-specific T-cell response. BiTEs are an off-the-shelf treatment and hence their use at the stage of treatment where lymphocytes numbers have recovered should be feasible in all high-risk patients. BiTEs have the potential to improve MRD treatment with less associated toxicity. We have generated a panel of human GD2/CD3 BiTes and their murine counterparts. Current work focuses on selection of the optimal BiTE design and approach to incorporate these into treatment for high-risk neuroblastoma.

**References**


Dr Karin Straathof

*Wellcome Trust Clinician Scientist, UCL Great Ormond Street Institute of Child Health, London*

Karin Straathof graduated with both a medical degree and BSc from Leiden University, the Netherlands. Her PhD at Baylor College of Medicine (Houston, USA) was on adoptive T-cell immunotherapy for Epstein-Barr virus associated malignancies. During her paediatric clinical training in London she continued her academic training as NIHR Academic Clinical Lecturer in paediatric oncology. She is currently a Wellcome
Trust Intermediate Clinical Fellow at UCL Great Ormond Street Institute of Child Health. Her research interest is in the development of T cell-based immunotherapy for childhood solid tumours. Dr Straathof has developed and is investigator on a phase I/II clinical trial of GD2-directed CAR T-cell therapy for patients with refractory or relapsed neuroblastoma. Her current research includes identification and validation of new target antigens for childhood solid tumours, assessment of the composition of the tumour microenvironment and mechanism employed to evade anti-tumour immunity, generation and testing of next generation T-cell based immunotherapeutics including CAR T-cells and bi-specific T-cells engagers, and pre-clinical models to study the optimal approach to translate these treatment strategies into clinical studies. Dr Straathof is member of the Innovative Therapies for Children with Cancer (ITCC) Biology Group and the Experimental Cancer Medicine Centres (ECMC) Paediatric Strategy Group. She is co-chair of junior faculty at the UCL Great Ormond Street Institute of Child Health Biomedical Research Centre and Lead for Childhood Cancer of the Cancer Research UK City of London Centre.
Immunotherapy of Acute Leukaemia

Chimeric antigen receptor (CAR) T cells targeting CD19 demonstrate unparalleled responses in relapsed/refractory acute lymphoblastic leukemia (ALL) but toxicity including Cytokine Release Syndrome (CRS) and neurotoxicity limits broader application. Moreover, 40-60% of patients relapse due to poor CAR T cell persistence or emergence of CD19- clones. CAR design can have a profound effect on CAR T cell function and persistence. However, little is known about the impact of CAR binding affinity. We therefore generated a novel CD19CAR (CAT) with a lower affinity than FMC63, the high affinity binder utilised in many clinical studies. In a clinical study (CARPALL, NCT02443831), 12/14 patients with relapsed/refractory pediatric B-ALL treated with CAT CAR T cells achieved molecular remission. CAR T cell expansion was enhanced compared to published data and persistence was demonstrated in 11 of 14 patients at last follow-up. Toxicity was low with no severe Cytokine Release Syndrome. One year overall and event-free survival were 63%/46%.

Further challenges in the field are to improve patient outcomes by reducing relapses post CAR T cell therapy, to improve access to CAR T cell therapy by facilitating scalability of CAR T cell manufacture and universal CAR T cell approaches, as well as trying to apply CAR T cell therapy to other haematological malignancies.

References


Dr Sara Ghorashian
Consultant Paediatric Haematologist, Great Ormond Street Hospital for Children, London

Sara Ghorashian is Consultant Paediatric Haematologist at Great Ormond Street Hospital for Children, London, UK, and an honorary Senior Lecturer at UCL. After receiving her medical degree from the University of Oxford, Dr Ghorashian undertook a PhD in TCR gene engineering of therapeutic T cells for at the University College London Institute of Immunity and Infection.

Dr Ghorashian’s research focuses on T-cell immunobiology and the use of cellular immunotherapies for children with haematological malignancies, having translated a novel CD19-targeted CAR-T cell product from the laboratory to the clinic. She is an investigator on 4 CAR-T cell studies at GOSH, is UCL lead PI for a CRUK accelerator grant to investigate factors involved in therapeutic efficacy of CAR T cells with a view to improving patient outcomes and finally is investigating approaches for AML CAR T cell therapy.
Day One: Invited Speakers

16:35

Professor Juan Pedro Martinez-Barbera

Tumour-Promoting Effects of Stem Cell Senescent in Pituitary Tumours

It is increasingly evident that paediatric brain tumours are developmental disorders, and as such, their study can benefit from using combination or cancer biology and developmental approaches. I will present data showing that applying a developmental perspective, we have revealed the molecular aetiology and pathogenesis of adamantinomatous craniopharyngioma (ACP), a clinically aggressive brain tumour in children. These studies have revealed a critical role of Ctnnb1 (encoding beta-catenin) mutations in ACP and uncovered a novel role of pituitary stem cells in cancer, which is conceptually different to the cancer stem cell paradigm. Specifically, we showed that when SOX2+ pituitary stem cells are targeted with oncogenic beta-catenin they do not form the tumour mass, rather they induce tumours in a paracrine manner. I will discuss current data suggesting a critical role for cellular senescence in paracrine tumourigenesis.

References


Professor Juan Pedro Martinez-Barbera

Professor of Development Biology and Cancer, UCL Institute of Child Health, London

Professor Juan Pedro Martinez-Barbera is the Head of Developmental Biology and Cancer at the Great Ormond Street Institute of Child Health. His research aims to reveal the mechanisms underlying normal development and the pathology associated to the forebrain and pituitary gland in mice and humans, with the ultimate goal of improving disease treatment and management of patients. His recent research focuses on childhood brain tumours, in particular paediatric craniopharyngioma. Combining murine and human studies he has identified cellular senescence as a tumour-inducing mechanism in the pituitary. His studies have provided a molecular rationale for the histological similarities between craniopharyngioma and tooth development, resulting in the identification of novel targetable pathways.
Day Two
Tuesday 10th September 2019

Session 5
Survivorship

Session 6
Early Diagnosis, Screening and Monitoring

Session 7
Causation and Epidemiology

Session 8
Low and Middle Income Countries - The Future

Session 9
Oral Presentation of Abstracts

Session 10
Keynote Plenary Lecture
Day Two: Invited Speakers

9:00

Dr Rod Skinner

Clinical prediction of late effects in childhood cancer

The continuing improvements in contemporary treatment for childhood cancer have resulted in 5 year survival rates of over 80% but this success has been tempered by a high prevalence of late adverse effects due to the cancer or its treatment, particularly with radiotherapy and / or chemotherapy. Several large cohort studies have shown that 60-70% of childhood cancer survivors suffer from at least one chronic medical condition, 25% suffer from two or more, and 25% from life-threatening or life-changing conditions.

This presentation will describe examples of clinical risk factors (eg drug or radiotherapy exposure and dose, patient age at treatment) that have been identified for several important late toxicities, including cardiomyopathy, nephrotoxicity, male and female gonadotoxicity, bone, auditory and neurocognitive toxicities, and secondary malignancies. However, these patient and treatment risk factors only explain some episodes of late toxicity. The observation that some patients of the same age may develop severe late toxicity despite receiving identical treatment to the majority who do not develop such toxicity highlights the important need to investigate genetic and other factors that may allow improved risk prediction and understanding of the pathogenesis of late effects.

Professor Rod Skinner
Consultant Paediatric Haematologist, Great North Children’s Hospital, Newcastle

Professor Rod Skinner has been a Consultant in Paediatric and Adolescent Oncology / BMT at the Great North Children’s Hospital, Royal Victoria Infirmary, Newcastle upon Tyne since January 1996, and is an Honorary Professor of Childhood Cancer, Northern Institute of Cancer Research, Newcastle University. He qualified in Birmingham in 1983, trained initially in general paediatrics and then in paediatric haematology/oncology and BMT in Newcastle, with further BMT experience in the Pediatric BMT Program, Minneapolis, USA. He was awarded a PhD by Newcastle University in 1995 for his research thesis into chemotherapy-induced nephrotoxicity in children with cancer. He is the clinical lead in Newcastle for paediatric haematology allogeneic haemopoietic stem cell transplantation, long-term follow-up (LTFU), and bone marrow failure, and has maintained a busy practice in paediatric / adolescent leukaemia.

His research interests include late adverse effects of childhood cancer treatment, especially nephrotoxicity, and LTFU of childhood cancer survivors. He has played a leading role in the development of evidence-based, internationally-harmonised LTFU surveillance guidelines. He is a member of the UK Children’s Cancer and Leukaemia Group, with leading roles in the Late Effects Group (current chair), Supportive Care Group (chair 2003-08) and BMT Group (chair 2008-2012). He was a National Clinical Advisor in the NHS National Cancer Survivorship Initiative (2008-2012). He was one of the three instigators and founding members in 2008 of PanCare (Pan-European Network for Care of Survivors after Childhood and Adolescent Cancer), and served on its Executive Board until 2019.
Day Two: Invited Speakers

09:25

Dr Debbie Hicks

Molecular survivorship: future opportunities in understanding and managing late-effects

Cancer ‘Survivorship’ is defined as starting at the time of diagnosis and lasts throughout the lifespan and as such encompasses both treatment-related acute toxicities and long-term sequelae (‘late effects’) involving reproductive, cardiac, respiratory, endocrine, metabolic and neurological systems. Despite the huge advances in molecularly-driven risk-stratification, classification, and therapeutics in childhood cancers, equivalent advances have not been made with reference to survivorship outcomes, with a particular paucity in paediatric brain tumour research. The huge unmet need of these childhood cancer survivors (~300,000 – 500,000 across Europe) represents one of the most urgent disease priorities. To date, the focus of cancer survivorship research has largely surrounded quality-of-life issues (eg, emotional well-being) and physical health (eg, fertility) with little reference to biological or genetic factors. Without full consideration of the molecular aspects that influence cancer survivorship, our ability to understand the potential mechanisms of risk for long-term and late effects of cancer and the impact of interventions to address these effects remains limited. I will review the current landscape of molecular studies in childhood cancer survivorship, and focus on initiatives in medulloblastoma that implicate relationships between host genotypes, tumour molecular subgroups and neurocognitive / health-related quality of life outcomes.

I will also look ahead to our potential future ability to address the question of whether the impact of late-effects can be reduced, whilst maintaining survival rates, using lower-intensity treatments in favourable-risk disease subgroups of medulloblastoma.

References


Dr Debbie Hicks

Newcastle University Research Fellow of Molecular Cancer Survivorship

Dr Debbie Hicks is a Newcastle University Research Fellow of Molecular Cancer Survivorship. She graduated with Honours in Molecular Biology (Edinburgh University, 2003) and a PhD in Molecular Genetics (Newcastle University, 2009). Following a post-doc at Newcastle University’s Institute of Genetic Medicine, where she worked within a multidisciplinary translational research group on large, multi-centre, European projects, Debbie was awarded a Faculty Fellowship to develop an original research idea that centred around the use of next-generation sequencing approaches in rare-disease diagnostics and etiology.

Recognising the benefit of applying of these cutting-edge omic’s technologies to cancer, Debbie moved to the Northern Institute for Cancer Research to work within Prof. Clifford’s paediatric brain tumour research programme (25 scientists/clinicians) and was later appointed to a Newcastle University Research Fellowship (NURF) position. Her work involves molecular pathology studies in medulloblastoma to enable biomarker discovery and improved risk-stratification and aims to reduce the disease/treatment-associated burden in medulloblastoma survivors by understanding the key clinico-molecular correlates.
of late effects, developing/assessing reduced intensity therapies, and advancing pharmacological intervention strategies.

Debbie is a member of national (CCLG, BACR) and international (EACR) professional bodies, and sits on the International Society for Paediatric Oncology (SIOPE) Brain Tumour (1) Quality of Survival, (2) Embryonal Tumours and (3) Biology working groups. She has published a book chapter and over 60 peer-reviewed papers and conference proceedings. Debbie has supervised 4 PhD candidates and >25 MRes, MSc, UG and Erasmus studentships.
The impact of cranial radiotherapy (CRT) in childhood upon future intellectual development is well established. Whilst late cognitive effects vary between individuals, mean loss of IQ post treatment in children with medulloblastoma is reported at 1-2 standard deviations below the mean\textsuperscript{1,2,3,4}. A dose dependent relationship between amount of CRT and level of IQ deterioration has been identified\textsuperscript{5}. The long-term effect of failure to make intellectual gains at the normal rate results in eventual mean IQ scores in the borderline learning disability range or lower for many children with medulloblastoma\textsuperscript{4}, resulting in poor academic attainment, high rates of unemployment, and low self-reported social-emotional satisfaction.

Earliest emerging treatment effects are identified in speed of processing, attention, and working memory (cognitive proficiency domains). Impairment in these domains negatively affect the acquisition of new learning, such that paediatric survivors do acquire further academic and general intellectual ability but at a greatly reduced rate comparable to healthy peers\textsuperscript{6}. The pattern of impairment is similar to that of children with acquired hydrocephalus secondary to brain tumour, in which failure to make intellectual gains over the developmental period results in a profile of generalised intellectual impairment with relative greater deficits in cognitive proficiency skills in the early years post treatment. Children in the post --CRT and post-hydrocephalus populations show reduction of normal appearing white matter volume, this being associated with slowed speed of cognitive processing\textsuperscript{7}. Structured equational modelling studies show a direct relationship between processing speed and future childhood cognitive development, suggesting that the remediation of impaired speed of processing may have the potential to reduce long term loss of overall intellectual ability in children with post-brain tumour cognitive deficit\textsuperscript{8}. Clinical research has considered the potential utility of psychostimulant medications used in children with ADD/ADHD to the paediatric oncology population for this purpose\textsuperscript{9}. This research team found short acting methylphenidate hydrochloride to be well tolerated by this group\textsuperscript{10}, offering improvement to children with brain tumour or acute lymphoblastic leukaemia in terms of increased attentional ability, when measured over a one-year period\textsuperscript{11}.

In March 2017 the paediatric neuro-oncology team at GNCH established a survivorship clinic for the neuropsychological assessment of attention and processing speed in children post-CRT and those with acquired hydrocephalus secondary to brain tumour. Appropriate candidates were provided with short-acting methylphenidate hydrochloride and entered to a long-term follow up programme of neuropsychological assessment. Results of preliminary analyses will be presented and discussed.

References


**Dr Sarah Verity**  
*Clinical Psychologist in Paediatric Neuro-oncology, Great North Children’s Hospital, Newcastle*

Dr Sarah Verity is a Chartered Clinical Psychologist with specialist training in Paediatric Neuropsychology. She is a Practitioner Full Member of the Division of Neuropsychology of the British Psychological Society. Sarah works in paediatric neuro-oncology at the Great North Children’s Hospital, Newcastle, within which she leads a project exploring the use of psychostimulant medication in preserving intellectual function in children with brain tumour. She is neurocognition lead on the SIOP-E International prospective trial on high-risk medulloblastoma.

Sarah also teaches onto the paediatric neuropsychology stream of the Doctorate in Clinical Psychology at Newcastle University, provides elective year neuropsychology placements to clinicians in training, and supervises Masters and Doctoral projects in Clinical Psychology and Medicine. She is lead for research and clinical innovation in the department of health psychology at Newcastle Upon Tyne Hospitals NHS Foundation Trust. Sarah remains disappointed not to have been awarded a Blue Peter badge in childhood.
Day Two: Invited Speakers

10:45

Dr Anupama Rao

Myelodysplastic syndromes: what lies beneath

The aim of my talk is to give a broad overview of our current molecular understanding of paediatric myelodysplastic syndromes. The natural history of each of the well-described genomic disease entities highlights the need for early diagnosis and pursuing a surveillance strategy which is personalised for each patient. I shall address the clinically relevant questions that arise when presented with genomic information and how this information influences clinical care. I shall present data from the NIHR MDS study to exemplify the impact of early diagnosis, referral pathways and surveillance outcomes in children within the heterogeneous cohorts of paediatric MDS, JMML and Transient Abnormal Myelopoiesis leading to ML-DS.

Dr Anupama Rao
Consultant in Paediatric Haematology, Great Ormond Street Hospital for Children

Anupama Rao is a Consultant Paediatric Haematologist at Great Ormond Street Hospital London. His research interests align with paediatric translational clinical research in rare pre-malignant myelodysplastic/myeloproliferative diseases in children and adolescents. He is Chief Investigator on a portfolio adopted NIHR Translational Research Collaboration Project in Paediatric Myelodysplastic Syndromes. This project is a collaboration between Great Ormond Street Hospital and Weatherall Institute of Molecular Medicine, Oxford where Prof Irene Roberts and Dr. Adam Mead have been the lead academic researchers in stem cell biology of children with rare pre-malignant haematological disorders. Information from genomic panels delivered via next generation sequencing platforms and combining this data with a detailed phenotypic characterisation of children has led to the identification of leukemia pre-disposition syndromes, GATA2, SAMD9 and SAMD9L, FPD/AML to name a few. Important questions relating to penetrance and expressivity of leukemia pre-disposition genes within families and the estimated risks of transformation to myeloid leukemia can vary based on the gene involved and therefore is unique to each condition. His focus of research is two-fold: to describe and interrogate the patterns of molecular heterogeneity in myelodysplasia and myeloproliferative disorders (JMML) and the utility of this genomic information when interrogating the cellular architecture of myeloid diseases. He is keen to empower and partner with patients and their families, explaining scientific information, in lay terms, regarding our current understanding of diseases affecting them and the knowledge gaps that exist. This serves as fertile ground to identify key questions, relating to treatment strategies and long term outcome, for future translational research projects.
Day Two: Invited Speakers

11:10

Dr Franck Bourdeaut

Detection of Cell-Free DNA

Liquid biopsies are revolutionary tools to detect tumour-specific genetic alterations in body fluids. While plasma is the fluid of choice for extracranial tumors, it seems not to be so relevant for malignancies from the central nervous system (CNS). Hence, isolating circulating tumor DNA (ctDNA) in Cerebral Spinal Fluid (CSF) is of potential interest to circumvent this limitation. We will describe our preliminary experience with pediatric embryonal tumors of the CNS. With as few as 4-5 lumbar puncture droplets, we have been able to isolate useful quantities of catena (mean 30ng). This ctDNA can first be used to generate whole exome sequencing, allowing comparisons between ctDNA, the primary tumor and the matched constitutional DNA. In medulloblastoma a mean of 416 commons SNVs were observed between the cfDNA and the primary tumor, including clinically relevant variations in SMO or SMARCB1 for instance. Interestingly, several SNVS were observed either in the tumor only (mean 50), or in CSF only (mean 58) suggesting a clonal heterogeneity. Such an heterogeneity may provide useful tools to monitor the tumor evolution upon treatment. Depending on the quantities of ctDNA, Copy Number Variations can also be searched for, allowing for instance the detection of MYCN amplification in medulloblastomas, or 19q13 miRNA cluster amplification in ETMR. Beyond whole exome sequencing, specific captures on ctDNA may also allow to infer the open status of the chromatin and thereby the regions that are actively transcribed. This technic has been used to define diagnostic signatures in the ctDNA. Altogether, we illustrate the feasibility of high throughput sequencing approaches on CSF with a low input of ctDNA. These results may pave the way for new tumor monitoring tools.

Dr Franck Bourdeaut

Cancer of the Child & Adolescent, Institute Curie, Paris

Franck Bourdeaut has been trained in Paris as a pediatric oncologist, and is sub specialized in neuro-oncology. After a PhD exploring the links between normal development of the sympathetic system and neuroblastoma in Olivier Delattre’s lab, curie Institute, he has focused his research interest on rhabdoid tumors, addressing issues ranging from basic science to translational and clinical research. His lab is mainly endeavoring to set up mouse models for the various subtypes of rhabdoid tumors and to develop preclinical studies using various screening methods and therapeutic experiments. As a neuro-oncologist trained in a laboratory of genetics, he is also involved in the molecular diagnosis of brain tumors, including ATRT, medulloblastomas and other embryonal tumors of the central nervous system. He has recently be elected chair of the SIOP Europe ATRT working group.
Day Two: Invited Speakers

11:35

Dr John Moppett

NGS detection of MRD in ALL

Measurable Residual Disease (MRD), as assessed by real time quantitative (RQ) PCR of Immunoglobulin and T-cell receptor gene rearrangement is recognised as the gold standard for risk stratification in childhood Acute Lymphoblastic Leukaemia. Dr Moppett will discuss recent developments in high throughput sequencing and how they have been applied to MRD analysis to help solve some of the remaining limitations of the assay.

Dr John Moppett
Bristol Children’s Hospital

Dr. John Moppett studied medicine at Jesus College Cambridge and St. Bartholomew’s Hospital London. He trained in both Paediatric Oncology and Haematology in the UK, Australia and Canada, and obtained his PhD in 2003 from the University of Bristol. He was appointed to his current role of consultant Paediatric Haematologist at Bristol Royal Hospital for Children in 2007. His clinical practice covers Paediatric Malignant Haematology, Bone Marrow Transplant and Benign Haematology – in particular cytopenias. In Bristol, he is local Principal Investigator for several early and late phase trials in Childhood Leukaemia.

His research interests are Measurable Residual Disease and clinical trials in Childhood Acute Lymphoblastic Leukaemia (ALL). He is clinical lead for the United Kingdom Childhood Measurable Residual Disease Laboratory Network and is currently leading a project developing Next Generation Sequencing (NGS) based MRD analysis in the UK. He is also leading a project to implement Copy Number Alteration (CNA) based risk stratification for Childhood Acute Lymphoblastic Leukaemia using SNP array technology. He is a member of the National Cancer Research Institute Children’s Clinical Studies Group and its Leukaemia subgroup. He is national co-investigator for the current de novo Childhood ALL trial UKALL2011 and is UK Lead Investigator for the upcoming international de novo Childhood ALL study ALLtogether (Chief Investigator Mats Heyman, Karolinska, Sweden). He is Chief Investigator for the Bloodwise Childhood Leukaemia cellbank.
Day Two: Invited Speakers

12:00

Alasdair Philips

**Highlights of Childhood Cancer 2018**

The main charitable aims of Children with Cancer UK are to understand what causes children to develop cancer, to develop improved treatments and to help to provide care for affected children and their families. To this end, over the past 31 years we have raised and used over £250 million.

Our research aims can be broken down into the following objectives:

- To improve knowledge of the genetic and environmental causes and the underlying biological mechanisms of that cause and promote childhood cancers;
- To identify diagnostic and prognostic biomarkers for childhood cancers;
- To optimise and develop more effective and less toxic treatments for children with cancer, with a special focus on those forms of cancer that still carry a poor prognosis;
- To understand the long-term health implications of childhood cancer and its treatment;
- To promote the dissemination of research findings to achieve maximum impact.

The 2018 conference was focused on issues related to our understanding the reasons why children develop cancer, to find causal explanations and to establish whether prevention is a possibility. We want to drive forward the development of novel approaches to diagnosis, identification of markers of likely outcome and improved treatments for childhood cancer in order to tackle those forms which still elude successful treatment. We also need to minimise the short and long-term risk of adverse, treatment-related effects. There are now over 30,000 childhood cancer survivors in the UK, many with long-term adverse effects on their health and well-being.

This year’s Newcastle conference focuses on Precision Treatment which is part of Precision Medicine (PM). This is an area that we currently support and we intend to continue to do so.

The NIEHS definition of PM is:

> “Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.”

We must also gain better insights into the biological, environmental, and human behavioural or lifestyle influences on the initiation and development of cancer. The age-standardised incidence rate for all cancers is increasing in both the 0—14 and, especially, the 15–24 year-old age groups that Children with Cancer UK’s remit covers. 287 non-natural potentially toxic substances, including known carcinogens, have been identified in cord-blood samples taken from newly born children.

Child cancers are rare and so epidemiological studies are difficult and expensive. We need to tie the more detailed knowledge that genomics and proteomics give us about neoplasms, to environmental and lifestyle information recorded at the time of diagnosis. Achieving that will start to enable a more forensic approach to determining likely causal factors and mechanisms.

My short talk will explore the above issues, especially those covered at our 2018 conference, and how our understanding will guide our funding decisions.
Alasdair Philips  
*Trustee, Children with Cancer UK*

A professional engineer and scientist, qualified in electrical & electronic engineering and also in agriculture, Alasdair has worked on the design of a wide range of environmental monitoring and analysis systems for most of his working life. Alasdair takes a holistic approach to scientific, environmental and health issues. He has spoken at many conferences and made many appearances on radio and TV. He was a founder member of UK Department of Health’s Advisory Group on ELF EMF (SAGE, from 2004-2010) and a member of the UK Health Protection Agency’s EMF/RF Consultation Group Chaired by HPA Chairman Sir William Stewart (2006-2010).

In 2004 Alasdair Philips and Denis Henshaw instigated the Children with Cancer UK international conferences held in Westminster every four years since. Alasdair was also a co-organiser of the 2008 Radiation Research Trust conference held at the Royal Society “EMF & Health - A Global Issue - exploring appropriate precautionary approaches”. He was a key programme organiser of the Children with Cancer 2012, 2016, 2017 and 2018 International Scientific Conferences.

Alasdair and colleagues recently reported a significant rise in the incidence of glioblastoma multiforme (GBM), an aggressive form of brain tumours, in England between 1995–2015, suggesting involvement of an adverse environmental or lifestyle factor¹.

**Reference**

Day Two: Invited Speakers

12:30

Professor Denis L Henshaw

2018/9 £2.5 million Grant Call: causal and promotional factors and possible preventative actions regarding childhood and young person cancer

We need to understand more about why children develop cancer, to find causal explanations and to establish whether prevention is a possibility. Accordingly, one of the stated Aims and Objectives of Children with Cancer UK is: “To improve knowledge of the genetic and environmental causes and relevant biological mechanisms of childhood cancers”.

Epidemiological investigation into environmental and lifestyle factors potentially affecting childhood cancer risk poses severe challenges, some well-known, others not well appreciated. Recent years have seen progress in addressing the issue of resolving power in investigating this rare disease, with the execution of large studies and their pooled analyses, notably for leukaemia, brain tumours and neuroblastoma.

At the same time, less progress has been made in some areas with respect to the relevant exposure metric. A prime example is air pollution. My own estimate is that in urban areas up to 40% of the above mentioned childhood cancers may be attributed to air pollution notably from motor vehicle exhausts. However, the majority of epidemiological studies conducted to date have used nitrogen dioxide, NO₂ as the exposure metric when this is neither a known carcinogen nor an adequate surrogate of air pollution’s carcinogenic components such benzene, 1,3-butadiene, PAHs and particulates. There are also issues of temporality, that the relevant exposure needs to be measured at or over the period of risk – in utero, in infancy and childhood per se.

Such considerations also apply to purported causes generally, be it chemical exposures, ionising or non-ionising radiation and infections, as well as reported protective factors such as a healthy maternal diet in pregnancy or infant breast-feeding beyond six months.

To address the above issues, in 2018 Children with Cancer UK announced a £2.5 million grant call focussing on: “Causal and promotional factors and possible preventative actions regarding childhood and young person cancer.” To encourage applicants to address specific issues of priority concern to the Charity, the grant call was accompanied by a six-page Tender Document which listed a number of suggested research areas.

The standard of Outline Applications received in response to this grant call was high. Thirty five were received to a total value of £8.2 million. This is more than three times the available funding and some difficult decisions had to be made as to which proposals to select for progression to the next stage.

In general, priority was given to proposals that best addressed the specific areas of interest laid out in the Tender Document, together with evidence of scientific excellence and ability to complete the project successfully. Even here, competition was intense.

Children with Cancer UK’s causes and prevention grant panel met on the 18th July 2019. It is intended that by the time of this conference, the Charity will be in a position to have announced the awards under this call, so that the details can be presented.
Professor Denis L Henshaw
Children with Cancer UK

Denis is Scientific Director at Children with Cancer UK and Emeritus Professor of Human Radiation Effects at the University of Bristol and Fellow of the Collegium Ramazzini.

As an MRC Programme Grant Holder, using newly developed techniques, Denis researched low-level alpha-radioactivity in the human body, principally in the lung and the skeleton, but more especially the accumulation of polonium-210 in Children’s teeth and transplacental transfer of alpha-radionuclides to the foetus. The same techniques were also used widely in the environment for analysing naturally occurring radon gas and radioactivity in contamination zones such as the area around Chernobyl.

In 1990, he published a link between domestic radon exposure and childhood leukaemia which, in high radon areas, could be an important contributive cause.

He later studied the mechanisms by which exposure to electric and magnetic fields from powerlines and the electricity supply in general may lead to increased risk of childhood leukaemia and other illnesses.

Denis has published over 260 scientific papers and served on a number of Government Committees. He was for ten years an Associate Editor of the International Journal of Radiation Biology.

His current interests centre on a range of environmental exposures that may contribute to childhood cancer risk and in fostering International collaboration to maximise the research effort.
Day Two: Invited Speakers

14:00

Professor Simon Bailey

Developing protocols in resource challenged countries

Eighty percent of children with cancer live in low or low middle-income countries (LMIC). Differential levels of care and lack of basic healthcare provision make treatment of children with cancer challenging. Modern treatment protocols for children with cancer are challenging to deliver in both their complexity and the management of treatment toxicity and as such protocols designed and used in high income countries are unsuitable for use in LMIC. This talk will highlight some of the challenges and potential solutions using the Queen Elizabeth Hospital in Blantyre, Malawi as an example. This will include the development of a treatment protocol for children with acute lymphoblastic leukaemia who had not had any treatment options previously, the evolution of treatment of endemic Burkitt lymphoma and challenges in managing children with brain tumours. Discussion of international efforts especially through the International Society of Paediatric Oncology (SIOP) to provide a framework through risk adapted protocols will also be discussed.

Professor Simon Bailey
Consultant, Paediatric Neuro-Oncology, Newcastle

Simon Bailey is a Professor of Neuro–Oncology and consultant paediatric oncologist based at the Great North Childrens Hospital and University of Newcastle upon Tyne. He is head of the Paediatric Oncology Department at the Great North Childrens Hospital.

His undergraduate medical training was at the University of Cape Town. Initial clinical experience was gained at Groote Schuur and Red Cross Childrens Hospitals before postgraduate Paediatric training in the United Kingdom and a year in New Zealand. He trained in paediatric oncology in Newcastle upon Tyne and became a consultant in 2001. He has a PhD awarded in 1999 and is a Fellow of the Royal College of Paediatrics and Child Health.

His research interests include molecular biomarkers in medulloblastoma, diffuse intrinsic pontine gliomas and delivery of risk adapted protocols to resource challenged countries. He is the European lead for high risk medulloblastoma and the chair of the NCRI brain tumour group.
Day Two: Invited Speakers

14:15

Dr Vikki Rand

Lessons from the genetics of sporadic & endemic B-NHL

There is huge disparity between survival for children with cancer in high income and low and middle income countries, which is unacceptable (Molyneux et al, 2012). Survival of children with sporadic Burkitt lymphoma (BL) has significantly improved in the last three decades due to the introduction of intensive multi-drug chemo-immunotherapy regimens. This, however, requires expensive infrastructure and supportive care, which is not available in sub-Saharan Africa were endemic BL is the most common childhood cancer and outcome remains unsatisfactory. Whilst intensive multi-agent immunochemotherapy now results in greater than 90% survival for children diagnosed with BL in the UK cure comes at the cost of frequent and debilitating toxicities. Moreover, outcome for patients with disease progression (relapsed or primary refractory disease), remains dismal with less than 30% salvaged by current treatment protocols. There is a clinical need to determine those patients who might be adequately treated with lower intensity protocols to reduce treatment related toxicity in the UK. Conversely, it is essential to determine those patients who will not respond to current frontline therapy and develop new treatment protocols to salvage these patients quickly in different healthcare settings.

Recent studies have begun to elucidate the key mutational changes and co-operating events leading to the pathogenesis of BL. Our knowledge of the clinical relevance and utility of these abnormalities, however, is limited (Moffit et al, 2017). To address this, we have performed high-resolution omics characterisation of the largest cohort of clinically annotated paediatric BL patient samples diagnosed and treated in the UK and Malawi. Our analyses has identified recurrent abnormalities associated with relapsed/refractory disease and highlighted similarities and differences in biomarkers and potential therapeutic targets in endemic and sporadic BL. For example, we have recently reported a high frequency of FOXO1 mutations in both sporadic and endemic BL (Zhou et al, 2019). We observed that the most frequent FOXO1 mutations were located within or immediately adjacent to the AKT recognition motif (RxRxxS/T). We demonstrated persistence of FOXO1 mutation in paired presentation/relapse sample, consistent with a role throughout the disease course, but there was no correlation between FOXO1 mutations and clinical outcome. CRISPR/Cas9 knockout of FOXO1 in an endemic cell line produced a significant decrease in cell proliferation, supporting an oncogenic role for FOXO1 in endemic BL. With small molecule inhibitors in development, FOXO1 may represent an emerging therapeutic target for a substantial proportion of paediatric BL patients at initial diagnosis and for the currently hard-to-treat children with relapsed disease in both high and low-income countries.

Using integrated omics data we have also shown that TP53 abnormalities are important prognostic markers in paediatric BL. Using integrated copy number array, exome sequencing and Sanger sequencing data we showed that TP53 abnormalities at presentation are associated with disease progression and a poor outcome. Moreover, we demonstrated that biallelic TP53 abnormalities are either maintained or gained at the time of relapse, implicating loss of TP53 function in the development of treatment resistance. Due to the acute toxicity associated with current front-line therapies, dose-reduction is a key objective. Importantly, the absence of TP53 abnormalities was associated with an extremely low risk of relapse, irrespective of bone marrow/CNS status and despite the absence of rituximab from the substantial majority of treatment schedules. We are currently investigating the clinical impact of TP53 abnormalities in endemic BL and exploring the genome further to understand the clinical relevance of other genomic alterations in paediatric BL in both high and low income healthcare settings.

References


Zhou et al (2019) Sporadic and endemic Burkitt lymphoma have frequent FOXO1 mutations but distinct hotspots in the AKT recognition motif Blood Advances in press

Dr Vikki Rand
Bloodwise Bennet Senior Fellow at Newcastle University

Vikki is a recent Bloodwise Senior Bennett Fellow and has established a paediatric lymphoma research group at Newcastle University. Her research team focuses on the identification of prognostic biomarkers and therapeutic targets in B-cell non-Hodgkin lymphoma (B-NHL) with the aim to improve survival and develop effective, kinder treatment strategies for both children and adults.

She holds a PhD in Genomics and Bioinformatics from the University of Cambridge and The Wellcome Trust Sanger Institute. Building on the foundation of her PhD, where she contributed to the Human Genome Project, she has successfully developed and applied cutting edge computational and omics approaches in several types of cancer, including acute lymphoblastic leukaemia, glioblastoma and ependymoma. Vikki’s group applies integrated omics approaches to investigate the biological and clinical relevance of genomic alterations in B-NHL patient cohorts from the UK and Malawi. In the UK, it is striking while primary B-NHL is chemosensitive and has an extremely high cure rate (>90% 5 year event-free survival), relapse is almost always fatal. Current treatment for paediatric B-NHL is one of the most acutely toxic treatment regimens and identifying those patients who could respond to reduced treatment protocols is a key objective. Moreover, the delivery of intensive multi-agent chemo-immunotherapy in low-income countries, such as Malawi, is prevented by high costs and insufficient supportive care facilities. Her current research is firmly focused on omic analysis of aggressive lymphomas, development of personalised therapies and further cancer genome discovery in both low- and high-income countries.

https://www.ncl.ac.uk/nicr/staff/profile/vikkirand.html#background
Day Two: Invited Speakers

15:45

Professor Christine Harrison

Evolution of Cancer Cytogenetics

It became known in 1956 that the human karyotype comprised 46 chromosomes. This discovery was rapidly followed in 1959 by the finding of the constitutional gain of chromosome 21 in Down syndrome. Throughout the 1960’s, chromosomes were identified by their size and position of the centromere only. The development of chromosomal banding techniques in the 1970’s greatly improved their identification. The first association between chromosomal abnormalities and cancer was the finding of the Philadelphia chromosome (Ph) as the hallmark of chronic myeloid leukaemia in 1960. The name came from the discovery having been made in the city of Philadelphia. A little recognised fact is that the same observation had been made in Edinburgh at the same time. Janet Rowley in 1973 identified that the Ph chromosome originated from a translocation between chromosome 9 and 22, t(9;22)(q34;q11). There rapidly followed a series of important workshops: “International Workshops on Chromosomes in Leukaemia” throughout the 1980’s. Eminent cytogeneticists from around the world collected large numbers of leukaemia cases that highlighted the association between cytogenetic abnormalities and diagnosis. Importantly, they were the first to demonstrate a link between karyotype and outcome in acute lymphoblastic leukaemia (ALL). Notable examples, still used today for risk stratification, are high hyperdiploidy associated with a good outcome and the MLL rearrangement from the translocation, t(4;11)(q21;q23), as well as the Ph chromosome, linked to a poor outcome. The cytogenetic study of leukaemia increased exponentially over the coming years, with thousands of chromosomal rearrangements and their relationship to outcome reported, notably in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, currently containing data on 69,134 cases and 21,477 gene fusions.

The advent of molecular biology techniques in the 1980’s threatened the use of cytogenetics as a diagnostic tool, however the molecular approaches facilitated the development of fluorescence in situ hybridisation (FISH), now widely used for screening for known chromosomal abnormalities. Today the detection of genome wide copy number abnormalities, using array based approaches, complements FISH and cytogenetic analysis, while the current state-of-the-art is the integration of a range of sequencing approaches into routine practise.

Today, genomic abnormalities have defined distinct subgroups of patients which play an important role in their risk stratification for treatment within clinical treatment trials, particularly in childhood ALL. In addition to those which have been known for some time, the more recent characterisation of specific subgroups have made a significant impact to clinical practice. Two important examples include: the discovery of iAMP21-ALL, in which patients harbour a grossly abnormal chromosome 21. It was shown in both in UK and USA that these patients had a dismal prognosis when treated on standard therapeutic regimens. Transfer to more intensive treatment arms has greatly improved their outcome, leading to changes to their risk stratification and subsequent therapy. A second relates to a subgroup of ALL with rearrangements involving ABL-class genes, among which many patients are refractory to conventional chemotherapies. This collection of genetic changes activates kinase signaling, as known for Ph positive ALL, and ABL-class patients have been successfully targeted with tyrosine kinase inhibitors (TKI) to achieve complete remission.

As a result of integrated genomic analyses worldwide, the numbers of childhood ALL patients not assigned to a specific subtype is now small. The search continues with ever advancing technologies to study non coding sequences, epigenomes and proteomes to identify further genomic abnormalities of biological and clinical relevance with the future view to personalised precision medicine for all.

References


Professor Christine Harrison
Professor of Childhood Cancer Cytogenetics, Northern Institute for Cancer Research, Newcastle University

Christine is a Manchester University graduate in Genetics and Cell Biology, with PhD from the Medical School in Manchester. For many years she was Director of the Oncology Cytogenetic Service at the Christie Hospital, Manchester, which she established in the 1980’s. In her current role she has moved her group from the Royal Free Hospital, University of London, to University of Southampton and now to the Northern Institute for Cancer Research, Newcastle University, where she is Professor of Childhood Cancer Cytogenetics. Christine has an international reputation for her translational research in leukaemia genetics, which has directly contributed to changes in treatment. She is one of the most highly regarded leukaemia cytogeneticists worldwide. She has directly contributed to improvements in outcome of childhood acute leukaemia by the discovery of novel genetic changes for which appropriately modified treatment has significantly improved outcome, notably her discovery of the iAMP21 chromosome in childhood acute lymphoblastic leukaemia. She develops state-of-the-art technologies for rapid integration into routine practice. Her approaches have been adopted internationally. In her talk she will provide a historical overview of the evolving role of cytogenetics in childhood leukaemia.
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Identification and Characterisation of DNA Methylation Changes within Homeobox Genes in Relapsed Medulloblastoma

Adam Darnley1, Stacey Richardson1, Rebecca Hill1, 2, Christopher Kui1, Yura Grabovska1, Matthew Selby1, Ed C Schwalbe1, 3, Stephen Crosier1, Simon Bailey1, 2, Steven C Clifford1

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The single most adverse event in Medulloblastoma (MB) is relapse, occurring in 30-40% of patients and accounting for 10% of childhood cancer deaths. Recent studies have highlighted the genetic divergence of recurrent tumours from their diagnostic counterparts, with high-frequency mutations acting as poor prognostic markers at the relapse stage (Hill et al. 2015). Changes in the epigenetic landscape are yet to be characterised, and no study to date has investigated the alterations in DNA methylation observed in relapsed MB. For the first time, we aimed to identify genes whose methylation status became altered at recurrence in the Sonic Hedgehog (SHH) subgroup of MB.

Using the largest paired cohort of diagnostic and relapsed MB samples to date (n = 84), the methylation status of CpG probes at each stage were interrogated genome-wide using Illumina 450K/EPIC arrays (Illumina Inc., USA). A subgroup-specific approach, based on the seven molecular subgroups of MB (Schwalbe et al. 2017) was taken, focusing on MB\textsubscript{SHH-Infant} (n = 15) and MB\textsubscript{SHH-Child} (n = 22). A bespoke pipeline was created to identify CpG probes and their respective genes where tumour specific methylation was aberrantly acquired in the relapsed state and correlated strongly with gene expression. To assess the prognostic value of these aberrations, univariate analysis was undertaken using methylation status of probes at diagnosis in an independent cohort of 615 samples and at relapse in the paired cohort. Gene expression was validated in selected genes of interest using a well-curated tissue microarray cohort (n = 96) to stain multiple MB\textsubscript{SHH} samples (n = 9) with antibodies according to manufacturer’s protocol.

Our pipeline identified 107 genes demonstrating acquisition of tumour-specific DNA methylation changes at relapse in MB\textsubscript{SHH-Infant} and 74 genes in MB\textsubscript{SHH-Child}. Interestingly, the most frequent group of genes represented in both SHH subgroups was the HOX family of genes (MB\textsubscript{SHH-Infant}, n = 6; MB\textsubscript{SHH-Child}, n = 3), which play a key role in embryonal development. Notably, methylation of CpG probes within these HOX genes all correlated positively with gene expression, suggesting an activating role of acquired methylation in the relapsed setting. Individual HOX probes were then interrogated: the frequency of aberrant methylation at relapse was compared to the controlled cohort of diagnostic samples, showing significant enrichment of all MB\textsubscript{SHH-Child} HOX probes at recurrence (n = 6; p <0.05, Chi-squared test). Two probes in HOXC4 predicted poor overall survival when aberrantly methylated at diagnosis in MB\textsubscript{SHH-Child} (p = 0.039; Log rank-test). Combining probes in HOXA3 and HOXA6 predicted time to death at relapse using all samples in the paired cohort (p = 0.026; Log rank-test). Staining for HOXA3 and HOXA6 in 2 MB\textsubscript{SHH-Infant} samples acquiring methylation at relapse demonstrated increased protein expression at recurrence compared to their diagnostic counterparts.

This is the first study to analyse changes in methylation between diagnosis and relapse in MB. We have identified the HOX family of genes, normally involved in embryonal development, to frequently acquire DNA methylation at recurrence, which correlates positively with expression. Probes within these genes can predict survival at both stages of the disease, indicating their potential for use as prognostic biomarkers, whilst protein overexpression at relapse could offer novel targets for therapy.
References

Myc-dependent Proteomic Signatures in a Cellular Model of Group 3 Medulloblastoma: identifying Protein Targets for Novel Therapeutic Strategies

Florence Burté, Janet C. Lindsey, Shanel J. Swartz, Amanda K. Miles, Daniel Williamson, David J. Boocock and Steven C. Clifford

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Background
Medulloblastoma (MB) is the most common malignant paediatric brain tumour. This highly heterogenous disease is characterised by four distinct groups according to their pathological, clinical and molecular features. Group 3 has the worst outcome with 40 to 50% survival. Amongst Group 3 patients, MYC amplification is a risk factor associated with marked metastatic potential and with the poorest prognosis (<20% 5 year overall survival). Current treatments consist of radiotherapy and non-target-specific cytotoxic chemotherapies, which result in significant neurological and intellectual defects in survivors. Novel targeted therapeutic strategies are urgently warranted.

Methods
Our group has developed a collection of inducible MYC-knockdown cell lines that allows the investigation of MYC-dependent pathways specific to Group 3 MB. The next-generation quantitative proteomic method SWATH-MS was employed to detect MYC-specific changes in these models. Proteomic data was further analysed for pathway analysis alongside matched transcriptomic data.

Results
Over 4,000 quantitative proteins were detected with hundreds showing statistically significant changes upon MYC knockdown. Integration with transcriptomics data showed a good correlation between gene and protein expression for over 60% of matched entities accompanied with few post-transcriptional divergences. MYC druggable protein targets were further investigated for suitability for therapeutic targeting.

Conclusion
Integrative multi-omic approaches allowed the detection of MYC-specific protein targets, as well as MYC-dependent pathways that warrant further investigation for novel therapeutic strategies in MYC-driven MB.
Preclinical investigation of PARP and ATR inhibitors in models of high risk neuroblastoma

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Background
Neuroblastoma (NB) is the commonest extra-cranial malignant solid tumour of childhood and one of the most difficult to cure. Around 50% of high-risk NBs have MYCN oncogene amplification (MNA) that promotes rapid DNA replication, leading to errors and replication stress (RS). Cells with RS are acutely dependent on the DNA damage sensor kinase ATR. PARP inhibition results in unrepaired single strand DNA breaks progressing to replication, further increasing RS. We hypothesise that combining PARP and ATR inhibition will lead to greater cytotoxicity due to increased RS.

Aim
To assess synergism between PARP inhibition and ATR inhibition in high risk NB cell lines and to measure RS.

Materials and Methods
Human NB cell lines: SHSY5Y and SKNAS (non-MNA), and NGP and N20_R1 (MNA). The PARPi olaparib and the ATRi VE-821 were used. CHK1S345 and H2AXS129 phosphorylation was assessed using Western blotting to determine ATR activity and RS respectively. RS was also determined by γH2AX foci formation using immunofluorescent microscopy. Cytotoxicity was assessed by XTT cell proliferation assay (Roche) and colony formation assay.

Results
Olaparib (5 µM) treatment increased CHK1S345 and H2AXS129 phosphorylation after treatment for 24 hours in all cell lines. H2AXS129 phosphorylation was further increased with the addition of VE-821 (1 µM). ATR inhibition prevented CHK1S345 phosphorylation, as expected. The number of γH2AX foci exhibited in the cell lines by immunofluorescence increased after treatment with olaparib (1 µM) which was further increased with the addition of VE-821 (1 µM). In cytotoxicity assays, combination index analysis (Calcusyn) showed that ATR inhibition by VE-821 is synergistic with olaparib at sub lethal concentrations (<1 µM) (CI value 0.04-0.89), although this effect is lost at higher concentrations.

Conclusion
ATR inhibition by VE-821 is synergistic with olaparib at sub lethal concentrations (<1 µM) and further increases the replication stress caused by PARP inhibition.
Early initiating events in childhood leukaemia: Exploring the role of DNA methylation

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Leukaemia, the most common childhood cancer, arises from genetic abnormalities occurring in utero. However, these are not sufficient for disease manifestation and additional ‘second hits’ are needed for childhood leukaemia to develop. Changes in DNA methylation is one mechanism by which genes are regulated. Aberrant methylation can lead to gene dysregulation, such as inactivation of tumour suppressor genes, and is observed in all cancers, including childhood leukaemia. Gene dysregulation via aberrant DNA methylation may be an important contributor to childhood ALL aetiology.

To investigate if abnormal DNA methylation is an early event in disease development, detectable prior to diagnosis, we carried out a pilot study to test the feasibility of accessing, locating and measuring DNA methylation in blood spots taken 5-8 days after birth in childhood leukaemia cases and healthy controls. The Great North Biobank houses neonatal blood spots and associated identification records from 1984-1994. Leukaemia cases born between 1984-94 were identified using the Northern Region Young Persons’ Malignant Disease Registry (NRYMDR). Blood spots for age-matched controls were selected based on date of birth, and cross-referenced with the NRYMDR to ensure controls had no reported malignancies in childhood or early adulthood. DNA was extracted from blood spot samples and methylation assessed using the Illumina Infinium EPIC arraying platform. Differences in methylation were assessed between leukaemia cases and controls at individual CpG sites (Differentially Methylated Probes - DMPs) and across differentially methylated regions (DMRs).

Blood spot samples for 28 leukaemia cases and 28 controls were identified, located and successfully underwent methylation assessment. On prediction of sex of case/control samples utilizing the methylation data, two cases were removed from downstream analysis as the predictions for these samples did not correspond with data held in the registry. No differences in methylation were observed between cases and controls of statistical significance using FDR correction. Previous studies with modest samples sizes have used lower thresholds to find DMP/DMRs of interest (Irwin et al., 2019). Consistent with this, DMPs and DMRs with a raw p<0.05 for significance, and a minimum methylation change of 5% between cases and controls were considered of interest. Using this threshold, 32 DMPs and 11 DMRs were different between leukaemia cases and controls, with the maximum mean methylation difference between groups being 17% for a DMP and 13% for a DMR.

We successfully demonstrate the feasibility of accessing and utilizing historically collected neonatal blood spot samples to measure DNA methylation prior to disease diagnosis in childhood leukaemia patients. Furthermore, we demonstrate that modest changes in methylation can be identified between cases and controls at birth, some occurring in genes with potential aetiological connections to leukaemia. Better understanding of these early events in the development of childhood leukaemia will aid the development of preventative interventions or provide biomarkers for disease screening. Future studies will require assessment of additional cases to provide sufficient power to enable the development of robust biomarkers and further understand the role of DNA methylation and potentially modifiable risk factors in childhood leukaemia development.

Reference
Identification of synthetic lethal genes as novel therapeutic targets in cancer

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Introduction
Genomic level data has significantly increased the depth of our understanding of genetic and epigenetic changes occurring in cancer development. However, utilising this complex data to improve patient management is challenging. We have developed a novel method integrating genome-wide DNA methylation and expression data to identify cancer sub-type specific synthetic lethal (SL) genes. SL genes (which are only required for growth/survival of cancer cells and not normal cells), represent ideal targets for the development of more targeted, less toxic and more effective cancer treatments.

Materials and Method
We developed a novel bioinformatics approach that combines DNA methylation and expression data to identify candidate SL genes. The identified genes lack any apparent genetic/epigenetic alterations, and thus most of the candidates have not previously been implicated in cancer. Initially, we used acute lymphoblastic leukemia (ALL) as a model using publicly available datasets, comprising 517 samples from six ALL subtypes.

To assess the applicability of the approach more broadly to different cancer types, it was extended to assess medulloblastoma and neuroblastoma. We utilised paired methylation and expression data for medulloblastoma (n=763). For neuroblastoma, methylation data from three studies (n=196 total) was integrated. As established subtypes have not been defined in neuroblastoma, this data was initially used to define neuroblastoma subgroups (using the t-SNE/dbSCAN method) and then subsequently for SL gene identification.

Functional validation of identified candidate SL genes was performed using siRNA-mediated knockdown of the candidate genes, coupled with cell proliferation (MTT assay) and apoptosis (annexin-V staining) assays.

Results
22 candidate SL genes were identified across six ALL subgroups, ranging from one in iAMP21 to nine in the TCF3-PBX1 subgroup. Functional work using siRNA-mediated knockdown of the ETV6/RUNX1-specific SL gene TUSC3 in ETV6/RUNX1 cells resulted in a 60% reduction in cell proliferation (p <0.01) and induction of apoptosis (p <0.01). In contrast, no impact was seen on cell growth or apoptosis in ETV6/RUNX1 negative cells. Similarly, we demonstrate that siRNA mediated-knockdown of TCF3-PBX1–specific SL gene FAT1, results in induction of apoptosis and reduced growth specifically in TCF3-PBX1-positive cells. In medulloblastoma, analysis of the well-defined WNT and SHH subgroups identified seven and four candidate SL genes respectively, while identification of SL genes was limited in group 3 and group 4 tumours, which lack a known group-defining molecular defect. However, using the recently defined further subtyping of Group3/Group4 tumours, three, four and two candidate SL genes were identified in subtypes II, VII and VIII respectively. In neuroblastoma, we identified three methylation-dependent subgroups, which correlate with molecular and clinico-pathological data. Six candidate SL genes were identified in methylation cluster 3, which consists almost exclusively of high-risk MYCN amplified cases.

Discussion and future work
We have demonstrated that SL genes, specific for defined molecular subtypes of cancer, can be identified utilising a novel approach combining methylation/expression data in multiple cancer types. We are currently developing robotic and fluorescent based approaches to facilitate rapid functional confirmation of the identified candidates.
Defining the genomic landscape of relapsed medulloblastoma

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Medulloblastoma relapse occurs in 30-40% of patients, is almost universally fatal, and accounts for ~10% of childhood cancer deaths. Cancer evolution is an adaptive, non-linear process. Treatment applies selection pressure which, in the context of this adaptive landscape, promotes the development/selection of therapy-resistant clones. We therefore undertook a comprehensive investigation of the genomic landscape (mutational and copy number variations) of relapsed medulloblastoma using a unique, unrivaled cohort of tumours sampled at disease recurrence and paired with their diagnostic counterpart (n>60 pairs). We hypothesise that integrative, multiomic analyses of this paired relapsed tumour cohort will identify converging biological mechanisms responsible for disease evolution, treatment resistance and medulloblastoma recurrence.

Illumina 450K and EPIC array datasets (Illumina Inc., USA) were utilised to interrogate molecular subgroup and copy number variation. Molecular subgroup was assigned as previously described to identify the four major consensus groups witnessed in medulloblastoma (MB\textsubscript{WNT}, MB\textsubscript{SHH}, MB\textsubscript{Group3} and MB\textsubscript{Group4}), alongside novel sub-structures within MB\textsubscript{Group3} and MB\textsubscript{Group4} (Sharma, Schwalbe \textit{et al.} 2019). Copy number analyses was undertaken with Conumee (Bioconductor). Genomic datasets were generated using the SureSelect\textsuperscript{XT2} Target Enrichment gene panel and the SureSelect\textsubscript{XHS} whole exome sequencing (Agilent, USA) and analysed utilising Burrows-Wheeler Alignment Tool and the Genome Analysis ToolKit for variant calling, and annotated with the variant effect predictor tool.

Overall, subgroups remained stable between diagnosis and relapse. Importantly focal and arm-level copy number aberrations were both acquired and maintained at disease recurrence. For example, acquired and maintained focal and arm level losses of the tumour suppressor gene \textit{PTEN}, were observed across molecular subgroups, strongly suggesting a driving role of the PI3K/AKT signalling pathway in disease recurrence. Similarly, predicted damaging candidate mutational events, both acquired and maintained, were witnessed in the majority of recurrent tumour samples. Importantly, a number of these mutational events converged on DNA damage response pathway genes. Evidence for the importance of particular pathways such as p53 signalling and DNA damage response was witnessed across molecular subgroups suggesting a consistent mechanism for therapy resistance.

In summary, this study has identified key biological mechanisms which characterise the majority of medulloblastoma relapsed disease. Future work is now essential to validate these findings, and to explore their functional consequences and utility as prognostic biomarkers, or as a basis for novel therapeutic interventions at relapse.

Reference
Poster abstracts

Dose-dependence of galactose protection against asparaginase-induced acute pancreatitis

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Acute pancreatitis (AP), a human disease with a substantial mortality, currently has no specific therapy. The major causes of AP are alcohol abuse and gallstone complications, but it also can occur as an important side effect of the standard Asparaginase (ASNase) - based treatments for childhood acute lymphoblastic leukaemia (ALL) (Gerasimenko et al, 2018). Our previous findings proposed mechanism of Asparaginase-induced AP (Peng et al, 2016) and strongly suggested that ATP supplementation by providing energy supplements pyruvate and galactose (Peng et al, 2019) can effectively alleviate the pathology. Here we show that providing galactose supplementation in a dose-dependent manner prevents or markedly reduces the ATP depletion and necrosis in in vitro studies. Galactose has also effectively protected against Asparaginase-induced pathology in mouse models of AP by substantially reducing histological score, blood amylase and IL6 activity, as well as improving food consumption and prognosis. Based on these data, we suggest that galactose feeding can be used to protect against AP and therefore improve Asparaginase-based treatments for childhood ALL.

References


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Development of a bioinformatics pipeline for identifying focal copy-number aberrations in medulloblastoma

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Background
Medulloblastoma is a biologically heterogeneous cancer which comprises four molecular subgroups. Copy-number aberrations (CNAs) are frequently witnessed in the Sonic Hedgehog, Group 3 and Group 4 subgroups; recurrent focal CNAs include amplification of the oncogenes MYC and MYCN which are associated with a poor prognosis, or deletion of tumour-suppressor genes such as PTEN.

DNA methylation arrays are performed to identify molecular subgroups in medulloblastoma and can be additionally analysed for copy-number data. Development of an automated bioinformatic pipeline may facilitate accurate identification of established and new, clinically significant CNAs.

Methods
A diagnostic medulloblastoma cohort (n = 517) with available Infinium Human Methylation 450K BeadChip or Infinium MethylationEPIC 850K BeadChip array data (Illumina Inc., San Diego, CA, USA) was interrogated for focal CNAs. The Bioconductor package, Conumee (Hovestadt and Zapatka, 2017), was utilised to compare methylation data to a normal control cohort (n = 17) and generate copy-number plots in R Studio (RStudio Inc., Boston, MA, USA). Conumee plots were annotated with genes of interest (n = 137), which were selected for biological significance in medulloblastoma (Northcott et al., 2012)

An automated script was developed to analyse copy-number profiles of genes of interest. Focal CNAs were called by examining the copy-number status of each gene (Gene Score), size of the segment the gene was located on (Seg Width) and mean copy-number status of the respective segment (Seg Score). Different filtering parameters were tested and final criteria were visually verified against copy-number plots by two independent assessors. The criteria for identifying focal gains were further validated by matching MYCN calls with available fluorescence in-situ hybridisation (FISH) and multiplex ligation-dependent probe amplification assay (MLPA) data.

Results
The final parameters for calling focal CNAs are as follows: Seg Width < 12 Megabase pairs (Mb), Seg Score > 0.12 log2 score (Gain) or < -0.22 log2 score (Loss), Gene Score > 0.6 log2 score (Gain) or < -1.1 log2 score (Loss). MYCN gain was the most common focal CNA, detected in 36/516 (6.98%) samples, with a high sensitivity (92.86%) and specificity (99.47%) when compared to FISH and MLPA data. Other recurrent focal CNAs include MYC gain in 24/517 (4.64%) and GLI2 gain in 10/517 (1.93%). CSMD1 was the most frequent loss 25/516 (4.84%), with TP53 loss in 6/517 (1.16%). Therapeutically targetable focal CNAs include CDK6 gain in 2/517 (0.39%) and PTEN loss in 6/517 (1.16%).

Conclusions
We have developed a pipeline that can detect focal CNAs from a predetermined gene list. This pilot suggests prognostically significant amplifications such as MYCN can be detected with high specificity and sensitivity. Whilst methylation arrays are not the clinical gold-standard for detecting CNAs, as they lack
the ability to detect highly focal or low-level changes that occur in fewer cells, the automated and flexible nature of this pipeline has huge future potential for discovery analysis.

References
Poster abstracts

Inheritance of *PM20D1* epitypes as a risk factor for neuroblastoma

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Introduction

Inheritance of multiple genetic differences has been identified as altering susceptibility to the development of various types of cancer. Although somatic epigenetic changes are clearly associated with the development of all types of cancer, inherited epigenetic differences have not been widely studied as susceptibility factors in cancer development. This is in part because epigenetic changes that arise in an individual’s lifetime are usually not passed through the germline and most genes are not inherited in different epigenetic states that vary between individuals. Here we provide evidence that the *PM20D1* gene is unusual, if not unique, in human genes, as being inherited in different epitypes that are directly inherited. These epitypes result in the inheritance of either active or inactive forms of the gene and are associated with a differential risk of development of neuroblastoma.

Materials and Method

Initial identification of differential *PM20D1* methylation in germline DNA from neuroblastoma patients and confirmation of familial inheritance of different *PM20D1* epitypes was carried out using data derived from Illumina human methylation 450K beadchip arrays. Further analysis of germline methylation in neuroblastoma patients was carried out using COBRA methylation assays. Functional assessment of *PM20D1* in neuroblastoma cell lines was carried out utilising a lentiviral expression system.

Results

DNA methylation in constitutive germline DNA from childhood cancer patients (n=32) was assessed at a genome level using Illumina 450K beadchip arrays, as part of a study aimed at identifying alterations in DNA methylation induced by exposure to treatment of childhood cancer. Unexpectedly, this identified differential methylation of the *PM20D1* promoter region in individuals with solid (primarily neuroblastoma) vs haematological malignancies prior to initiation of treatment. Further analysis of datasets from healthy individuals confirmed that *PM20D1* methylation varies extensively across the population, is present in three distinct methylation groups (likely equivalent to 0, 1 or 2 methylated *PM20D1* alleles) and that these methylation epitypes are directly inherited in germline DNA. Furthermore, methylation of the *PM20D1* was associated with silencing of gene expression.

Analysis of additional blood samples from neuroblastoma patients (n=16) confirmed the differential *PM20D1* epitypes in this patient group. The heterozygous epitype was significantly less common in neuroblastoma patients (p=0.002), suggesting that this epitype may be associated with reduced risk of neuroblastoma development, with increased risk in both other epitypes. Re-expression of *PM20D1* in the IMR32 neuroblastoma cell line resulted in rapid downregulation of the *PM20D1* expression construct, followed by long term stable complete inactivation. In contrast, re-expression in cancer cell lines of other cancer types (leukaemia, pancreatic cancer, medulloblastoma) did not result in reduced *PM20D1* expression and expression was stable over 29 days.

Discussion and future work

Our results identify *PM20D1* as a gene with a highly unusual mechanism of inheritance, involving the inheritance of either highly methylated (and transcriptionally inactive) or unmethylated (and transcriptionally active) alleles. Furthermore, we provide evidence that different *PM20D1* epitypes are associated with differential risk of neuroblastoma and provide initial evidence for a neuroblastoma specific mechanism for down regulation and inactivation of *PM20D1* expression.
**Poster abstracts**

**A Novel Screening Strategy Identifies Compound Combinations for the Treatment of Paediatric Acute Myeloid Leukaemia**

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Paediatric acute leukaemia is the most commonly diagnosed cancer in children (~30% of cases). Around 20% of these patients are diagnosed with acute myeloid leukaemia (AML) although the majority are acute lymphocytic leukaemia (ALL). The prognosis for AML has improved significantly with overall survival now approaching 70% but is still behind that for ALL patients. The improvement can be accredited to intensification of chemotherapeutic regimes which are associated with substantial acute and chronic side effects, affecting patients into their adult years and beyond.

Differences between adult and paediatric AML in their mutational profiles have now been identified, which has highlighted the need for improved treatment stratification based on patient age and better targeted therapies to reduce unnecessary side effects.

To try and address these issues, we designed a novel screening strategy, using computational and in vitro resources that could identify compound combinations with potential to be used in the treatment of paediatric AML. An all-pairs testing algorithm, designed in-house, was used to identify all possible pairwise combinations from a given number of compounds and placing them into groups of ten. For this study, we screened all pairings of 80 apoptosis-inducing compounds (3,160), based on our in-silico analysis, in only 160 wells, alongside a screen of the single agents. The data from both experiments identified successful wells; these were considered to be those in which the agents in the well had little activity as single treatments but when in combinations of ten, cell death was observed using a fluorescent readout with CellTox™ Green. Interestingly, of the 160 wells tested only one well was considered successful and overlapped two cell lines (MV4-11 and CMK). When the 10 agents were deconvoluted into 45 pairs, the pairing of ABT-737, a Bcl-2 inhibitor and Purvalanol A, a CDK inhibitor, was the most successful combination for the MV4-11 cell line. Whilst the results for the CMK cell line were not as clear, with several combinations producing positive results suggesting that a combination of three or more of the compounds was at play in the combination well. When the combination of ABT-737 and Purvalanol A was screened against a panel of paediatric AML cell line models, only those with an MLL rearrangement and a FLT3-ITD were sensitive with several other non MLL or FLT3-ITD cell lines not showing any loss of viability.

Our *in vitro* screening strategy has identified novel therapeutic drug combinations for specific mutational sub-groups and highlighted the need for stratification of patients into treatment groups.
Does Benzene Exposure Influence Chromosomal Translocation Events Involved in Childhood Leukaemia?

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Leukaemia accounts for nearly a third of all childhood cancers with survival rates reaching close to 90% (Children with Cancer UK, 2019). Many leukaemias are thought to originate from chromosomal rearrangements in utero, as various abnormalities have been retrospectively detected at birth in leukaemia cases. Chromosomal translocations occur when a chromosome breaks and reattaches to a different chromosome leading to fusion proteins and misregulation of processes. Causes of translocations are unknown. However chemotherapy drugs, such as the topoisomerase II poison, etoposide, can induce chromosome breaks and are associated with therapy related leukaemias, with translocations similar to childhood leukaemias (Cowell et al, 2012). With leukaemia incidence increasing and long-term treatment effects contributing to secondary cancers, fertility and cardiac issues, understanding the causes and prevention of translocations is important. Epidemiological studies have identified various environmental factors associated with increased risk of childhood leukaemia i.e. parental smoking, maternal nutrition, caffeine intake, air pollution, paints, solvents and pesticides (Timms et al, 2016, Filippini et al, 2019). It is plausible these exposures in utero and in early childhood could trigger the initiating translocations of childhood leukaemia.

Benzene, one of the major carcinogens of cigarette smoke has long been linked to occupational acute myeloid leukaemia (AML) in adults (Snyder, 2012). A derivative of petroleum, benzene is also found in gasoline, air pollution and solvents used in inks and paints, which have all been associated with an increased risk of childhood leukaemia. Benzene and its metabolites are able to cross the placenta during pregnancy, and as infants have an underdeveloped excretion pathway and smaller body weight than adults, exposure to benzene may be more potent. Epidemiological studies have shown an association between childhood leukaemia and markers of benzene exposure (Carlos-Wallace et al, 2014). We aim to investigate if benzene, a potentially modifiable environmental factor associated with an increased risk of childhood leukaemia, may trigger the induction of chromosomal translocations associated with childhood leukaemia, utilising the topoisomerase II poison, etoposide, to induce susceptibility to translocations.

To determine the optimum etoposide concentrations to increase susceptibility to translocations, the leukaemic cell line NALM6 was exposed to a gradient of etoposide concentrations over varying times and allowed to recover. Cell viability and translocation events were assessed. Reverse transcription PCR and qPCR assays developed to detect the most common childhood leukaemia associated and etoposide related translocations were used. This will be repeated to determine the optimum exposure levels for benzene. Cells will then be exposed to benzene with and without the presence of etoposide, to identify if benzene alone or with etoposide treatment may increase the common translocation events associated with childhood leukaemia. COMET assays following exposure and then recovery will also allow DNA damage to be assessed.

Results show that cell viability is relatively unaffected at etoposide concentrations up to 100nM but is greatly reduced at higher etoposide concentrations found during chemotherapy treatment (10µM). Preliminary indications show that common childhood leukaemia translocations can be found at etoposide concentrations as low as 10nM over a 48 hour exposure period. Short bursts of high etoposide concentrations were also seen to induce translocations, i.e. 1µM over a 1 hour exposure. Further results on benzene exposures are to follow and will be presented.

References
Identifying Candidate Genes That Drive Epigenetically Dysregulated Paediatric Tumours: Medulloblastoma and Malignant Rhabdoid Tumours

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Introduction
Malignant rhabdoid tumours (MRT) and Group 3 Medulloblastoma (MB) are high-risk paediatric tumours with high mortality. Current treatments result in long-term morbidity, and poor outcome, 80% of children with MRT survive less than 1yr from diagnosis. More targeted treatments are required to reduce side effects and improve survival. Both tumours are epigenetically dysregulated, with the SWI/SNF subunit SMARCB1 being the most frequent mutation in MRT, leading to altered function of the chromatin remodeling complex. Group 3 MB cases with C-MYC mutation have poorer outcome, and additional epigenetic modulators are mutated in this subgroup. These aberrations in the epigenetic machinery result in widespread changes to the chromatin landscape in turn dysregulating gene expression.

Aims
To identify candidate driver genes through use of a bioinformatics pipeline, aiming to validate these genes with known targeting drugs to be taken forward for use in the clinic.

Methods & Results
Next-generation sequencing (NGS) data including ChIP-seq, RNA-seq, CRISPR and 450k generated in SMARCB1-reexpressing cell lines has been integrated along with publically available data through a bioinformatics pipeline to identify dysregulated genes that are potential drivers of SMARCB1-dependent MRT. Importantly cell-line and patient data has been intersected in order to find clinically relevant genes for future translation into new clinical therapies.

The four MRT data types were interrogated to find genes significantly dysregulated in terms of cell fitness and expression as well as epigenetically. Approximately 1400 SMARCB1-dependent driver loci were found. From this list using databases such as DrugBank, targetable genes were identified. These targetable genes were then ranked according to the most significant changes in the four data types.

There is a paucity of available ChIP-seq data for Group 3 MB, preliminary ChIP-qPCR performed in our cell-lines shows MYC knockdown causes a shift towards a more active chromatin state. ChIP-seq will be performed in our MYC-regulable cell-lines to understand the changes induced by MYC overexpression upon the epigenetic landscape of Group3 MB by exploring MYC and BRD4 binding as well as changes to histone modifications H3K27 and H3K4. Data collected from this experiment will add to RNA-seq, 450k/850k and CRISPR-screen data to identify additional therapeutic targets in Group 3 MB using the same bioinformatics pipeline as MRT.

A selection of candidate genes in each tumour type will be taken forward to validate the role of epigenetic regulation. CRISPRa/i/m will be used to modulate expression and methylation status. CRISPR validated loci will be explored further to understand whether gene-targeted or genome-wide approaches will be more appropriate for treatment.
Multi-omics Assessment of Group 4 Medulloblastoma for Improved, Biomarker Driven, Prognostication and Risk-Stratification

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Introduction
Despite being the largest of the medulloblastoma (MB) molecular subgroups (~35-40%), Group 4 (MBGrp4) has the least understood underlying biology. Recent advances have revealed clinically-relevant subtypes within MBGrp4 with distinct molecular features (e.g. second-generation Grp3/Grp4 subtypes (Sharma et al., 2019) and copy number (CN) signatures (Goschzik et al., 2018)). There is now a clear requirement for a dedicated, comprehensive study of MBGrp4, considering both established clinico-pathological features and emerging biomarkers to enhance patient-stratification models and identify novel therapeutic targets for MBGrp4.

Methods
To elucidate the specific molecular pathology of MBGrp4, we have assembled a comprehensive, clinically-annotated, discovery cohort (total n = 424; U.K (n = 216), Spain (n = 25) and France (n = 28) as well as from historic HIT-SIOP PNET3 (n = 56) and PNET4 (n = 81) clinical trials), in addition to validation cohorts from published datasets (Cavalli et al., 2017 and Northcott et al., 2017, n = 760). Molecular subgroup/subtype classifications and arm level CN estimates were determined by methylation array. Transcriptional (RNA-seq) and mutational (targeted panel sequencing) analysis of MBGrp4 was performed.

Results
Second generation Grp3/4 subtypes VIII (39%) and VII (30%) were predominant, whilst subtypes II, III, and IV were absent from MBGrp4 (p < 0.001). Correlation analysis identified positive associations between subtypes and biological features; MYCN amplifications with V (p = 0.003) and i17q with VIII (p < 0.0001), which was otherwise lacking CN aberrations. The favourable risk CN group established in standard-risk MB is recapitulated in this cohort (10yr PFS; favourable CN = 89% vs high-risk CN 57%, p < 0.0001) and is enriched in subtypes VI (p < 0.001). Mutational data has been generated for 228/424 (54%) and transcriptional data for 139/424 (33%), for further integrative investigation.

Conclusion: We have generated/assembled comprehensive MBGrp4 discovery and validation cohorts totalling >1000 tumours, enriched with multi-omics data. This will enable the investigation of improved biomarker driven patient-stratification models and novel therapeutic targets.

References
Temporal clustering of neuroblastoma in children and young adults from Ontario, Canada

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Background
The aetiology of neuroblastic tumours is likely to involve both environmental and genetic factors. A number of possible environmental risk factors have been suggested, including infection. If an irregular temporal pattern in incidence of cases of a disease is found, this might suggest that a transient agent, such as infection, is implicated. Previous work has found that such an agent might have been involved in the aetiology of neuroblastic tumours in children and young adults living in Northern England. We examined data from a population-based registry from Ontario, Canada to see if there is evidence of temporal clustering of neuroblastic tumours.

Methods
All cases of neuroblastic tumours diagnosed in children and adults aged 0-19 years between 1985 and 2016 were extracted from the Pediatric Oncology Group of Ontario Networked Information System (POGNIS). This population-based registry covers a population of 3.1 million young people resident in Ontario, Canada at the time of diagnosis. A modified version of the Potthoff-Whittinghill method was used to test for temporal clustering. Estimates of extra-Poisson variation and standard errors were obtained.

Results
876 cases of neuroblastoma were diagnosed during the study period. No evidence of temporal clustering was found between fortnights within months, between months within quarters or between quarters within years in the study period. However, significant extra-Poisson variation was found between quarters within the study period (extra-Poisson variation = 0.41 SE = 0.13; p = 0.002) and between years within the study period (extra-Poisson variation = 1.05 SE = 0.25; p = 0.005).

Conclusions
The findings are consistent with the possibility that a transient agent, such as an infection that is characterised by ‘peaks and troughs’ in its occurrence, might be implicated in the aetiology of neuroblastic tumours. Further analyses will be performed to explore potential differences between children diagnosed in infancy and those diagnosed later in childhood.
**Therapeutic Combination Approach Alleviates the Main Side Effect of Treatment for Acute Lymphoblastic Leukaemia**

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**Introduction**

Anti-leukemic drugs based on asparaginase are an essential element in treatments used in the clinic currently for childhood acute lymphoblastic leukemia (ALL) and are associated with its increasing survival rate. The most common reason for ending asparaginase treatment, however, is the development of acute pancreatitis (AP) which occurs in up to 10% cases (Raja *et al.*, 2012). AP is a life-threatening disorder which continues to increase in prevalence on a global scale. Despite considerable research over the past two decades, there is still no specific therapy available in the clinic (Petersen *et al.*, 2011; Gerasimenko 2018). Although asparaginase is a known cause of AP, excessive alcohol consumption and gallstone biliary disease are the leading causative factors. Sustained elevation of cytosolic Ca²⁺ concentration inside pancreatic acinar cells initiates AP, resulting in premature activation of digestive enzymes, mitochondrial dysfunction and cellular necrosis (Gerasimenko *et al.*, 2013). This in turn causes autodigestion of the pancreas and a potentially fatal inflammatory response. Excessive release of Ca²⁺ from intracellular stores and subsequent Ca²⁺ entry via Ca²⁺ release-activated Ca²⁺ (CRAC) channels in the plasma membrane as well as mitochondria malfunction and loss of ATP instigate cytosolic Ca²⁺ overload (Gerasimenko *et al.*, 2013). Currently, a novel selective CRAC channel inhibitor CM4620 (developed by CalciMedica) has reached phase II human trials (NCT03709342). However, long-term application of this inhibitor is doubtful due to its profound effects on immune cells (Waldron *et al.*, 2019). Recently, another approach has emerged where cells are supplied with energy supplement galactose which substantially reduces AP effects in vitro and in vivo (Peng *et al.*, 2018). Here we have used a combination of these two approaches in an attempt to alleviate AP-related Ca²⁺ toxicity and necrosis.

**Methods**

The effect of CM4620 on CRAC channel-mediated Ca²⁺ entry was investigated in vitro using freshly isolated mouse pancreatic acinar cells and cytosolic Ca²⁺ indicator, Fluo-4 AM as described previously (Peng *et al.*, 2018). The *in vitro* effect of CM4620 on the level of cellular necrosis, elicited by AP-inducing agents such as bile acids, alcohol metabolites and asparaginase, was also investigated using propidium iodide staining of dead cell nuclei. To investigate the protective effect of mitochondria energy supplements, cells were treated with galactose (1 mM) in the presence or absence of CM4620 (100 nM, 50 nM, 10 nM, 1 nM, 200 pM).

**Results**

CRAC inhibitor CM4620, at much lower concentrations than reported previously (i.e. 10 μM, Wen *et al.*, 2015; 3 μM, Waldron *et al.*, 2019), significantly reduced both sustained cytosolic calcium evoked by intracellular store depletion and near-physiological Ca²⁺ responses induced by acetylcholine. CM4620 has markedly protected against acinar cell necrosis, at nanomolar concentrations, following exposure to bile acids, alcohol metabolites and asparaginase. A combination of CM4620 (at 100 nM and 50 nM) and galactose (1 mM) was most effective at reducing necrosis induced by bile acids. Combining galactose with even lower concentrations of CM4620 (10 nM, 1 nM and 200 pM) provided a higher degree of protection against cell necrosis evoked by asparaginase or alcohol metabolites. In all cases, the combination approach reduced necrosis to near-control levels.
Conclusions
Inhibition of CRAC channels with novel compound CM4620 prevents abnormal elevations in cytosolic Ca\textsuperscript{2+} and necrosis in murine acinar cells. The protective effects of low, nanomolar concentrations of CM4620 reduces chances of potential side effects of asparaginase. Combining CM4620 with galactose increases the effectiveness of treatments and is therefore a very promising approach for the development of an effective combination therapy for AP as well as improving current treatments for childhood ALL.

References
Identification of anti-MYC therapeutic targeting strategies in Group 3 medulloblastoma using novel regulable cell-based models of MYC-dependent tumourigenesis

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Background
Medulloblastoma (MB) is the most common malignant paediatric brain tumour and accounts for almost 10% of childhood cancer deaths. Group3 MB is characterised by high levels of MYC expression and amplification of the MYC locus (in 17% of Group3 tumours). Patients with MYC amplification have a dismal prognosis (<20% 5 year overall survival) under current therapeutic regimes; therefore there is an urgent need for novel targeted therapies. MYC has disease-context specific effects, thus investigation in appropriate models relevant to MB is essential.

Methods
We have developed three independent Group 3 MB cell line models in which MYC expression can be regulated through the doxycycline-induced expression of a MYC targeting shRNA. We have used these cell lines to characterise MYC directed changes at the transcriptomic and proteomic level alongside investigation of the MYC-dependency of the response to a large panel of cancer therapeutics (>500 compounds).

Results
Transcriptomic and proteomic analysis following MYC knockdown highlighted a series of MYC regulated pathways and genes of relevance to primary Group3 MB, with key targetable molecules and pathways identified, such as PLK1, CHEK1 and AURKA/B. Genetic dependency on these targets was confirmed in siRNA knockdown and CRISPR/Cas9 knockout experiments. Our drug screen has identified drugs targeting these molecules as being among ~70 compounds, which have a greater effect on the proliferation of MYC expressing cells.

Conclusion
Integrative analysis identifies a number of reproducibly MYC-dependent pathways, which can be targeted therapeutically for further validation and investigation in vivo.
Poster abstracts

Inferred intra-tumoural heterogeneity of medulloblastoma

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Introduction
Medulloblastoma is the most common malignant brain tumour seen in the paediatric population. It has recognised inter-tumoural heterogeneity with different subgroups (MB_WNT, MB_SHH, MB_Group3, MB_Group4) having distinct molecular and clinical features. Despite biological and clinical advances there has been a plateauing of survival rates. Computational methods to infer intra-tumoural heterogeneity offer tools to predict the clonal evolution of tumours and assist identification of relevant subclonal populations which could impact the response to treatment.

Methods
A pilot cohort of 76 published medulloblastoma whole exome sequences were analysed using the EXPANDS bioinformatics method. Subpopulation analyses were utilised to interrogate their relationship with established clinicopathological correlates and inferred intra-tumoural heterogeneity.

Results
Our data analysis predicted a range of 0-8 subclones per tumour and a median value of 3 subclones; the higher the subclone number the greater the predicted heterogeneity. The mean number was highest in the MB_Group3 tumours (4.59 subclones per tumour) compared to 3 subclones in the remaining subgroups. Mutations of known medulloblastoma driver genes were mainly allocated to subclone sizes greater than 0.5 indicating these were fully clonal or early subclonal events. In the driver mutations allocated to subclone sizes less than 0.5, 4 of the 5 were in MB_Group3 and MB_Group4 tumours.

Conclusion
This initial pilot analysis of inferred intra-tumoural heterogeneity has developed methods to review the number and nature of inferred subclones and how this correlates with clinicopathological features. This data suggests the subgroup associated with the poorest outcomes, MB_Group3, had the greatest inferred heterogeneity. Review of the clonal evolution of medulloblastoma tumours indicated driver mutations appeared to mainly occur early in tumour development. Expanding this inferred heterogeneity analysis into larger cohorts will improve the understanding of the relationship between intra-tumoural heterogeneity and clinicopathological and molecular features, and disease outcomes. This could provide further insight into the biological basis of high-risk disease, assist with improved risk stratification and guide future treatments.
Establishing Methods for Analysing Low-Depth Cancer Whole Genome Bisulfite Datasets

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Introduction
Medulloblastoma is the most common paediatric brain tumour, representing ~20\% of diagnosed brain tumours annually (Khanna \textit{et al.}, 2017). Current consensus classifies medulloblastoma into four subgroups (WNT, SHH, Group 3 & Group 4), each differing in prognostic outcome and characterized by distinct pathological and molecular features (Taylor \textit{et al.}, 2012). The current gold-standard method of subgroup classification is the assessment of DNA methylation patterns using microarray, and it is now used as a routine diagnostic tool. However, since this method is proprietary, clinicians will be left without a robust alternative should the platform be withdrawn. Next Generation Sequencing (NGS) offers the possibility to interrogate methylation status across the genome at single base resolution. The gold standard method of interrogating methylation with NGS is whole genome bisulfite sequencing (WGBS). However, its adoption by researchers has so far been limited due to high library preparation costs and high starting material requirements. In addition, there is currently no consensus on the most appropriate bioinformatics pipelines for WGBS analysis. Now, various low-cost methodologies have been developed, enabling whole methylome research to become more accessible (Crary-Dooley \textit{et al.}, 2017; Suzuki \textit{et al.}, 2018).

Method
Raw sequence data is subject to quality control and trimming prior to alignment with a specialized bisulfite aligner tool. Additional quality control is performed before cytosine methylation information is extracted. Downstream analysis consists of a multi-faceted bioinformatics approach which begins with subgroup detection and clustering analysis. Differential methylation analysis is then carried out between subgroups alongside mutation analysis and chromosomal copy number detection.

Results
Here, we present an optimised analysis pipeline for analysing WGBS data and generating numerous data of clinical interest. This pipeline provides a reproducible template for performing the appropriate sequence quality control, trimming and alignment approaches to WGBS sequence processing. After processing, we show that diverse clinically-relevant molecular readouts can be generated, including differential methylation, sample subgrouping, aneuploidy detection and identification of mutations. We also model the analysis of data from poor-quality DNA derivatives by data down-sampling.

Conclusions
This pipeline provides a foundation for future research with samples sequenced via WGBS and will serve as the methodology for future analysis of WGBS subgrouping of medulloblastoma samples. The increased resolution afforded by WGBS may reveal additional molecular heterogeneity and can also provide readouts for numerous prognostic markers (e.g. chromosomal alterations, mutations). Ultimately, it is hoped that this methodology will serve to provide a platform-independent method of analysing clinical samples sequenced using WGBS.

References


**Poster abstracts**

**The clinical significance of extent of resection in medulloblastoma**

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**Introduction**

Medulloblastoma is the most common malignant brain tumour in children. Standard treatment consists of maximal safe gross-total resection (GTR), chemotherapy and radiotherapy. In most treatment protocols a sub-total resection (STR), defined by >1.5cm² tumour residuum, is a high-risk feature, resulting in more intensive treatment regimens which are associated with greater neurological morbidity. The impact of the extent of resection (EOR) on survival lacks clear consensus and until recently studies predated the discovery of the molecular subgroups (Thompson *et al*., 2016). Our aim was to identify the impact of EOR on survival within the four molecular subgroups (MB\(^{WNT}\), MB\(^{SHH}\), MB\(^{Group3}\), MB\(^{Group4}\)).

**Methods**

We collected clinical, pathological and molecular data from 419 paediatric patients in the Newcastle MB series who had neurosurgery between 1990 and 2015. This data was combined with data published in 2016 by Thompson *et al* which included 787 patients who had neurosurgery between 1997 and 2013. This combined cohort consisted of 1113 patients (96 MB\(^{WNT}\), 278 MB\(^{SHH}\), 247 MB\(^{Group3}\), 418 MB\(^{Group4}\)); the largest cohort ever constructed to assess the impact of EOR in medulloblastoma. We performed univariate and multivariate survival analysis using Kaplan-Meier log-rank and Cox proportional hazard modelling, analysing overall survival (OS) cohort-wide and in reference to the molecular subgroups and other clinico-pathological factors.

**Results**

Association analysis of the combined cohort evidenced that infant patients (<5 years of age) were more likely to have STR (p=0.02), as were those with metastatic disease (p=<0.001). EOR was prognostic for survival in the combined cohort in univariate analysis (HR 1.64, 95% CI 1.30-2.07, p=<0.001), in accordance with previous papers, but not in multivariate analysis (HR 1.29, 95% CI 0.97-1.70, p=0.077) unlike metastatic disease and MBGroup3 which showed prognostic significance in both univariate and multivariate analysis. STR was variably prognostic in sub-cohort analyses and significance depended on the clinico-molecular context; worse outcomes were observed in the under 5 age group in both MB\(^{SHH}\) (HR 1.87, 95% CI 1.02-3.43, p=0.044) and MBGroup4 (HR 2.56, 95% CI 1.03-6.38, p = 0.044), but not in other disease subgroups.

**Conclusion**

In this large cohort, STR is significantly associated with a lower OS in univariate analysis but is not independently prognostic; it is therefore recommended that surgeons should continue to pursue maximal safe resection in all disease subgroups. GTR significantly improves survival in patients under 5 years of age in MB\(^{SHH}\) and MB\(^{Group4}\) and whilst at present molecular subgrouping cannot be done prior to surgery, we suggest the classification of STR as a high-risk feature in these disease contexts persists.

**Reference**

Thompson *et al*., (2016) Lancet Oncology 17:484-95
Poster abstracts

Use Of SNP Array for Risk Stratification in B-Cell Acute Lymphoblastic Leukaemia

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Genetic abnormalities are widely used in the risk stratification of B-cell acute lymphoblastic leukaemia (B-ALL). Up-coming trials will incorporate copy number abnormality (CNA) profiling based on a sub-set of genes into risk stratification algorithms to determine the optimum therapy. For example, the UKALL-CNA risk profile uses CNA of IKZF1, PAX5, EBF1, RB1, CDKN2A/B, ETV6 and BTG1, as well as genes deleted from within the PAR1 region resulting in the P2RY8-CRLF2 fusion, to assign patients into three risk groups: good (CNA-GR), intermediate (CNA-IR) and poor (CNA-PR). When considered alongside other risk factors, patients with a CNA-GR profile may be eligible for treatment de-escalation in future trials. Currently the standard method for detection of the UKALL-CNA risk profile is Multiplex Ligation-dependent Probe Amplification (MLPA) targeting the specific genes mentioned above. More recently, SNP arrays, which provide genome wide copy number data, are increasingly being used in the diagnosis of B-ALL and will be used routinely in future trials.

Here we compare results obtained from the Illumina Infinium CytoSNP-850K array with those from MLPA to confirm that SNP arrays provide the same degree of accuracy in identification of the UKALL-CNA risk profile.

MLPA and SNP array were performed on the same 123 patients with B-ALL. CNA were called according to standard methodologies. There was good correlation between the techniques, with 97% of calls (959/984) being concordant. Both methods identified a full range of abnormalities, including whole gene and intragenic deletions, as well as focal amplifications. We observed 25 discordant calls. These discrepant results could be divided into 2 classes: A) sub-clonal CNA present in <20% of cells (n=13) and B) small focal deletions, often involving a single exon (n=12).

Class A discrepancies were typified by sub-clonal deletions of PAX5 and RB1, which were often associated with large deletions of chromosomes 9 and 13, respectively, present in <20% of cells by standard cytogenetic analysis. These deletions could be more confidently called by SNP array, due to the large number of probes within the affected regions, which were within the normal range by MLPA.

Class B was exemplified by deletions of ETV6, SNP array identified focal deletions of exon 2 (n=6) which is not covered by the MLPA kit and conversely MLPA identified loss of exon 1 (n=4) for which there is no coverage on the SNP array.

Despite these discrepancies, the UKALL-CNA risk group remained unchanged between the two techniques in 97% of patients (119/123). Four patients, classed as CNA-GR by MLPA, would be classed as CNA-IR or CNA-PR by SNP array and would therefore be ineligible for proposed treatment reduction.

We conclude that SNP array is an appropriate technique for calling the UKALL-CNA risk profile, which can easily be incorporated into routine diagnostic testing in B-ALL.
Non-genotoxic combination treatments for paediatric acute myeloid leukaemia

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Acute myeloid leukaemia (AML) is a rapidly progressing, heterogeneous malignant hematopoietic disease of the bone marrow and peripheral blood. The RUNX1/ETO fusion gene is the most frequent chromosomal rearrangement in childhood AML resulting in a block in differentiation, increased proliferation, and eventually to development of AML.

The mainstream treatments have not changed much for decades and the mechanisms underlying drug resistance in AML are poorly understood.

To identify additional therapeutic targets and pathways involved in drug resistance in AML, we performed genome wide CRISPR-Cas9 screens for mutants resistant to the potent CDK4/6 inhibitor palbociclib in human RUNX1/ETO cancer cell lines, Kasumi-1 and SKNO-1.

Using next generation sequencing, we identified 846 candidate genes in Kasumi-1 and 524 in SKNO-1 that were enriched under palbociclib treatment. Amongst others, this screen highlighted several members of the mTOR and autophagy pathways such as AMBRA1, GSK3B, and GABARAPL1, suggesting that inhibition of CKD4/6 increases the dependence of leukaemic cells on activation of mTOR. In subsequent experiments we explored potential synergistic effects between mTOR and CDK4/6 inhibition. Notably, combinations of palbociclib with different rapalogs show a substantially increased potency to inhibit leukaemic propagation when compared to single agents. We are now in the process of confirming the ex vivo efficacy of these combinations in patient-derived leukaemic cells and, subsequently, in vivo in mice transplanted with AML cells. These experiments in combination with further functional studies will provide an important next step towards our goal of a non-genotoxic therapeutic approach.
Clinical and Biological Factors associated with Refractory disease in UK patients with High Risk Neuroblastoma

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Background
Despite advances in neuroblastoma therapy, a third of cases have refractory disease, and fail to achieve an adequate response that allows consolidation with myeloablative therapy.

Aims
To investigate factors associated with refractory disease in neuroblastoma patients by linking epidemiological, clinical, genetic, and treatment data from 21 Paediatric Oncology Principal Treatment centres, in the UK and Ireland in patients treated on the European high risk neuroblastoma trial (SIOPEN HR-NBL-1).

Methods
This is a retrospective study. Refractory disease is defined as patients who came off the HRNBL-1 trial for insufficient response (> 3 positive bone lesions on mIBG scan or a positive bone marrow aspirate or trephine).

Results
Data from 10 centres open in the UK, are included in this interim analysis. There are 25 refractory disease cases, all stage 4, 29% MYCN amplified with a median age at diagnosis of 3.3 years (IQR 1.4-20 years). Treatment for refractory disease included topotecan / vincristine / doxorubicin in 16/25 (64%), topotecan / cyclophosphamide in 6/25 (24%), temozolomide / Irinotecan in 10/25 (40%), BEACON trial (a randomised phase IIb trial of BEvACizumab added to Temozolomide ± Irinotecan) in 6/25 (24%). The INRC response to second line treatments for refractory disease were 2/25 (8%) complete response, 6/25 (24%) very good partial response, 4/25 (16%) partial response, 2/25 (8%) mixed response, 4/25 (16%) stable disease and 7/25 (28%) progressive disease. Subsequently 3/25 (12%) patients received myeloablative therapy and 7/25 (28%) patients received immunotherapy. The median follow-up period was 3.3 years (IQR 1.3 – 4.5 years) with 9/25 (36%) alive disease free, 5/25 (20%) alive with disease and 11/25 (44%) cases have died from disease.

Conclusions
Refractory patients are more likely to be non-MYCN amplified and older at diagnosis compared with standard high risk patients (50% MYCN amplified, median age of presentation 18 months). This early data shows that some refractory patients can achieve subsequent response and complete standard treatment for high risk neuroblastoma. This data will form a useful historical comparator group for the forthcoming VERITAS trial for refractory neuroblastoma.
Preclinical Evaluation of the ATR Inhibitor AZD6738 Alone and in Combination with the PARP Inhibitor Olaparib in Neuroblastoma

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Introduction
Neuroblastoma (NB) is the most common extracranial solid tumour of childhood (Matthay et al, 2016) with the high-risk type having <50% survival at 5 years. MYCN is an oncogene and can drive replication stress in cells in NB. Cells with stalled/collapsed replication forks which cause replication stress in cells are dependent on ataxia telangiectasia and Rad3-related protein (ATR) signalling to stall progression into the cell cycle. Inhibiting ATR causes DNA damage to accumulate leading to cell death. Poly-ADP ribose polymerase (PARP) is a protein involved in the repair of single stranded breaks (SSBs). Inhibition of PARP increases replication stress in the cell causing more breaks in DNA and ultimately cell death (Delia et al, 2017).

Hypothesis
Combining a PARP inhibitor with an ATR inhibitor will be synergistic for growth inhibition in NB cell lines due to the increase in replication stress caused by the PARP inhibitor.

Aims
To determine the effect of the AZD6738 (ATR inhibitor) alone and in combination with olaparib (PARP inhibitor) in 4 human NB cell lines, 2 non-MYCN amplified and 2 MYCN amplified.

Methods
The XTT assay (Roche) was used to determine single and combination agent sensitivity after 72 hour drug treatments. Western blotting was used to determine expression of proteins important in the DNA damage response (DDR).

Results
SKNAS cells (non-MYCN amplified) were most sensitive to ATR inhibition alone with the lowest GI50 value (1.08±0.30µM) compared to N20_R1 (MYCN amplified) which were most resistant with the highest GI50 value (1.92±0.62µM). Comparing NGP and N20_R1 paired cells, N20_R1 (p53 mutant) is more resistant to both single and combination treatment, implicating p53 mutations as a potential feature causing resistance in cell lines. Combination index analysis of combination treatments showed that AZD6738 and olaparib were synergistic at lower concentrations (0.1/1µM) in all cell lines. Western blotting of N20_R1 cells showed drugs were acting by expected pathways with decreased ATR/CHK1/pCHK1 following AZD6738 treatment. With combined AZD6738 and olaparib treatment, expression of ATR/CHK1/pCHK1 decreased further. Olaparib treatment alone lead to increased levels of ATR/CHK1/pCHK1 showing an increase in replication stress on the cells.

Conclusions
AZD6738 and olaparib are synergistic at sublethal concentrations (≤1µM). p53 status presents as a feature that may determine sensitivity of cells to inhibitors, but more cell lines are required to test this further.

References
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Elucidating the Mutational Landscape of High-Risk Medulloblastoma

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Introduction
Medulloblastoma (MB) is the most common malignant paediatric brain tumour, and displays heterogeneous survival outcomes. Non-infant patients are assigned to the high-risk (HR-MB) group if one or more of the following features are apparent: sub-total surgical resection, metastases, LCA histology, MYC/N amplification, TP53 mutation (SHH subgroup). However, survival models for the group of patients with these features do not fully describe outcomes, indicating the need for further biomarker discovery in this clinical group. To address this need, we have assembled a multi-national cohort of HR-MB patients with full clinico-pathological data annotation, however mutational data is lacking.

Methods
394 HR-MB patients (UK n=198, French n=66, Spanish n=32, SIOP-PNET3 clinical trial n=98), aged 3-16 years old and who received full craniospinal irradiation were included. Tumour DNA samples were quantified using Qubit (Fluorometric dsDNA broad range assay). 72/394 (18.3%) were insufficient for next generation sequencing (NGS) library preparation (<100 ng). gDNA was sonicated to 150 bp fragments (30 sec on/off for 70 cycles, Bioruptor Plus) and the NGS libraries prepared using Agilent SureSelect XT Low Input protocol to target a panel of the 65 most commonly mutated genes in Medulloblastoma. Quality control assessments were made using Tapestation 4200 with gDNA, D1000 and High Sensitivity D1000 assays throughout. NGS libraries were pooled to equimolar concentration of 40 nM and concentrated in 10 µL using MinElute PCR Purification kit (Qiagen), then sequenced using Illumina HiSeq 2500.

Results
Very high quality sequencing data was obtained for 319/322 (99.1%) patients (average read depth = 289), with few samples producing a read depth <50 (3/322, 0.9%), consequently a large proportion of tested INDELs were validated via Sanger sequencing (87%). Data quality was significantly improved compared to a previously used platform, Agilent SureSelectXT2, where 1) far fewer samples could be attempted due to more stringent input DNA requirements (90/123, 73.2%); 2) fewer samples successfully passed library-prep QC (36/90, 40%) and validation rates were much lower (22%).

Conclusion
The Agilent SureSelectXT Low Input platform was highly suitable for targeted panel NGS analysis of the often FFPE-derived DNA from the HR-MB cohort. Data resulting from this approach will provide insights into the mutational landscape of HR-MB, offering potential for improved, biomarker-driven, risk-stratification models, elucidation of underlying biology and novel therapeutic target identification.
Utilising the Attune® Nxt Acoustic Focusing Flow Cytometer for Clone Tracking of EGFP Constructs in ALL Cell Lines

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Introduction
The Attune® NxT Acoustic Focusing Flow Cytometer (AFC; Invitrogen) uses acoustic-assisted hydrodynamic focusing to increase measurement precision and provide 10x faster sample throughput compared to traditional flow cytometric analysers (Cossarizza et al., 2017). Traditional flow cytometry has an abundance of limitations, namely longer sample preparation time and a markedly slower flow through of cells, thus limiting batch size and overall sample quantity due to time constraints. Simultaneous analysis is important to establish such quantities, yet these limitations have restricted the application of flow cytometry to relatively small sample sizes.

Methods
We utilised in vitro AFC clone tracking using a variety of acute lymphoblastic leukaemia (ALL) cell lines (n=5), each transduced with lentivirus expressing enhanced green fluorescent protein (EGFP) alone or in combination with one of five cDNAs (A-E) coding for leukaemia related genes. EGFP expression was monitored over a series of time points with AFC (488-nm blue laser, channel BL1), from time point 1 (TP1) at day 3 post-transduction up to the final time point at 27 days. Cell lines were transduced with each cDNA construct in triplicate (n=105). Percentage of cells expressing EGFP at each TP was analysed after flow cytometry (FlowJo v_10), with mean EGFP% of gated live cell population plotted for each cell line/TP (after normalisation to TP1).

Results
In Pre-B 697 cells, construct A showed the largest decrease in EGFP% up to TP4, after which expression plateaued (TP4=10%, TP7=6%). Constructs B, C, and D followed the same trend in expression (TP7=15%, 22% and 19% respectively). EGFP% expression of construct E showed little change, plateauing at 80% at TP4. In REH, EGFP% decreased gradually over the TPs for constructs A, B, C, and D (TP7=55%, 17%, 10%, and 25% respectively), with construct E EGFP% remaining high (TP7=95%). EGFP% for the vector control was consistently high (≥90%) in both REH and Pre-B 697 cell lines across all TPs. Little change was observed in all other cell lines. These results were consistent with previous methods of tracking of the same constructs using Illumine Amplicon Sequencing.

Conclusion
In this study, we highlight the power of AFC for rapid processing of samples in large-scale experiments. 105 samples per time point were analysed in this study, a batch size that could not practically be achieved using traditional flow cytometry. Such tools are vital in monitoring clonal expression changes, crucial for understanding gene activity in vitro.

Reference
Cossarizza et al., 2017. Guidelines for the Use of Flow Cytometry and Cell Sorting in Immunological Studies. European Journal of Immunology, 47(10), 1584-1797
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The Molecular Landscape And Clinical Experience In Infant Medulloblastoma

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Introduction
Infants with medulloblastoma (iMB; 0-5.0 years; ~30% of patients) are clinically distinct and commonly treated with radiation-sparing regimens aimed at minimising neuro-developmental late-effects. Outcomes remain poor (5yr OS <60%). Recent molecular advances have enabled improved biomarker-driven risk-stratification in non-infant MB clinical trials. However, in iMB, biological studies of large trials and/or retrospective cohorts have not been undertaken to support equivalent strategies.

Methods
We undertook comprehensive characterisation of the molecular pathology and associated historical clinical experience in iMB, encompassing discovery and validation in cohorts totalling 387 patients.

Results
iMB displayed distinct clinico-molecular features; iMB$_{SHH}$ (40%) and iMB$_{Grp3}$ (42%) predominated, each harbouring clinically-significant biological heterogeneity. iMB$_{Grp3}$ was enriched for second-generation molecular subtypes IV, III and II; the latter strongly associating with large-cell/anaplastic pathology (LCA; 23%) and MYC amplification (19%), defining a very high-risk group with common rapid progression on current therapies (0% 10yr OS vs 73% in non-LCA/MYC iMB$_{Grp3}$). In our primary discovery cohort, iMB$_{SHH}$ was strongly associated with DN/MBEN pathology (75% of iMB$_{SHH}$; p<0·0001) and harboured two subgroups with characteristic clinico-molecular features (iMB$_{SHH-II}$); we demonstrate these are reproducible in infant-specific analyses of large independent cohorts. iMB$_{SHH-II}$ DN/MBEN patients had better PFS, offering opportunities for stratification of radiation-sparing treatments. However, DN/MBEN and extent of surgical resection were the only significant independent risk-factors for OS, discriminating favourable- (60% of iMB$_{SHH}$) and very-high-risk (40%) disease groups in survival modelling (93% vs 23% 10yr OS). DN/MBEN iMB$_{SHH}$ tumours had greatest potential for rescue post-relapse (56% post-relapse survival). Survival relationships were reproducible in large validation cohorts and not influenced by treatment type (e.g. upfront radiotherapy), strongly supporting their adoption as independent risk-factors.

Conclusions
Our models outperform current risk-stratification schemes, enabling risk-status reclassification for >50% of iMB and personalisation of therapy. Routine molecular diagnostic sub-classification will be essential for the future management of iMB.
Medulloblastoma Survivorship Outcomes are Related to Tumour Molecular Subgroup; A Meta-analysis of the SIOP-UKCCSG-PNET3 and HIT-SIOP-PNET4 Trials

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Introduction
Reduced health-related quality of life (HRQoL) and cognitive outcomes are common in children treated for medulloblastoma (MB). The 2016 WHO classification of brain tumours recognises four consensus MB molecular subgroups (MBWNT, MBSHH, MBgrp3, MBgrp4) and limited evidence in small cohorts suggest molecular subgroup may influence MB survivorship.

Methods
Neurocognition measures (Wechsler Intelligence Scales, Kaufman Assessment Battery for Children, and Raven’s Progressive Matrices) were collected in the HIT-SIOP-PNET4 MB trial and HRQoL data (health status, HUI3; emotional and behavioural difficulties, SDQ; HRQoL, PedsQL) was additionally available for this and the previous SIOP-UKCCSG-PNET3 MB trial. Molecular subgroup was retrospectively determined in remnant tumour material and assessed for relationships to HRQoL and neurocognition alongside other treatment and clinico-pathological features, in univariate and multivariate analyses.

Results
In a combined cohort comprised of SIOP-UKCCSG-PNET3 and HIT-SIOP-PNET4 patients (n=150), tumour molecular subgroup was significantly associated with parent- and child-reported HRQoL, even after taking into consideration other significant and reported HRQoL predictors (e.g. treatment, gender, age etc); MBgrp4 predicted significantly worse outcomes than MBgrp3 and MBgrp4 (p<0.05). Furthermore, MBgrp4 predicted poorer patient- and parent-reported health status in HIT-SIOP-PNET4 (n=40/81, p<0.003). MBSHH showed a trend for exhibiting highest scores on all measures of cognitive performance, possibly related to tumour location. Within a restricted MBgrp cohort (n=35), HIT-SIOP-PNET4 treatment (p=0.041, B=-0.175) and classic tumour histopathology (p<0.001, B=0.516) were significantly associated with health status.

Conclusion
This combined analysis across two MB trials has identified relationships between tumour molecular subgroup and survivorship outcomes post-therapy (HRQoL, neurocognition), highlighting findings for assessment in larger series. Such cross-discipline, international trials-based studies have the potential to inform our current understanding of MB survivorship outcomes and could impact subgroup-directed disease management strategies in future.
HARMONIC: Health effects of cardiac fluoroscopy and modern radiotherapy in paediatrics

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Introduction
HARMONIC is a newly launched European Union funded project investigating the long term health effects of ionizing radiation exposure in children and adolescents. The study will focus on exposures not covered by existing European research programmes: (a) modern external beam radiotherapy techniques (proton beam therapy and intensity modulated radiotherapy) and (b) cardiac fluoroscopy. Here, the focus is just on the radiotherapy component of HARMONIC.

Rationale
Modern radiotherapy techniques ensure that the high dose region conforms to the target tumour volume. Such targeting may come at the expense of increased exposure of healthy surrounding tissues to low/moderate doses, or, in the case of proton beam therapy, unwanted neutron radiation. The long-term impacts of these modern radiotherapy on non-targeted tissues is unclear. Standardized patient registries are required, alongside international cooperation.

Methods
HARMONIC will involve creating the first European registry of children and young adults treated with modern RT techniques, in collaboration with the ESTRO/European Particle Therapy Network. It is
anticipated that about 2670 patients treated between 2000 and 2022 at centres in France, Germany, Belgium and Denmark will be included. Radiation doses to in-field regions will be estimated using the treatment planning systems. Doses to out-of-field regions will be estimated using a tool developed at the University of Zurich based on Monte Carlo computer modelling. Patient doses from image guidance will also be considered.

**Outcomes**

A framework will be set up to investigate the following outcomes relation to estimated organ doses: incidence of (1) second primary cancer incidence, (2) cardiovascular and neurovascular disorders, (3) endocrine dysfunction and (4) quality of life. HARMONIC is closely aligned with other initiatives to study the long-term effects of proton beam therapy, such as that implemented and coordinated in the US by the National Cancer Institute. We welcome expressions of interest for additional collaboration.
Integrated quantitative proteomics by SWATH- MS of Malignant Rhabdoid Tumours uncovers new therapeutically opportunities

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The functional consequences of SMARCB1 mutation in Malignant Rhabdoid Tumours (MRTs) have so far been characterised at the DNA and RNA level, however proteomic changes induced by SMARCB1 loss remain to be revealed. SMARCB1 is the key driving mutation in MRT without which MRT cells cease to proliferate. In this study we developed an integrative proteomics pipeline to ask to what extent the effects of SMARCB1 on gene expression also alter the composition of the MRT proteome. We performed MS-SWATH, a technique for unlabelled discovery proteomics, on MRT cells in which SMARCB1 was forcibly re-expressed using a lentivirus (5 cell lines, +/- SMARCB1 x 3 replicates x 3 fractions total, membrane, nuclear). MS-SWATH is used to capture a snapshot of all spectra within a sample. These spectra are then probed using a library of known spectra linked to known proteins and peptidoforms. To overcome the limited experimental information regarding the paediatric cancer proteome, we generated a custom spectral library for the analysis of the MRT proteome using a pH fractionation pool of total cell lysates, membrane, and nuclear fractions. These libraries cover >8000 individual proteins.

Our study shows that whilst gene expression and protein abundance are significantly related there are many instances whereby expression changes do not reliably predict protein abundances or their sub-cellular localisation. For instance > 100 proteins show significantly increased abundance with no concomitant change in RNA expression, as measured by RNA-seq. By integration with whole-genome CRISPR screening we can describe previously unappreciated but demonstrably functionally essential SMARCB1 dependent pathway/membrane biomarkers, evident at the protein but not the RNA level. We describe several which are druggable and may be therapeutically exploited, or membrane specific proteins that could conceivably form the basis of a CAR-T cell based immune therapy. Our analysis links, for the first time in MRT, genome-wide transcriptomic and proteome aberrations and reveals broad mechanisms underlying the effect of SMARCB1 mutation.
Relapse specific genomic alterations in UK relapsed neuroblastomas: evidence from whole exome sequencing and copy-number arrays of paired tumours

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Background
Relapsed neuroblastoma remains a major challenge. Identification of new genetic abnormalities at relapse is key to predicting response to existing targeted agents as well as identifying potential new treatment targets. By studying paired neuroblastoma tumours at diagnosis and relapse we aimed to determine the frequency of relapse specific mutations and new copy number abnormalities.

Methods
Thirty-five paired neuroblastomas from diagnosis and relapse were collected through Children’s Cancer & Leukaemia Group (CCLG) tissue bank. Collected samples were whole exome sequenced (WES) using Illumina Truseq Rapid Exome Library Prep Kit to a read depth of 100x. 31 pairs were run on SNP arrays using Illumina Infinium CytoSNP-850k v1.1 bead chip and analysed using Nexus 10 software. 16 pairs had matched germline exomes sequenced at 30x coverage used to subtract variants from matched diagnostic samples. Sequence reads were analysed using a GATK and MuTect pipelines. 29 pairs had matched data from SNP arrays and WES.

Results
WES data revealed increased numbers of mutations were present at relapse (mean = 11.1) vs. diagnosis (mean = 3.9) (P=0.01). Recurrent variants in at least five cases targeting genes such as ALK, CHEK2, OR6Y1 and KMT2C were observed. Two cases had multiple relapses spanning 10 years in one and 18 years in another with distinct tumour evolution (genes include IGFN and KMT2C). A total of 384 copy number abnormalities (CNAs) were detected in 31 pairs with an increase in CNAs at relapse mostly gains rather than losses (277 vs. 95). We have identified numerous CNAs that were not present at diagnosis. Chromothripsis was observed in two cases (chromosome 11 at diagnosis and chromosome 21 at relapse) in one case and (chromosome 11 at diagnosis and relapse) in the other. MDM2 amplification was detected (at diagnosis and relapse) in a single case as well as an intragenic deletion of ATRX at relapse in another.

Conclusions
Our data shows increased mutational burden and chromosomal abnormalities at relapse. Identified genes and pathways may provide new targets for drug development in neuroblastoma.
Identification and Characterisation of Candidate Oncogenes on Chromosome 21 in B-Cell Precursor Acute Lymphoblastic Leukaemia

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Introduction
Acute lymphoblastic leukaemia (ALL) is the most common paediatric cancer, with B-cell precursor ALL (B-ALL) accounting for around 80% of ALL diagnoses (O’Neill et al, 2012). Whole or partial copy number gain of chromosome 21 (CNG21) is the most common somatic chromosomal aberration across the differing cytogenetic subgroups (Heerema et al, 2007). The prevalence of these copy number gains imply that these aberrations are non-random, suggesting genes present on chromosome 21 potentially have an important, oncogenic role in leukaemogenesis and disease maintenance in patients who exhibit CNG21. Intrachromosomal amplification of chromosome 21 (iAMP21)-ALL is a distinct subgroup associated with a poor prognosis (Harewood et al, 2003, Moorman et al, 2007, Attarbaschi et al, 2008), which feature common regions of amplification and overexpression on 21q (Strefford et al, 2006, Rand et al, 2011, Li et al, 2014), offering a refined location for candidate oncogene exploration.

Methods
Clustered regularly-interspaced short palindromic repeats (CRISPR)-mediated gene editing technology was utilised as a whole genome CRISPR knock-out (GeCKO) screen to identify novel oncogenes in a B-ALL cell line panel (NALM16, 697, REH, HAL01). Model-based analysis of genome-wide CRISPR/Cas9 knockout (MAGECK) was used to analyse screening data, GeCKO data was integrated with cell line and patient RNA-sequencing data and copy number data (SNP6.0) to identify authentic candidates for functional investigation. Isogenic cell lines were developed using the lentIMPHv2 and lentISAMv2 transcriptional activation system for the overexpression of target genes. Protein levels of isogenic cell lines were measured by Western blot.

Results
GeCKO screening in CNG21 cell line NALM16 identified DYRK1A as a novel candidate oncogene within the common regions of interest on chromosome 21. DYRK1A is implicated in many pathways which are commonly dysregulated in cancer including the RB1 pathway and phosphorylation of FOXOs, and has an essential role in pre-B cell development (Thompson et al, 2015). Furthermore, DYRK1A has both oncogenic and tumour suppressive properties which are dependent upon cellular context (Fernandez-Martinez et al, 2015). RNA-sequencing of patient and cell line samples (CNG21 n=20, non-CNG21 n= 59) demonstrated DYRK1A overexpression, which occurred in direct association with chromosome 21 copy number. Isogenic cell line models were successfully developed showing increased expression of DYRK1A, validated at the protein level.

Conclusions
The identification of DYRK1A as a candidate oncogene by GeCKO screening in B-ALL represents a novel finding. DYRK1A is located within a refined genomic region of interest on 21q with potential functional consequence in CNG21 B-ALL. Overexpression of DYRK1A has been confirmed in CNG21 B-ALL patients, including iAMP21-ALL, in line with copy number gain. The development of isogenic cell line models with overexpression of DYRK1A provides a model system for investigating the functional consequence of increased DYRK1A expression in B-ALL, and for identifying candidate genes and pathways amenable to therapeutic targeting in B-ALL patients with CNG21.
References
Genomic Analysis of Burkitt-like Lymphoma with 11q Aberration

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Burkitt-like lymphoma with 11q aberration (BLL-11q) is a new subgroup of B-cell non-Hodgkin lymphoma (B-NHL) defined in the updated World Health Organisation classification of lymphoid malignancies. BLL-11q cases are morphologically similar to Burkitt lymphoma (BL) however, they lack the hallmark BL translocation between the oncogene \textit{MYC} and the immunoglobulin genes, and are defined by a recurrent pattern of proximal gains and telomeric losses on chromosome 11q. Recent studies suggest that the underlying genetics are vastly different between these B-NHL subtypes. A high proportion of BL patients harbour mutations in \textit{TCF3}, \textit{ID3} and \textit{CCND3}, however these variants were not detected in BLL-11q patient samples. Importantly, patients with BLL-11q have been shown to have good outcome and the 11q abnormalities potentially define a subgroup of patients who may benefit from treatment de-escalation in order to reduce treatment-related toxicities. Utilising an integrated approach we have characterised the genome of BLL-11q and BL patients with 11q abnormalities using copy number analysis (CN), fluorescence in-situ hybridisation (FISH) and whole exome sequencing (WES). Preliminary analysis of BLL-11q patients have identified a gain of 6q which is associated with good outcome in BL patients and an absence of 1q gains which are frequently seen in BL. In addition to these findings recurrently mutated genes have been identified. Abnormalities have been correlated with clinical factors, including outcome. Our findings have been integrated with cases reported in the literature and we present the most comprehensive genetic analysis of this potentially good prognostic subgroup of B-NHL.
Polyphenols have been shown to sensitize cancer cells from solid tumours to some alkylating agents such as cisplatin, and induce apoptosis and/or cell-cycle arrest. This sensitization could enable a reduction of alkylating agent dose and decrease off-target side effects; whilst still maintaining treatment efficacy. However, actions in leukaemia cells have not been previously determined. Here, we assess the effects of five polyphenols alone and in combination with three alkylating agents (cisplatin, cyclophosphamide and chlorambucil) in lymphoid and myeloid leukaemia cells lines, and non-tumour control cells. The effects of combined treatments were investigated on ATP levels, glutathione levels, cell-cycle progression, DNA damage and apoptosis.

In lymphoid leukaemia cell lines, quercetin, apigenin, emodin and rhein synergistically enhanced cisplatin and cyclophosphamide activity, reducing ATP and glutathione levels, causing cell-cycle arrest, DNA damage and apoptosis. Similarly, apigenin and rhein acted synergistically when combined with chlorambucil in lymphoid leukaemia cell lines. In myeloid leukaemia cell lines, all three alkylating agents had differential effects. Synergistic effects were observed when alkylating agents were combined with quercetin, apigenin and emodin; whilst antagonistic effects were observed with some or all alkylating agents when combined with emodin, rhein and cis-stilbene. The observed synergistic effects were associated with a decrease in glutathione levels, DNA damage and apoptosis; whilst during antagonism the contrary effect was observed, glutathione levels were increased, and DNA damage was reduced.

In conclusion, this study suggests that combination of alkylating agents, in particular cisplatin with polyphenols could be promising for the treatment of lymphoid leukaemias, with apigenin showing the greatest synergistic effects with all alkylating agents. However, emodin, rhein and cis-stilbene were shown to antagonise the alkylating agent in myeloid leukaemia cell line. However, the use of apigenin with all alkylating agents could also prove beneficial for myeloid leukaemia patients.

**Keywords**
Leukaemia, Cisplatin, Cyclophosphamide and Chlorambucil, Polyphenols
Medulloblastoma (MB) is the most common malignant brain tumour in children. Molecular stratification has divided MBs into four consensus subgroups (WNT, SHH, Group 3 and Group 4) based on molecular and clinical features. Group 3 represents around 25% of all MB tumours. Amplification and elevated expression of MYC is a notable abnormality in this group and also correlates with poorer clinical outcomes. Metabolic reprogramming is a well-recognised hallmark of cancer. Alterations in bioenergetics provide supportive mechanisms for aberrant growth and survival. The role of MYC as a transcription factor regulating multiple cellular pathways, in particular metabolism, offers opportunities to exploit metabolic dependencies upon which MYC amplified MBs rely.

To better understand the role of MYC in MB metabolism, we engineered three independent MYC amplified Group 3 MB cell lines (D425Med, D283Med, HDMB03), to each harbour doxycycline-inducible anti-MYC shRNAs (two independent species) or a non-silencing shRNA control. We utilised $^1$H high resolution magic angle spectroscopy (HRMAS) and stable isotope resolved metabolomics to assess changes in intracellular metabolites and pathway dynamics when MYC expression was modulated. We probed whether these changes revealed metabolic vulnerabilities in MYC amplified cells through pharmacological manipulation.

Downregulation of MYC resulted in a marked reduction in proliferation and cell cycle progression analogous to MYC-dependent cancer phenotypes. Metabolic profiling revealed changes in metabolites involved in energy metabolism and amino acid metabolism across the three MYC amplified cell lines. Clustering and integrative analysis revealed cell line specific and common metabolite changes. Notably, glycine was found to be accumulated following MYC knockdown (KD) in all three cell lines. 13-C-glucose labelling showed a reduction in serine and glycine synthesis following MYC knockdown. Expression of PHGDH, the rate limiting enzyme in de novo serine synthesis, correlates with MYC amplification and poorer survival outcomes. Furthermore, MYC expressing cells showed greater sensitivity to pharmacological inhibition of PHGDH compared to MYC KD (Group 3) and SHH subgroup cell lines. Together, these findings provide insights into MYC-dependent metabolic alterations and reveals de novo serine/glycine pathway as a novel and clinically relevant therapeutic target in Group 3 MYC amplified MB.
Poster abstracts

Senolytics: a Potential Novel Therapeutic Concept in Paediatric Low Grade Gliomas

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Pilocytic astrocytoma (PA), WHO grade I, is the most common paediatric brain tumour, characterized by constitutive activation of the MAPK pathway. PA tumours present a slow growth, without tendency to progress to high-grade malignancies; however, a high-risk group of patients for whom a total resection is not feasible requires additional therapy. The typical proliferative index of a PA tumour, measured by Ki-67 staining, is 1-2% whereas a large part of the tumour is Ki-67 negative and expresses markers of oncogene-induced senescence (OIS) such as SA-β-Gal positivity and up-regulation of the cell cycle inhibitors p16INK4a and p21Cip1. Conventional treatments (i.e. chemotherapy and radiotherapy) and new molecularly targeted therapies (e.g. MAPK and PI3K/mTOR pathway inhibitors) tend to target only proliferative cells. Here, we discuss the opportunities to combine these therapies with new compounds targeting the senescent cells, referred to as senolytics.

In this study, we have used two different PA models: (1) in vitro culture of human PA tumours (i.e. explant cultures); and (2) the DKFZ-BT66 PA human cell line (BT66), carrying the oncogenic driver KIAA1549:BRAF-fusion. This cell line expresses the SV40 large T antigen in a doxycycline (dox) dependent manner resulting in the reversible inhibition of two major senescence-maintenance pathways, p16INK4a and p21Cip1. Conventional treatments (i.e. chemotherapy and radiotherapy) and new molecularly targeted therapies (e.g. MAPK and PI3K/mTOR pathway inhibitors) tend to target only proliferative cells. Here, we discuss the opportunities to combine these therapies with new compounds targeting the senescent cells, referred to as senolytics.

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Our research demonstrates that BT66 senescent cells exhibit an increased sensitivity to senolytic compounds, such as Navitoclax, a clinically approved BCL2/XL inhibitor, relative to proliferative controls (IC50 = 40nM for senescent and 300nM for proliferative BT66 cells). Additionally, we reveal a strong synergistic effect on BT66 cells when combining conventional chemotherapy with senolytics, resulting a significant decrease of IC50 from 300nM to 6nM. We show that the mechanism underlying this synergistic effect involves increased apoptosis mostly due to the inhibition of BCL-XL. Moreover, we show that combination therapy is also effective in human PA explant cultures and result in elevated apoptosis.

Together, our research provides a strong rationale supporting the combined use of senolytics and current conventional therapies against human PA, a concept that needs to be further validated in preclinical and clinical research.

References
Poster abstracts

Replication inhibitors as single agents and combination agents in Neuroblastoma

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The effects of MYCN expression in Neuroblastoma (NB) on the DNA damage response and directly on DNA replication fork dynamics were analysed both in the presence and absence of the ATR inhibition. MYCN amplification or MYCN expression resulted in significantly increased cell death in response to several ATR inhibitors compared to the response seen in non-expressing NB cells. ATR inhibition also sensitized to the PARP inhibitor olaparib regardless of MYCN status. MYCN expression resulted in slowed replication fork speed, increased replication fork stalling and increased origin firing an effect that was amplified by PARP inhibition. Further ATR inhibition in MYCN expressing cells or following PARP inhibition increased DNA damage to a level where could not be repaired. Our working hypothesis is that under MYCN induced (or PARP inhibitor induced) replication stress ATR becomes essential, thus inhibition of ATR is lethal in these conditions. In addition ATR inhibition also sensitized NB cells to classical chemotherapeutics that cause replication stress; this effect was greater in MYCN expressing cells, suggest that both single agent or combination therapy have potential for clinical translation.
Pediatric Pan-CNS Tumor Analysis of Immune-cell Infiltration Identifies Correlates of Antitumor Immunity

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Immune-therapy is an attractive alternative therapeutic approach for targeting CNS tumors. Tumor Immune Microenvironment (TIME) is a key factor likely to dictate approach to immune-therapy and predict patient response. Here, we describe the TIME of >6000 primarily pediatric CNS tumors using a deconvolution approach based on the methylCIBERSORT algorithm. We produced and validated a custom reference signature defining 11 non-cancer cell types and used this to estimate relative proportions of infiltration in a pan-CNS tumor cohort spanning 80 CNS tumor subtypes. We identified infiltrating cell profiles which could be grouped into three broad immune clusters associated with particular subgroups of CNS tumors and demonstrating either relatively increased monocytes, CD4+ T-cells or CD8+ T-cells. We further applied our analysis to a cohort of medulloblastomas (n=2325), malignant rhabdoid tumors (MRT, n=229) and pediatric high-grade gliomas (pHGG, n=401). We show TIME in each tumor type is associated with specific molecular subgroups/subtypes and identify tumor-specific immune clusters with phenotypic characteristics relevant to immunotherapy response (i.e. Cytolytic score, PD1, PDL1, CD276 expression). In medulloblastoma, we further show immune cell infiltration estimates (e.g. monocytes in Group 4 patients) add significant independent prognostic value beyond existing molecular subgrouping schemes. In MRT, we show monocyte and Natural Killer (NK) cell infiltration is related to tumor location and that infiltration of B-cell and CD8+ T-cells is significantly related to outcome. In pHGG, we show subgroup WT-A is particularly strongly infiltrated by monocytes and that estimates of immune cell infiltration are significantly prognostic even within molecular subtypes (i.e. WT-A, WT-C and GBM-G34). Our analysis provides a widescale description of TIME within pediatric CNS tumors and a first indication of the potential future therapeutic and prognostic possibilities of immuno-methylomic profiling in pediatric CNS tumor patients that may ultimately inform approach to immune-therapy.

Keywords
Pediatric; Brain Tumors; Immune Cell Infiltration; Immunotherapy
Targeting the intra-tumour heterogeneity in paediatric ependymoma: an integrated omics study towards patient-tailored therapy

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Ependymoma (EPN) is the second most malignant paediatric brain tumour, with a five year survival rate at relapse of only 25%. First line therapy consists of maximal resection of the tumour and concomitant radiotherapy, however prognosis remains poor with the role of chemotherapy being ill defined, emphasizing the urgent need of novel therapies. Multi-omic profiles provide rich information about the tumor and are likely to reveal dysregulated pathways that may be predictive of patient specific biomarkers. Given the close association between gene expression and metabolism, it is of scientific interest to determine whether the dysregulated gene expression in EPN correlates with aberrant metabolic pathways.

In this context, we present a multi-omics integration of the expression data at transcriptomic and metabolomic levels, comparing two distinct subgroups of paediatric ependymoma. We homogenised surgically resected ependymoma tissue from two epigenetic subgroups, posterior fossa A (n=10) and supratentorial RELA fusion (n=5), and extracted polar metabolites, lipids and RNA. Using liquid chromatography-mass spectrometry (LC-MS) we have identified 115 metabolites and 430 lipids significantly altered between the two subgroups, with 53 metabolites being distinguished between grade II and III of posterior fossa-A tumours. RNAseq identified 1580 upregulated genes between the two subgroups with 1341 genes mapping onto metabolic pathways. Furthermore, 71 genes have been identified to be dysregulated between posterior fossa-A grade II and III tumours. Integration of genes and metabolites using pathway based network analysis uncovered 59 dysregulated pathways between the two subgroups, with large numbers of interactions occurring on the purine and tyrosine metabolic pathway.

Although gene expression studies have been conducted before to investigate the heterogeneity in EPN, this is the first instance where multi-omic data integration and metabolic heterogeneity has been investigated for this tumour. In addition, data integration will be conducted on multi-regions collected from 8 paediatric ependymoma patients to better understand the intra-tumour heterogeneity of gene-metabolite correlations.
**Modelling the clonal evolution of sporadic Burkitt lymphoma**

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**Introduction**

Sporadic Burkitt lymphoma (sBL) is a highly proliferative germinal centre (GC) derived B cell malignancy driven by Ig-MYC translocations and tonic-B cell receptor signaling. Clinically, children affected with this disease have an excellent prognosis, although for the small proportion in which the disease relapses, therapy resistance is common and outcomes are very poor. However, the drivers of this dramatic behavioural switch are unknown.

This project used patient samples and their derived xenografts (PDX) to investigate two potentially critical factors in development of therapy resistance, intratumoural heterogeneity and clonal evolution, in untreated conditions. By developing a detailed understanding of how models behave in the absence of therapy, we are able to design more effective and efficient experiments to study the impact of treatment on clonal evolution and the development of therapy resistance.

**Methods**

Viable, cryopreserved pleural fluid samples (n=4) were engrafted into immunocompromised NSG mice by intraperitoneal injection. Exome and RNA sequencing of original patient samples underpinned RNAseq and Sanger sequencing studies used to track clonal evolution through multiple generations of untreated PDX models.

**Results**

RNA sequencing data from PDX models expressed a metagene consistent with that of published BL cases (Dave et al., 2006). Mutations within 15 candidate genes, previously described as being recurrently mutated in sBL (Love et al., 2012; Schmitz et al., 2012), were identified in exome sequencing of patient material. These mutations were confirmed in RNAseq data from patients and one or two generations of PDX. Some mutations, including in MYC and ARID1A were stable across multiple generations of mice. However, others amongst the candidate driver genes showed marked variation. Multiple, mutually exclusive sub-clonal mutations in ID3 were identified. In two cases, the recurrent L64F mutation achieved clonal dominance, with evidence of ongoing mutational evolution required to achieve this. In contrast, two subclonal mutations of TP53 offered no survival advantage in these untreated models.

**Discussion**

Patient-derived xenograft models are frequently used for pre-clinical studies of potential novel therapies. Here we demonstrate an additional value of such models in studying natural intra-tumoural heterogeneity and evolution, two features critical to the development of therapy resistance but not recreated by in vitro cell models. These studies will underpin future investigation of treatment resistance, a poorly understood feature of this disease.

**References**


Germline Copy Number Variant risks of developing Medulloblastoma: A whole genome sequencing approach

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Introduction
In medulloblastoma, epigenetic and transcriptional alterations have been identified alongside a variety of somatic single nucleotide variants (sSNVs), and somatic copy number variants (sCNVs), which are associated with specific medulloblastoma disease subgroups. So far, most sSNVs and sCNVs have been called from exome sequencing data, and all used paired analysis methods (e.g. Parsons et al. 2011, Morrissy et al. 2016). Whole genome sequencing (WGS) produces relatively stable read depths whilst reducing the technical variation associated with exome sequencing, as well as allowing variants to be called across the breadth of the genome. One of the major advantages of WGS data is that CNVs can be called on individual samples in an unpaired manner producing absolute CNVs (aCNVs), thereby avoiding ploidy and batch specific biases that can influence ratios and cut-offs. This work aimed to determine whether un-paired analysis methods could reduce technical noise resulting from different read depths in samples from different sequencing runs, avoiding batch effects and produce copy number calls for all patient samples.

Methods
101 WGS bam files (Morrissy et al. 2016) were downloaded from ICGC, converted to shuffled fastq files using Samtools 1.2, realigned with the b37 decoy genome, base quality scores recalibrated and variants called using GATK 3.7. Copy numbers were called from recalibrated bam files using paired (sCNV) and un-paired methods (aCNV) with control-FREEC 11.3 with the same settings as Morrissy et al. (2016).

Results
Using unpaired analysis methods, we catalogued multiple aCNVs of various sizes and magnitudes in both germline and tumor samples. Unusually we found a germline WGS sample that contained aCNVs implicated in the development of Grp4 medulloblastoma with trisomy 17q and trisomy 4 called in germline and recurrent (Grp4) samples, but neither were called in the paired analysis. A WNT and a SHH showed erratic germline copy number changes alongside non-synonymous variants in TP53 and interacting proteins. In medulloblastomas; the WNT isochromosome 6 and 11q were identified using paired and unpaired analysis, while the SHH showed multiple consistent broad copy number gains and losses with paired and un-paired analysis. Since no diagnostic samples were available for these patients, it is possible germline samples were taken following radiotherapy, therefore the noise observed in the germline WNT and SHH could be treatment related DNA damage, especially considering their TP53 pathways may have been compromised. Overall using paired analysis methods fewer arm level copy number change events were identified in diagnostic and recurrent samples, whereas unpaired analysis on these samples removed technical noise identifying more arm level events; some of the CNVs originated from germline samples and went undetected using paired methods.

Conclusions
Unpaired methods improved the sensitivity and reliability of CNV calling using WGS data identifying previously overlooked germline aCNVs and SNVs that may contribute to the development of medulloblastoma and risk of relapse. The results indicate the need to stratify pre and post treatment germline samples, the analysis will be repeated using a panel of normals derived from pre-treatment germlines identified as copy number neutral in an un-paired assessment, to filter and validate those which may be of biological significance.

Reference
Poster abstracts

Single-cell sequencing dissects intra-tumoural heterogeneity in childhood medulloblastoma

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Background
Medulloblastoma is one of the most aggressive childhood brain cancers. Recent studies involving immunohistochemistry and iFISH show that medulloblastomas possess high levels of intra-tumoral heterogeneity for key biomarkers (e.g. MYC/MYCN, β-catenin, TP53, etc.). This is proposed to be a major cause of treatment failure. Intra-tumoral heterogeneity is not reflected by conventional bulk tumour sequencing and needs studying on a single-cell level. We hypothesise that the identification of biologically and clinically significant intra-tumoural heterogeneity will improve the understanding of medulloblastoma progression and will promote the development of novel therapeutic strategies.

Methods
Methodologies for frozen tissue processing and single-cell isolation were developed. DNA was extracted from 756 single medulloblastoma cells (12 patients, 2 tumour regions per patient) and subjected to low-pass whole genome sequencing. Tumour purity was assessed by THetA software. Intra- and inter-tumoral heterogeneity patterns of cellular chromosomal copy number variations were identified using hierarchical clustering approaches.

Results
750/756 single-cell DNA samples passed data quality control, emphasizing the reproducibility of the developed methodologies. Copy number changes identified in single-cells matched the expected changes in the tumour bulk. Intra- and inter-tumoral heterogeneity patterns of copy number variation were dissected. Driver clones were identified in all single cells. Sub-clones were detected in the smaller proportions of cells (e.g. a gain of chromosome 8 in ~30% of cells of the WNT subgroup patient).

Conclusion
Intra-tumoral heterogeneity is a general feature of medulloblastoma. However, different tumours vary in patterns and sub-clones. Dissected genomic heterogeneity patterns could provide a basis for better biomarker selection and medulloblastoma diagnostics (e.g. patient subdivision into subgroups), and enhance the development of novel targeted therapies.
The Northern Region Young Persons’ Malignant Disease Registry (NRYPMDR) is hosted by the Institute of Health & Society at Newcastle University. The NRYPMDR has been in existence since 1968 and is the second oldest specialist registry of its kind in the world. All patients living in North East England and North Cumbria who are diagnosed with a primary cancer aged less than 25 years are eligible for inclusion. Furthermore, all patients living elsewhere in Great Britain who have received treatment for cancer in one of the hospitals operating within the registry catchment area are also included. As well as providing an excellent resource for epidemiological studies, all patients are followed up long term via their treating hospital or registered GP practice meaning it can also function to monitor co-morbidities and survival.

The information landscape has changed dramatically over the last 5 years and therefore the registry has been radically overhauled to ensure its administration is up to date with national information governance legislation, particularly General Data Protection Regulation (GDPR) compliance and the Data Protection Act 2018. Evidence of patient and public involvement (PPI) was required as part of the application for ethical and Confidentiality Advisory Group (CAG) Approvals.

A PPI survey was carried out to gather opinions with regards to the appropriateness of the registry operating procedures. The first four questions focused on gathering opinions about the registry methodologies and their suitability: (1) operating procedures in general; (2) data security methods; (3) recruitment methods; (4) attitudes towards data retention, particularly after a patient dies. The final five questions created a demographic profile of the survey population.

203 respondents took part in the survey: 104 people on a paediatric cancer ward filled in the paper questionnaires with another 10 respondents recruited through a Young Patients Advisory Group meeting. A further 99 respondents completed an online version of the questionnaire. Respondents were categorized as a “person living with cancer” (PLWC) (N=102) or “general public” (N=101). The ethnic make-up of the surveyed population was as expected given the survey was run in the North East of England. That is, the majority (87.7%) of the respondents categorised themselves as White British. There was sufficient representation from all age groups (N= (<=25 years), N= (25-49 years), N= (>50 years)). There were more females (N = ) than males (N= ) primarily because over half of the respondents were recruited from the ward visits and mothers are more likely to be caring for children in a hospital setting.

The vast majority of the respondents believed the registry was run appropriately. The largest number that disagreed were from the general public population in response to question 3 (N=5): recruitment methods. 96% agreed that automatic registration after a diagnosis of primary cancer was an appropriate way to recruit patients. However, the free text responses also showed there were some people who did not understand the question or had not read the information leaflet and privacy notice that explained the reason behind automatic registration.

In conclusion, as long as a registry adopts a transparent approach and ensures patients are informed about the registry operating procedures and its opt out procedures, automatic registration is the most ethical approach to recruitment. The resulting database will be timelier and more cost effective than one with an opt-in recruitment strategy and therefore extremely useful in genomic medicine through linkage with datasets from for example, the UK Biobank.
Poster abstracts

An Excellent Neurological Prognosis Following Severe Nelarabine-related Neurotoxicity


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Introduction

Nelarabine is a guanosine nucleoside analogue licensed in 2005 by the European Medicines Agency for the treatment of refractory or relapsed T-cell lymphoblastic leukaemia and T-cell lymphoblastic lymphoma. Kuhlen et al 2017 demonstrated nearly 70% response rate, and almost 60% remission rate in treated individuals¹. However peripheral and central neurotoxicity, sometimes profound or even lethal, have made its use contentious. Berg et al 2005 claimed up to 18% patients experiencing ≥grade 3 neurological adverse events². The side effects in the paediatric population, nor the anticipated recovery, are not widely described.

Case Study

We present an 11-year old boy with progressive lymphoblastic lymphoma, unresponsive to first line therapies, treated with intensified consolidation Nelarabine. After 3 days he went on to develop lower limb weakness, personality changes and hallucinations as well as a sensory level. This progressed over 4 weeks to a severe ascending neuropathy with encephalopathy. MRI showed lumbar-sacral nerve root thickening and abnormal EEG. He continued to deteriorate until eventually ventilated on PICU due to respiratory embarrassment and unsafe airway.

The boy was treated with IV methylprednisolone as well as 5 plasma exchanges, was intubated for 5 days in total before being successfully extubated and weaned down to CPAP and eventually into air. He demonstrated a slow reversed recovery of motor function with the aid of physiotherapy and occupational therapy, until he was able to walk independently again.

Remarks

We present this boy as an interesting case of profound nelarabine-related neurotoxicity, where the current literature regarding young people is relatively sparse. His example shows how intensive support and multidisciplinary input has yielded an excellent prognosis in the face of life-threatening adverse chemotherapeutic effects.
Poster abstracts

Childhood Craniopharyngioma Research Consortium (CCRC): From developmental biology to novel therapies

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Adamantinomatous Craniopharyngiomas (ACPs) are clinically challenging sellar region tumours, frequently resulting in poor long-term quality of life, visual impairment, endocrinopathy and hypothalamic damage. Current treatments are predominantly surgery and radiotherapy, but these are non-curative and associated with significant morbidity and tumour recurrence. The clinical course is often chronic and variable and there is an urgent need to understand the biology underlying ACP and to develop novel therapies.

In 2015, Children with Cancer funded the Childhood Craniopharyngioma Research Consortium, bringing together experts from across Europe, with the aims of improving understanding of tumour pathogenesis and revealing potential novel targeted therapies against ACP.

Over the last four years, work through collaboration within and outside the consortium has provided important insights into tumour biology and identified targetable pathways, which are currently being tested in preclinical and clinical trials. Some of the achievements have been:

- Revealing the genomic and transcriptomic landscape within specific tumour cell compartments in ACP (Apps et al., 2018).
- Providing a molecular rationale explaining the close relationship between ACP and the developing tooth, including the molecular equivalence between nucleo-cytoplasmic accumulating β-catenin cell clusters (‘clusters’) and the enamel knot (Apps et al., 2018).
- By combining research on human tumours and ACP mouse models, demonstrating that cluster cells in both species are senescent and activate the release of soluble factors that create a tumourigenic microenvironment fueling tumour growth and invasion (Gonzalez-Meljem et al., 2017).
- Identifying the activation of multiple gene pathways in human and mouse ACP, including: the MAPK, Sonic Hedgehog (SHH), Ectodysplasin, TGFβ and BMP pathways.
- Characterizing the inflammatory environment within solid and cystic ACP tumour components, highlighting the activation of inflammasome mediated inflammation (Apps et al., 2018).
- Testing of SHH pathway inhibitors in human ACP explant cultures, genetically engineered ACP mouse models and human ACP patient-derived xenografts revealing SHH pathway inhibition if pro-tumourigeneic (Carreno et al., 2019).
- Demonstrating that MAPK pathway in human and murine ACP explants results in reduced proliferation and increased apoptosis, suggesting a therapeutic benefit (Apps et al., 2018).
- This has led to the development and design of a trans-Atlantic clinical trial to test the efficacy of IL6 inhibition and/or MAPK inhibition by using clinically approved drugs in paediatrics.
These results have given promise of novel targeted therapies for patients, particularly through targeting the MAPK pathway and inflammation. The results, particularly with regard to the SHH pathway have highlighted the need for appropriate pre-clinical testing prior to use in humans. Additionally, innovative therapies such as using senolytic drugs (i.e. drugs killing specifically senescent cells) are being tested preclinically. Building on the success of the Childhood Craniopharyngioma Research Consortium, we are working with our international partners to initiate new clinical trials.

References
Thank you for helping us find ways to reduce the burden of cancer on parents and children.