Implementation of a Comprehensive Streamlined Next Generation Sequencing (NGS) Test for Glioma including Detection of the 1p/19q Codeletion

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AWMGS

Introduction

The All Wales Medical Genomics Service (AWMGS) has been delivering precision medicine molecular pathology services for cancer patients since 2008. There is increasing demand to molecularly characterise tumour samples for a wider range of cancer types, and for an expanding range of genes. To meet with the continuous increase in demand, and expand the current service provision, the AWMGS introduced a bespoke multi-gene Next Generation Sequencing (NGS) panel, covering a greater number of genes, relevant to a wider range of tumour types.

The most recent editions of the WHO Classification of Tumours of the Central Nervous System (CNS) (published in 2016 and recently updated in 2021) increasingly integrates molecular diagnostics alongside histology and immunohistochemistry. The NGS panel was designed to incorporate molecular markers (SNVs only) in IDH1, IDH2, H3F3A, BRAF, TERT, PTEN, CDKN2A, ATRX, TP53, and EGFR, as well as the ability to detect 1p/19q codeletion, to widen the repertoire of testing for CNS tumours. Thus tumour types such as 'Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted' can be identified in a single test, replacing multiple tests performed on the same sample, providing a more streamlined, cost – effective approach.

Aims

- Validate the specificity and sensitivity of the NGS test in detecting 1p/19q codeletions using 1p/19q codeletion positive/negative samples analysed previously using FISH
- Establish the reproducibility and repeatability of the test
- Determine the limitations of the NGS test for the identification of 1p/19q codeletion

Methods

The NGS panel was designed with probes to target polymorphic SNP's along the full length of chromosome arms 1p and 19q to assess loss of heterozygosity (LOH).

Region	No. Probes	Size Covering (bp)	Probe Spacing (Avg)
1p	228	45600	530Kb
19q	59	11800	527Kb

Table 1: Panel Design

FFPE-extracted DNA from glioma samples (n=66) previously characterised for 1p/19q status by fluorescent in situ hybridization (FISH) were used to validate the NGS panel's utility in detecting 1p/19q codeletion. The workflow consists of an optimised SeqCap EZ HyperCap (Roche) protocol and sequencing on the Nextseq550 (Illumina).

CNVKit software was used to infer copy number from NGS data. CNVKit generated $\log_2$ ratios by comparing the depths of on target and off target reads, in the sample of interest, and the pooled reference of all the other samples on the run. Results are presented as a final table of $\log_2$ copy ratios and displayed as scatter plots.

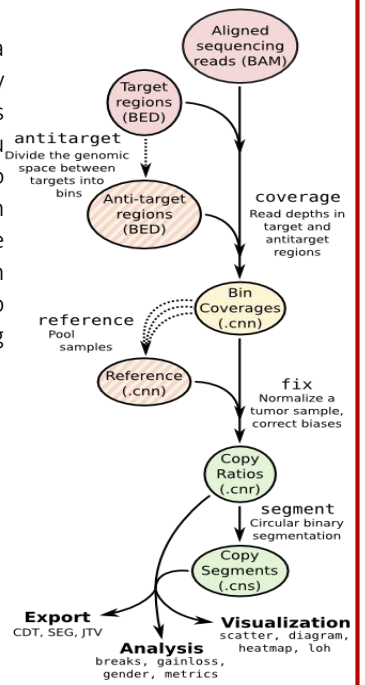

Figure 1: Copy number calling pipeline

Talevich, E., et al., (2014). CNVkit: Genome - wide copy number detection and visualization from targeted sequencing. *PLOS Computational Biology* 12(4).

Results

This study demonstrated a good correlation between the detection of a 1p/19q loss by FISH and by the NGS test. A $\log_2$ threshold of ≤-0.4 over the FISH probe regions of 1p and 19q was established for detecting 1p/19q codeletion, when the tumour content was >50%. The panel was able to detect evidence of losses of the whole chromosome arm and partial chromosome arm losses, and detect relative loss of 1p and/or 19q in samples showing evidence of polysomy.

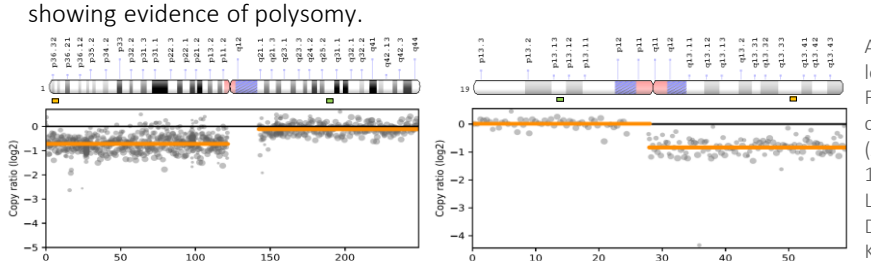

Figure 2: 1p/19q Codeletion Result. $\log_2$ values of -0.7 and -0.8 for 1p and 19q respectively

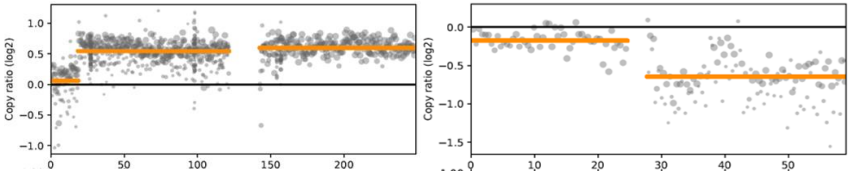

Figure 3: Impact of polysomy. A discordant result between FISH and NGS was observed. FISH was reported as consistent with 1p/19q codeletion (polysomy noted). NGS scatter plots revealed loss of the whole of 19q ($\log_2$ -0.6) as well as partial-loss of 1p (1p36 FISH probe region) relative to increased copy numbers of chromosome 1.

Conclusions

Validation of 1p19q co-deletion detection by NGS established the following:

Performance parameters: *Specificity = 100%, Sensitivity = 97.5%, Accuracy = 98.5%, Good inter- and intra-run reproducibility*

Limitations:

- For the pooled reference; sequencing run must contain minimum of 19 samples, comprising a maximum of 30% glioma samples in total
- As there were limited samples with ≤50% tumour available, a $\log_2$ threshold for such cases wasn't established (concordance between NGS and FISH observed but $\log_2$ -0.4 not met)
- In service, reflex FISH performed if $\log_2$ ratios suggest loss, but threshold -0.4 not met, or if complex patterns observed

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I have no conflicts of interest to declare

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