Next Generation Sequencing of Gliomas

Overview

Glioma are classified by location, cell type and grade, and represent the most common group of primary brain tumours in adults and children. Brain, other central nervous system and intracranial tumours are the 9th most common cancer in the UK, affecting slightly more females than males. Molecular analysis is used alongside histopathological evaluation to characterise tumours, assisting diagnosis as well as providing information about prognosis and treatment options. Molecular analysis supports the correct classification of tumour type and grading in line with World Health Organisation (WHO) guidance. [(1)].

AWGL will utilise the TSO500 NGS panel alongside 1p/19q FISH and MGMT methylation assay to provide a comprehensive glioma service.

Some important genes in glioma classification, prognosis and treatment response are:

IDH1* and *IDH2 genes encode dehydrogenase enzymes that are involved in cellular glucose metabolism and oxidative damage control. Variants in these genes are seen in most low grade gliomas and secondary high grade gliomas, and are associated with an improved prognosis compared to gliomas with non-mutated IDH genes. Oligodendroglioma is now classified as a glioma with either an *IDH1* or an *IDH2* variant and 1p/19q codeletion.[(2,3,4)]

TERT gene encodes the catalytic subunit of telomerase, an enzyme complex that regulates telomere length. *TERT* promoter variants have primarily been identified in adults, with highest frequencies in oligodendroglioma, primary glioblastoma, and medulloblastoma[(5)].

BRAF variants occur in about 3% of gliomas and provide diagnostic and prognostic information and access to targeted treatment e.g. BRAF inhibitors. *KIAA1549-BRAF* fusions are the most common driver variant in pilocytic astrocytomas, a low grade, predominantly paediatric tumour[(6)].

NTRK1, **NTRK2**, and **NTRK3** fusion testing is available by FISH as required for eligible patients, please refer to the clinical guidance and use the NTRK FISH request form.

Test Information

The All Wales Genomics Laboratory utilises the Illumina TruSight Oncology 500 High Throughput DNA/RNA assay for next generation sequencing using the Illumina NovaSeq 6000™ to identify nucleotide variants and gene rearrangements (fusions) in patients with solid tumours. More information on this service is available here.

Table 1. Glioma DNA Gene Panel

Gene	Hotspots/Screen	Regions covered
IDH1	Hotspots	Exon 4 (covers, p.R132)
IDH2	Hotspots	Exon 4 (covers: p.R172)
BRAF	Hotspots	Exon 15 (covers, p. V600E)
EGFR	Hotspots	Exon 18, 19, 20, 21
ATRX	Screen	Whole Gene
H3F3A	Hotspots	Exon 2 (covers p.K28, p.G35)
TERT	Promoter Variants	Promoter Regions (c.-124 and c.-126)
PTEN	Screen	Whole Gene
TP53	Screen	Whole gene sequence
CDKN2A	Screen	Whole gene sequence

Table 2. Glioma RNA Gene Panel

Gene	Regions covered
BRAF	Whole gene
EGFR	Exons 1-8 for EGFR vIII structural variant
NTRK1 **NTRK2** **NTRK3**	Whole gene

Table 3. Non-NGS Glioma Testing

Gene/Region	Test
1p36.31 / 19q13.32	FISH to detect 1p / 19q loss of heterozygosity, presence of co-deletion confirms oligodendroglial classification and predicts better response to adjuvant therapy.
MGMT	Bisulphite conversion and pyrosequencing to obtain promoter methylation status, which is associated with increased tumour sensitivity to the cytotoxic effects of alkylating chemotherapy.

Please note validation is ongoing for the DNA-NGS assay regarding potential to analyse copy number variants.

Interpretation

A non-interpretative NGS report is issued by AWGL for glioma, this provides only genetic variant information with no diagnostic, prognostic or treatment implications.

This test is performed to evaluate somatic variants within tumour samples and is not designed to assess for germline variants within the targeted genes.

DNA assay sensitivity/specificity may be reduced in specimens containing <10% tumour nuclei.

RNA assay sensitivity/specificity may be reduced in specimens containing <30% tumour nuclei.

Specimen Requirements

For information on sending FFPE samples refer to the CYSGODI service information sheet.

Please use the FFPE solid tumour request form and complete all fields.

References

1) Louis DN, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131(6):803–20
2) Horbinski C. Something old and something new about molecular diagnostics in gliomas. Surg Pathol Clin. 2012 Dec 1;5(4):919-939.
3) Vigneswaran K, Neill S, Hadjipanayis CG. Beyond the World Health Organization grading of infiltrating gliomas: advances in the molecular genetics of glioma classification. Ann Transl Med. 2015 May;3(7):95.
4) Horbinski C. What do we know about IDH1/2 mutations so far, and how do we use it? Acta Neuropathol. 2013 May;125(5):621-36. doi: 10.1007/s00401-013-1106-9.
5) Lee, Y., Koh, J., Kim, S. I., Won, J. K., Park, C. K., Choi, S. H., & Park, S. H. (2017). The frequency and prognostic effect of TERT promoter mutation in diffuse gliomas. Acta neuropathologica communications, 5(1), 62.
6) Faulkner, C., Ellis, H. P., Shaw, A., Penman, C., Palmer, A., Wragg, C., Greenslade, M., Haynes, H. R., Williams, H., Lowis, S., White, P., Williams, M., Capper, D., & Kurian, K. M. (2015). BRAF Fusion Analysis in Pilocytic Astrocytomas: KIAA1549-BRAF 15-9 Fusions Are More Frequent in the Midline Than Within the Cerebellum. Journal of neuropathology and experimental neurology, 74(9), 867–872. https://doi.org/10.1097/NEN.0000000000000226

Links for further information
Orphanet www.orpha.net
EDDNAL www.eddnal.com
OMIM www.omim.org
Genetic Test Directory
https://www.england.nhs.uk/publication/national-genomic-test-directories/

Any cancer specific links
https://www.cancerresearchuk.org/about-cancer/brain-tumours
https://www.thebraintumourcharity.org/

Consent for genetic testing and DNA storage is assumed when a test request and samples are received.