



## Molecular subgrouping of medulloblastoma:

### Bridging the divide between research and the clinic using low-cost DNA methylomics

NewGene, working in collaboration with researchers at Newcastle University and Agena Biosciences, have contributed to development and validation of a new disease subgrouping assay utilising the MassARRAY® platform. This has a significantly faster turn-around-time, reduced sample quantity requirement and lower cost than the previously used test system.

The assay is now in routine clinical use, improving the care delivered to patients across the UK. As the only Agena certified service provider in the country, NewGene is uniquely placed to provide this service.

Effective and efficient collaborative clinical assay and diagnostic service development can be challenging.

In this project NewGene have facilitated effective migration of a multi-analyte diagnostic and prognostic biomarker assay from a high-complexity, low throughput research technology platform to a lower-complexity, high-throughput system better suited to routine use. Research initiated by an academic group has been successfully translated into a clinical diagnostic service, which is now available to healthcare providers nationwide.

NewGene is well placed to carry out similar work in a cost-effective and timely manner which is responsive to the needs of research projects, such as development of a new clinical diagnostic assay service based upon a known biomarker or panel of biomarkers identified by a partner. Such services could, for example, be integrated into routine clinical practice, or developed to support a CTIMP, based upon pre-clinical studies.

#### Project background

Medulloblastoma is the most frequently occurring malignant brain tumour in children. It has an approximate incidence of 1.5 cases per million, rising to 6 per million in children aged 1 to 9 years. It also occurs in adults, although in this group it is around ten times less common<sup>1</sup>.

Cases of the disease can be placed into one of four distinct subgroups on the basis of characteristic patterns of gene expression which can be determined by promoter methylation state analysis; WNT, SHH, Group 3 and Group 4<sup>2</sup>.

Each group has distinct molecular, clinical and pathological features and classification is an important step in determining the most appropriate course of treatment and follow-up for individual patients<sup>3</sup>.

Historically, subgroup classification has been carried out using techniques such as DNA microarrays. Whilst suitable for use in research projects these systems are expensive, require comparatively large amounts of high-quality sample material and are impractical to use in a diagnostic laboratory processing large volumes of samples, due to the amount of hands-on operator time needed for each analysis.

To overcome these limitations this project was instigated, with the objective of developing a faster and lower cost subgroup assay, capable of being used routinely in a high-throughput diagnostic laboratory to analyse clinical samples.

### Development of a new genetic testing service

A collaborative research team consisting of Professor Steve Clifford and his group at Newcastle University, the Agena Biosciences MassARRAY® technical development team in Hamburg, Germany, and NewGene staff was established.

The Clifford group, internationally recognised as experts in medulloblastoma research, created the initial subgroup classification assay. A minimal, multiply-redundant, 19 locus methylation signature based upon Illumina Infinium® HumanMethylation450 BeadChip array DNA methylation data and subgroup calls from 225 medulloblastoma cases was identified and a cross-validated machine-learning classifier capable of assigning subgroup using these loci developed.

To replace microarray analysis, the Agena Biosciences technical development team then advised upon creation of an adapted iPlex® assay, utilising multiplexed PCR.

In order to determine methylation status at each of the 19 chosen loci, bisulfite treatment of DNA is used to induce methylation-dependent SNPs suitable for analysis.

Reaction products are then detected by MassARRAY® MALDI-TOF mass spectrometry.

As specialists in high-complexity genetic diagnostic services and the only Agena certified analysis service provider in the UK, NewGene have made this new iPlex® assay available as a routine clinical diagnostic service, analysing and reporting results for patient samples and supporting ongoing research.

Technical validation of the assay using molar ratios of methylated to unmethylated DNA has demonstrated close concordance between the known methylation ratios in these synthetic samples and the assay-determined methylation proportion estimates at all loci.

Clinical performance was assessed using DNA extracted from samples (n=101) of fresh-frozen, FFPE and cytospin (<30,000 nuclei) tumour material, representing all disease subgroups.

Subgroup assignments determined by MassARRAY® assay were compared to the established gold standard of Illumina Infinium®

HumanMethylation450 BeadChip array calls. Subgroup allocation by the two assays was 98% (99/101) concordant with a high degree of confidence and 100% concordant if a more relaxed confidence threshold was applied.

A publication containing complete details of this project is in preparation and is expected to be available in the near future.

### Project impact

Following conclusion of this research project, NewGene have added the medulloblastoma subtyping assay to the portfolio of diagnostic tests offered by the company. Currently around 5 samples are processed per month, with a mean turn-around-time from sample receipt to reporting of results of 8 working days.

Prior to development of the assay, molecular subtyping of disease samples was carried out on the basis of DNA methylation data produced by another supplier; this service had a 25% higher cost and substantially longer turn-around-time of 25 days. Furthermore, the MassARRAY® assay only requires 100 ng of sample DNA, a 2.5-fold lower amount than the previous platform.

Introduction of this new service has brought meaningful improvements to the standard of care which can be provided to medulloblastoma patients.

The length of time required to perform subgroup analysis has been more than halved and the quantity of biopsy-derived sample material needed reduced 2.5-fold, whilst the cost of the assay has also been substantially reduced.

### References

1. Smoll NR & Drummond KJ (2012) 'The incidence of medulloblastomas and primitive neuroectodermal tumours in adults and children.' *J. Clin. Neuroscience*. **19**: 1541-1544.
2. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC *et al.* (2012) 'Molecular subgroups of medulloblastoma: the current consensus.' *Acta. Neuropathol.* **123**: 465-472.
3. Ramaswamy V, Remke M, Bouffet E *et al.* (2016) 'Risk stratification of childhood medulloblastoma in the molecular era: the current consensus.' *Acta. Neuropathol.* **131**: 821-831.

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