CCLG/TOM GRAHAME TRUST GRANT

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

FUNDING PERIOD:
1st September 2013 for three years.

GRANTHOLDERS:
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And on behalf of the UK PNET 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 TWO:
The project has built upon the foundations laid in year one and is progressing well, as outlined below:

Cohorts:
Infants: In year one the project focussed on instigating investigations in infant medulloblastoma (i.e. <5 years at diagnosis). We have extended and completed sample collection in this cohort to increase its total size from 180 to 225 frozen and/or FFPE tumour biopsies, all with associated clinical data. Initial analysis revealed the particular importance of tumour pathology with regard to prognostication in infant medulloblastoma; as such the

146 cases with available material have undergone a further central pathology review this year, in anticipation of a final data analysis, which is now underway.
Non-infants: In a similar manner, we undertook in year two an equivalent preparation of a non-infant high-risk patient cohort. These number approximately 250 and will be equivalently fully reviewed in terms of clinical, pathological and biological data in year 3.

Data collection: DNA has been extracted from all infant and high-risk medulloblastoma biopsies with available material. The analysis of DNA methylation patterns in these samples by Illumina 450K methylation (450,000 CpG residues) has now been completed successfully for tumours from 189 infant and 210 non-infant high risk patients. In year 2, we expanded our biological data collection to include the next generation sequencing technology, RNAseq. This allows the analysis of gene expression, single nucleotide variation and fusion gene detection and, to date, 78 and 128 RNA samples respectively from the infant and non-infant high risk cohorts have been sequenced. We have developed methods to assess medulloblastoma subgroup status using these data [1] 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*[2]) medulloblastoma biomarkers have been assessed in our cohorts using specific assays.

Data analysis: This project has developed the largest collected series of infant and high risk medulloblastomas to date for biological analysis. We are currently completing the first phase of data analysis in the infant cohort, in preparation for a publication in which we identify the critical biological features which could be used to improve our ability to stratify treatment and predict risk. A detailed analysis of the clinical, pathological and biological features of high risk disease is underway and we anticipate the initial reporting of this wave of data analysis in the coming year. Given that in year 2 we generated multidimensional datasets on a range of platforms, the challenge going forward for our experienced and expanding team of bioinformaticians and statisticians is to develop innovative approaches to elucidate the features of infant and high risk disease highlighted by these methods that would otherwise be obscured, and this work is ongoing.

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 have recently developed a subgrouping assay for medulloblastoma that is rapid, inexpensive and robust and therefore suited to clinical use. 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.

REFERENCES:

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Janet C. Lindsey, Ed. C. Schwalbe, Sandeep Potluri, Simon Bailey, Daniel Williamson, Steven C. Clifford (2014). TERT promoter mutation and aberrant hypermethylation are associated with elevated expression in medulloblastoma and characterise the majority of non□infant SHH subgroup tumours. Acta Neuropathol (2014) 127:307–309.