CCLG/TOM GRAHAME TRUST GRANT

REPORT, FEBRUARY 2016.

TITLE:
Biomarker and target discovery for the improved therapy of high-risk medulloblastoma.

FUNDING PERIOD:
1st September 2013 for three years.

GRANTHOLDERS:
Principal Investigator:
Professor Steve Clifford,
Northern Institute for Cancer Research (NICR),
Newcastle University,
Sir James Spence Institute Level 5,
Royal Victoria Infirmary,
Newcastle upon Tyne,
NE1 4LP, U.K.

Co-investigators: Professor Simon Bailey, Newcastle University; Professor David Ellison, Memphis, USA; Dr. Tom Jacques, UCL Institute of Child Health; Dr. Antony Michalski, Great Ormond Street Hospital; Dr. Andrew Peet, University of Birmingham; Professor Barry Pizer, Alder Hey Children’s Hospital, Liverpool; Dr. Keith Robson, Queen Elizabeth Hospital, Nottingham; Professor Roger Taylor, South West Wales Cancer Centre; Dr. Stephen Wharton; University of Sheffield; Dr. Dan Williamson, Newcastle University.

And on behalf of the UK Embryonal Tumour group (ex- CCLG CNS Tumours Division).

PROJECT AIMS AND OBJECTIVES:
Aim: To discover biomarkers which can direct individualised therapy for high-risk medulloblastoma.
Hypothesis: Biomarkers required for the improved management of high-risk medulloblastoma are unique and distinct from standard-risk medulloblastoma.
Objectives: To undertake the largest and most comprehensive biological investigations of high-risk medulloblastoma patients (infants (n>160) and older children (n>200)) to date, using cohorts based on recent clinical trials.

PROGRESS IN YEAR THREE:
The project has built upon the foundations laid in years one and two and is progressing well in two high risk cohorts 1) infants 2) older children, as outlined below:

1) Infant medulloblastoma

Data analysis: Data analysis in the past year has focussed on this group. We assembled 208 infant medulloblastomas with full central clinical and pathological review, subgroup
assignation, and critical biological features in the largest collected series of its kind to date. We have completed the first phase of data analysis, and are preparing a manuscript for publication in which we characterise the molecular pathology of infant medulloblastoma with particular consideration to the consensus molecular subgroups and outcomes, to inform contemporary treatments, risk-stratification and clinical trials (1). We show differences in the distribution of in critical medulloblastoma features in infant compared to non-infant medulloblastoma cohorts, and offer novel, sub-group specific, survival models that will be essential to direct improved risk-stratified therapies, and novel approaches for these very high-risk patients.

This work has been submitted for presentation at the bi-annual ISPNO meeting, the premier worldwide meeting for paediatric neuro-oncology (June 2016, Liverpool).

Research summary:

The molecular pathology of infant medulloblastoma (iMB) has not been systematically characterised, particularly in relation to the consensus molecular subgroups or outcomes, to inform contemporary treatments, risk-stratification and clinical trials. We assembled 208 iMBs (0-5yrs) with full central clinical and pathological review, subgroup assignation, and comprehensive profiling of copy-number and mutational features. iMB represented a three-subgroup disease with MB\textsubscript{SHH} and MB\textsubscript{Grp3} predominant (41% each; MB\textsubscript{Grp4}, 17%). MB\textsubscript{SHH} significantly associated with DN/MBEN pathology (72% (50/69); \(p=6.8\times10^{-19}\)), but also contained classic (CLA; \(n=15\)) and LCA (\(n=4\)) tumours (28%; 19/69) throughout the age-range. Multivariate survival analysis identified sub-total resection (STR; HR 6.3, \(p=3.1\times10^{-5}\)) and DN/MBEN (HR 0.1, \(p=0.004\)) as independent prognostic factors, however metastatic (M+) disease and other established biological features were not associated with outcome. A novel MB\textsubscript{SHH} survival model defined CLA/LCA and/or STR tumours as very high-risk (44% (27/61); 10yr OS, 24%), with 18.8-fold relative-risk compared to favourable-risk totally-resected DN/MBEN disease (56% (34/61); 10yr OS, 93%). MB\textsubscript{Grp3} was strongly associated with LCA (23%, 14/62) and \(MYC\) amplification (19%, 12/62). Presence of either feature defined a very high-risk group (27% (18/62); 10yr OS, 23%), with common rapid progression on current therapies and an 11.7-fold relative-risk than remaining MB\textsubscript{Grp3} tumours (73% (45/62; 10yr OS, 74%; standard-risk). Only MB\textsubscript{SHH} DN/MBEN tumours showed potential of rescue at relapse following initial therapy (56% survival post-relapse); other relapses were almost universally fatal. Combined diagnostic assessment of iMB subgroup, pathology and molecular biomarkers will be essential to direct improved risk-stratified therapies, and novel approaches for very high-risk patients.

2) Non-infant medulloblastoma

In year 3, we further expanded our non-infant high risk cohort (presently \(n>300\)), which are currently undergoing full review in terms of clinical, pathological and biological data.

Data collection: DNA has been extracted from all high-risk medulloblastoma biopsies with available material. The analysis of DNA methylation patterns in these samples by Illumina 450K methylation (450,000 CpG residues; recently replaced by the MethylationEpic array with >850,000 CpG residues) has now been completed successfully for tumours from 240
non-infant high risk patients. RNAseq data, to allow the analysis of gene expression, single nucleotide variation and fusion gene detection has been collected for 128 of these cases. We have developed methods to assess medulloblastoma subgroup status using these data (2) and applied these to determine subgroup status (WNT, SHH, Group 3 or Group 4) in our cohort. Copy number variation data from >200 of these tumours has been collected using the genome-wide Human SNP Array 6.0 and we have further developed methods to derive the copy number status of key loci using the Illumina 450K data. In addition, the status of established (e.g. MYC and MYCN amplification, TP53 pathway and mutation, and chromosome 17) and newly identified (e.g. TERT(3)) medulloblastoma biomarkers have been assessed in our cohorts using specific assays. The cohort will be further expanded to include 80 risk cases from the recent French trial in high-risk medulloblastoma (Drs. Christelle Dufour and Jacques Grill, Paris, collaborators), which will be analysed as previously described. Finally, we are developing methods for next-generation sequence analysis of these cohorts, to complement other analyses.

Data analysis: Once data collection is complete, we will undertake an equivalent analysis to the infant cohort in the non-infant high-risk patients. A detailed analysis of the clinical, pathological and biological features of non-infant high risk disease and initial reporting of this wave of data is anticipated in the coming year.

ANTICIPATED CLINICAL IMPACT:
Rather than being a single entity, medulloblastoma comprises four distinct biological subgroups, each with its own methylation signature, and this biomarker is key in current prognostication models. The gold standard assay for methylomic-subgrouping is the 450K methylation array; however, this is not appropriate for routine clinical use. We are about to submit for publication a novel subgrouping assay for medulloblastoma that is rapid, inexpensive and robust and therefore suited to clinical use (4). We hope this will deliver a significant patient benefit in that it allows for better access to molecular subgrouping in a timely fashion, a feature that is built into the next generation of medulloblastoma clinical trials.

Biomarkers discovered over the course of this grant will support the development of new treatment strategies, including molecular disease-risk stratification and delivery of targeted therapeutics. Findings will be incorporated into planning future medulloblastoma trials, through our leading roles in National and European trials, and our ongoing biological research programme, together aimed at delivering improved outcomes.

In particular, our findings in infant medulloblastoma are being used to plan biological and treatment stratification approaches for the forthcoming pan-European clinical trial planned for the infant disease, which we are playing a leading role in developing as part of the SIOP-Europe PNET group (Clifford, Biology lead; Bailey, U.K. CI). Additionally, we will play a leading role in the non-infant high-risk European medulloblastoma trial currently under development (Bailey, trial CI; Clifford, Biology lead), which our data will similarly inform.
References


