Next Generation Sequencing of Metastatic Melanoma

Overview

Melanoma is a type of skin cancer and one of most common forms of cancer in the UK. In Wales, there is an increasing trend in the incidence of melanoma particularly in women.

Genetic testing of melanomas informs diagnosis, prognosis and treatment options. In particular, analysis focuses on DNA changes linked to targeted *tyrosine kinase inhibitor* (TKI) therapies. TKI treatments slow or stop the cancer growth by blocking chemical messengers that promote cell growth and division.

BRAF (OMIM 164757) variants are found in approximately 50% of malignant melanomas. Over 90% of detected BRAF variants are c.1799T>A p.(Val600Glu) (often referred to as V600E), which is the most common variant in *BRAF*. NICE recommends the use of BRAF kinase inhibitors for locally advanced or metastatic melanoma patients with the BRAF p.V600E variant. BRAF variants can be characterised in to three classes including those that have impaired kinase activity or are kinase-dead[1]. Treatment recommendations will vary subject to the class of variant.

KIT (OMIM 164920) variants are found in 5-10% of melanoma samples. Some evidence from case reports and phase II clinical trials suggests that KIT-mutated melanomas may confer sensitivity to treatment with KIT TKIs.[2,3,4]

NRAS (OMIM 164790) variants are found in 15%–20% of melanomas. NRAS targeted therapies are still in the clinical trial stage. Co-occurring NRAS and BRAF Class III variants may have an impact on treatment recommendations [1,5].

Test Information

Next Generation Sequencing

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. Metastatic Melanoma DNA Gene Panel

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Gene	Hotspots/Screen	Regions covered
NRAS	Hotspots	Exon 2, 3, and 4 (covers: p.12, p.13, p.59, p.61, p.117, p.146 mutations.
BRAF	Hotspots	Exons 11 and 15 (covers: p.599, p.600 and p.601 variants)
KIT	Hotspots	Exons 9, 11, 13, 14 and 17

NTRK1 (OMIM 191315), NTRK2 (OMIM 600456), and NTRK3 (OMIM 191316) genes fusion testing is also available by RNA NGS.

Next Generation Sequencing Interpretation

Reports will provide information on NICE approved treatment options, specifically BRAF- or MEK-targeted therapies.

Please be aware that variants of uncertain significance (VUS) may be identified with this test, these will be further investigated if they are in clinically relevant gene regions and reported as appropriate.

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

URGENT Pyrosequencing

FFPE

Please note that clinically urgent melanoma samples can be referred to the laboratory for BRAF pyrosequencing upon request. This test covers BRAF codons p.599, p.600, and p.601 only. The turnaround time is 7 calendar days, this is to guide treatment specifically for BRAF-targeted therapies.

ctDNA

BRAF pyrosequencing using ctDNA derived from a blood sample is available for exceptionally urgent cases (Stage IV or rapidly deteriorating) when a suitable FFPE sample is not available. Please see PD-GEN-ReqctDNABRAFPyro and PD-GEN-MelanctDNAInfo for further information and specimen requirements.

Sample Information

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.
For information on sending ctDNA samples refer to the

Links for further information:

Orphanet www.orpha.net
EDDNALwww.eddnal.com
OMIM www.omim.org
GeneticTestRegistry www.ncbi.nlm.nih.gov/gtr
CancerResearchUK www.cancerresearchuk.org
MelanomaUK www.melanomauk.org.uk

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References

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

Please note that this NGS panel is not yet accredited by UKAS.