Somatic Mutation Testing in Solid Tumours

Supporting treatment decisions and improving outcomes for cancer patients
**What Do We Test?**

* Somatic Mutation in Cancer

The advent of molecular profiling overcame the limitations of traditional solid tumour classification methods, which relied on the morphology of tumour cells and the surrounding tissue. Today, molecular profiling is a standard technique for classifying solid tumours. In turn, genomic technology has evolved to meet molecular profiling needs.

The presence or absence of activated therapeutic driver mutations or gene targets (e.g., \(\text{BRAF}\) in melanoma, \(\text{KRAS}\) in colorectal cancer and \(\text{EGFR}\) mutation or \(\text{ALK}\) rearrangements in non-small cell lung cancers [NSCLC]) is currently employed to guide treatment decisions.

In breast cancer, for example, somatic \(\text{PIK3CA}\) mutations occur in ~25% of cases\(^\text{1,2}\). As such, mutated \(\text{PI3K}\) has become an attractive therapeutic target in breast cancer therapy and a number of agents targeting the \(\text{PIK}\) pathway are currently in clinical development. In Thyroid Cancer, \(\text{BRAF p.V600E}\) mutation occurs in ~40% of patients with papillary thyroid carcinoma (PTC) and is associated with a more aggressive disease\(^\text{3,4}\). In pancreatic cancer, mutation in codon 12 of \(\text{KRAS}\) is a very common event, occurring in up to 90% of pancreatic cancers which can predict a poorer prognosis.

Mutations in tumour suppressor genes, \(\text{APC}\) and \(\text{p53}\), and proto-oncogene, \(\text{KRAS}\), are all implicated in colorectal carcinogenesis. \(\text{KRAS}\) mutations are detected in up to 40% of colorectal cancers (CRC)\(^\text{25}\).

**Precision Medicine**

Cancer, a leading cause of mortality, is associated with mutated genes. Precision or Personalised Medicine harnesses genomic knowledge banks to tailor individualised treatments based on patients’ or their tumours’ genetic signatures. Analysis of tumour-associated genetic alterations is increasingly used for diagnostic, prognostic and treatment purposes.

An FFPE sample of 5-10 μm thickness from the tumour tissue. NGS can detect variants using inputs of ~20ng of FFPE extracted DNA, and from specimens with a tumour cell content less than 50%, for which we recommend, where possible, micro detection.
Next Generation Sequencing at Clinical Labs

Australian Clinical Labs uses a high-quality genomic analysis such as Next Generation Sequencing (NGS) testing panels designed to investigate multiple relevant actionable mutations in formalin fixed paraffin embedded (FFPE) tumour samples.

NGS provides a comprehensive method for assessing the majority of genes associated with solid tumours. NGS also delivers high sensitivity to detect rare mutations and tumour subclones, offering increased visibility into important driver mutations in cancer, which other approaches often miss. Tumour profiling using NGS analyses select a set of genes, gene regions, or amplicons based on known involvement with solid tumours. The assay uses targeted NGS on an Illumina MiSeq instrument. Average amplicon coverage is >x2000 with an acceptance criteria being >x500.

Somatic Mutation Testing Panels At Clinical Labs

1) TruSight Tumour 26 Gene Panel

This Comprehensive Tumour Multi-Gene Panel detects mutations within 26 oncogenes and tumour suppressor genes (see right) that are frequently mutated in solid tumours, aiding clinicians to select the most appropriate treatment for their cancer patients. This panel can be helpful in elucidating the genetic profile of multiple gene pathways of a given tumour which is potentially useful in designing tailored treatment regimens that avoid unnecessary toxic therapy, resistance or overtreatment. It is also of valuable use if the tumour is poorly differentiated or of unknown origin.

Genes sequenced in this panel include:
- AKT1
- ALK
- APC
- BRAF
- CDH1
- CTNNB1
- EGFR
- ERBB2
- FBXW7
- FGFR2
- FOXL2
- GNAQ
- GNAS
- KIT
- KRAS
- MAP2k1
- MET
- MSH6
- NRAS
- PDGFRA
- PIK3CA
- PTEN
- SMAD4
- SRC
- STK11
- TP53
2) Colorectal Cancer Gene Panel

The Colorectal Cancer (CRC) Gene Panel detects mutations within oncogenes that are frequently mutated in CRC. Mutations in proto-oncogene, KRAS, are detected in up to 40% of CRC which can confer resistance to treatment with EGFR antibodies and only patients with wild type KRAS tumours obtain benefit from these agents. It is therefore vital that the KRAS mutation status of a patient's colorectal tumour can be detected to allow patients access to treatment to which there is increased likelihood of benefit.

3) Lung Cancer Gene Panel

Mutation in EGFR occurs in ~35% of NSCLC patients of East Asian origin and ~16% in Western populations. Multiple in-frame deletions in exon 19 and the p.L858R missense mutation in exon 21 comprise 90% of the mutations detected. Studies have confirmed EGFR mutations as a predictive biomarker of treatment response to tyrosine kinase inhibitors (TKIs), Gefitinib and Erlotinib. As such, screening for EGFR mutations in NSCLC patients is deemed necessary before offering these drugs to patients. Most of the patients who initially responded to Gefitinib and Erlotinib will eventually develop resistance to the drugs. Recently, a third-generation EGFR TKI, which is effective in tumours harbouring the p.T790M EGFR mutation (~50-60% of lung cancer patients), was approved in Australia for patients with NSCLC harbouring the EGFR T790M mutation following progression on an EGFR TKI. Studies have confirmed EGFR mutations as a predictive biomarker of treatment response to tyrosine kinase inhibitors (TKIs), Gefitinib and Erlotinib. As such, screening for EGFR mutations in NSCLC patients is deemed necessary before offering these drugs to patients. Most of the patients who initially responded to Gefitinib and Erlotinib will eventually develop resistance to the drugs. Recently, a third-generation EGFR TKI, which is effective in tumours harbouring the p.T790M EGFR mutation (~50-60% of lung cancer patients), was approved in Australia for patients with NSCLC harbouring the EGFR T790M mutation following progression on an EGFR TKI. Recently, Australian recommendations along with NCCN Guidelines (2017) were made to test for resistance mutations using plasma ctDNA testing in NSCLC (currently available) followed by a guided tissue biopsy if blood results are negative or indeterminate. It is also important to check for KRAS mutations in NSCLC. The presence of KRAS mutations in NSCLC patients is considered a negative predictor of response to anti-EGFR treatment and is associated with poorer survival. Interestingly, BRAF mutation can also trigger resistance to TKIs in EGFR-mutant lung cancer.

4) Melanoma Gene Panel

Targeted therapy with Anti-BRAF remains the first-line treatment for melanoma tumours which harbour a BRAF mutation, particularly in Australia. In cutaneous melanoma, the BRAF gene is mutated in ~60% of cases and p.V600E (c.1799TA) accounts for more than 90% of BRAF mutations. Detection of cKIT mutations may guide the selection of KIT TKIs (imatinib and sunitinib) for the melanoma treatment. It has been shown that melanoma patients with cKIT mutations treated with imatinib may have a better outcome compared to BRAF melanoma patients treated with BRAF inhibitors.
Medicare Eligibility Criteria

EGFR Mutation Test Funding Information

Medicare Eligibility Criteria (Item 73337)

A test of tumour tissue from a patient diagnosed with non-small cell lung cancer that is shown to have non-squamous histology or histology not otherwise specified, requested by or on behalf of a specialist or consultant physician. The test to determine if the requirements relating to epidermal growth factor receptor (EGFR) gene status for access to erlotinib or gefitinib under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.

NEW Medicare Eligibility Criteria (Item 73351)

A test of tumour tissue that is derived from a new sample from a patient with locally advanced (Stage IIIb) or metastatic (Stage IV) non-small cell lung cancer (NSCLC), who has progressed on or after treatment with an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI). The test is to be requested by a specialist or consultant physician, to determine if the requirements relating to EGFR T790M gene status for access to osimertinib under the Pharmaceutical Benefits Scheme are fulfilled.

KRAS Mutation Test Funding Information

Medicare Eligibility Criteria (Item 73338)

A test of tumour tissue from a patient with metastatic colorectal cancer (stage IV), requested by a specialist or consultant physician, to determine if the requirements relating to rat sarcoma oncogene (RAS) gene mutation status for access to cetuximab or panitumumab under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.

BRAF Mutation Test Funding Information

Medicare Eligibility Criteria (Item 73336)

A test of tumour tissue from a patient with unresectable stage III or stage IV metastatic cutaneous melanoma, requested by, or on behalf of, a specialist or consultant physician, to determine if the requirements relating to BRAF V600 mutation status for access to dabrafenib under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.

ALK Rearrangement Test Funding Information

ALK IHC – Medicare Rebate/Private Payment **

ALK IHC will be available as a screening test for samples that return a negative EGFR mutation result. This test may be rebated by Medicare; a separate request will be required following the EGFR reporting. If the test is not rebated by Medicare, a private fee of $75 may be applied. If ALK over expression is detected by IHC, confirmation of an ALK rearrangement by FISH is recommended.

ALK (FISH) – Medicare Eligibility Criteria (Item 73341) (referred out test)

Fluorescence in situ hybridisation (FISH) test of tumour tissue from a patient with locally advanced or metastatic non-small cell lung cancer, which is of non-squamous histology or histology not otherwise specified, with documented evidence of anaplastic lymphoma kinase (ALK) immuoneactivity by immunohistochemical (IHC) examination giving a staining intensity score >0, and has documented absence of activating mutations of the epidermal growth factor receptor (EGFR) gene. The test is requested by a specialist or consultant physician to determine if requirements relating to the ALK gene rearrangement status for access to crizotinib under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.

References:
Why Choose Australian Clinical Labs

- Excellent analysis using the most advanced NGS technology
- Medical expertise in the interpretation of complicated cases
- Affordable prices and short turnaround times
- Highest level of quality & accuracy

Our Expert Pathologists

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