Carcinoma of Unknown Primary

Carcinomas of unknown primary (CUPs) are histologically confirmed secondary malignancies for which the originating primary cancer is yet to be determined on the basis of standard evaluation and imaging studies. These cancers may be either confirmed CUPs (where further evaluation of all diagnostic tests has been unable to identify the likely primary site of origin) or site-specific CUPs (in which further evaluation of the tumour is suggestive of a possible primary site, e.g. biopsy of bone metastasis suggests likely breast carcinoma but no breast mass detected). CUP comprises 2-5% of all diagnosed cancers worldwide, with approximately 8,600 CUP diagnoses being made each year in the UK. CUP is the fifth most common cause of cancer death in the UK.

Genetic testing of CUPs may inform diagnosis, prognosis and treatment options. The genetic testing on offer is determined by whether the CUP is a confirmed CUP or a site-specific CUP (see test information below). Tumour-agnostic treatment options, such as TRK-inhibitors, larotrectinib and entrectinib, are available for adult and paediatric patients with NTRK gene fusion-positive solid tumours (refer to PD-GEN-NTRK-Gene-Fusion-Testing-Clinical-Guidance-Document-Sept-2022-2.pdf (medicalgenomicswales.co.uk) and, as such, NTRK gene testing may be appropriate for all CUPs.

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: Microsoft Word - PD-GEN-CYSGODIinfo (medicalgenomicswales.co.uk).

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

Test Information: confirmed CUPs

For confirmed CUPs, multi-target panels are used to provide analysis of a selection of cancer-related genes (tables 1 and 2). Both DNA and RNA NGS should be requested for each confirmed CUP case.

For confirmed CUP, a non-interpretative report will be issued for all genes with the exception of NTRK1/2/3 (for which full interpretation of the NTRK1/2/3 results will be provided).

Table 1. DNA Gene Panel

Genes Covered (detection of small variants)			
Androgen receptor	ARID1A	ATRX	BRAF
BRCA1	BRCA2	CDKN2A	EGFR
ESR1	ERBB2	H3F3A	HRAS
IDH1	IDH2	KIT	KRAS
NRAS	PDGFRA	PIK3CA	PTEN
RET	TERT	TP53	

Note: if <40ng of DNA is obtained from the FFPE sample provided, an alternative NGS panel will be utilised for testing, which provides a less comprehensive gene analysis.

Table 2. RNA Gene Panel

Genes Covered (detection of structural variants)			
ALK	BRAF	EGFR	MET
NTRK1	NTRK2	NTRK3	RET
ROS1			

Note: if <30ng RNA is obtained, the FISH salvage pathway will be used in place of RNA-based NGS for the delivery of NTRK1/2/3 testing. Should this salvage pathway be required and insufficient material be available to perform all relevant analyses, tests will be performed in accordance with the published gene fusion frequencies within the tumour type, with the most frequently rearranged gene being the first to be tested.

Test Information: site-specific CUPs

For site-specific CUPs, tumour of origin-specific multi-target panels (determined by referrer based on all available clinical information) are used to provide analysis of all tumour-specific clinically actionable genes. This is relevant to the tumour types listed in table 3. Both DNA and RNA NGS should be requested for these referrals.

The CYSGODI service information sheet (Microsoft Word - PD-GEN-CYSGODIinfo (medicalgenomicswales.co.uk)) provides more information on which genes will be analysed according to the likely primary site. Fully interpretative reports will be provided for these site-specific CUPs.

Table 3. Tumour types

✓ Breast	✓ Glioma
✓ Colorectal	✓ Melanoma
✓ Endometrial	✓ Non-small cell lung cancer
✓ Gastrointestinal Stromal Tumour (GIST)	✓ Thyroid

Site-specific CUPs for which a tumour-specific multi-target panel is not available at AWMGS (tumour types not listed in table 3) are analysed using multi-target panels to provide analysis of a selection of cancer-related genes (tables 1 and 2); both DNA and RNA NGS should be requested for these referrals.

For these site-specific CUPs, a non-interpretative report will be issued for all genes with the exception of NTRK1/2/3 (for which full interpretation of the NTRK1/2/3 results will be provided).

Specimen requirements

For information on sending FFPE samples refer to the CYSGODI service information sheet: Microsoft Word - PD-GEN-CYSGODIinfo (medicalgenomicswales.co.uk).

Request forms

For site-specific CUPs listed in table 3, please use the relevant solid tumour request form (available at AWMGS - Home (medicalgenomicswales.co.uk)), and complete all fields. Use of these referral forms for site-specific CUPs will ensure a subset of clinically relevant, tumour-specific genes are analysed.

For confirmed CUPs and for any site-specific CUP not listed in table 3, use CUP request form (available at AWMGS - Home (medicalgenomicswales.co.uk)), and complete all fields.

Links for further information

Cancer Research UK www.cancerresearchuk.org
https://www.nice.org.uk/guidance

All Wales Genetics Laboratory (AWGL)
Phone: 02920 742 641 Fax: 029 2074 4043
Email: lab.genetics@wales.nhs.uk
Website: http://www.medicalgenomicswales.co.uk

References

Cancer of unknown primary | The BMJ

Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up - PubMed (nih.gov)

Cancer of Unknown Primary: A Review on Clinical Guidelines in the Development and Targeted Management of Patients with the Unknown Primary Site - PMC (nih.gov)