Patient ID / MRN	Patient Name		Birth Date	Gender	Age
	LAST, FIRST		dd-0m-yyyy	Male	
Accession Number	Account Name		Ordering Physician		
	Clinic / Hospital		LAST, FIRST		
Sample Type	Collected	Received	Reported		
Formalin Fixed Paraffin Embedded (FFPE) tissue 10-SP-24-#####, Block A1	DD Mmm YYYY	DD Mmm YYYY	DD Mmm YYYY		
Test		Test Indication			
1723123 - BRAF Somatic Mutation, Tumor		Non-Small Cell Lung Cancer			

BRAF Somatic Mutation, Tumor

RESULT: Oncogenic mutation at the codon p.Val600 - DETECTED.

INTERPRETATION

Normal Gene/Protein Function: The BRAF gene encodes a protein called B-Raf, which is a serine/threonine kinase involved in the MAPK/ERK signaling pathway, a critical regulator of cell growth, differentiation, and survival. B-Raf functions as part of a cascade that transmits signals from cell surface receptors to the nucleus in response to extracellular stimuli, such as growth factors. It acts by phosphorylating MEK, which in turn activates ERK, leading to changes in gene expression. Under normal conditions, B-Raf activity is tightly regulated to maintain proper cell function and prevent uncontrolled proliferation.

Mutation Effect: A BRAF mutation at codon p.Val600 (V600E, V600E2, or V600D) was detected. The p.Val600 mutation in the BRAF gene results in a substitution of valine (V) with glutamic acid (E) at codon 600 (V600E), located within the kinase domain of the B-Raf protein. This mutation leads to constitutive activation of the MAPK/ERK signaling pathway, independent of upstream signals. The hyperactivation of this pathway promotes uncontrolled cell proliferation, survival, and tumorigenesis.

Disease Associations: BRAF p.Val600 mutations are frequently found in human cancers and are highly recurrent in melanoma, lung, and thyroid cancer among others. In addition, the presence of the V600 mutation is of diagnostic importance for hairy cell leukemia, Langerhans cell histiocytosis, and Erdheim-Chester Disease. Over 90% of the mutations that affect the codon p.Val600 of the BRAF gene are due to a single DNA change c.1799T>A which leads to amino acid change p.Val600Glu, commonly known as V600E.

Therapeutic Implications: The presence of a BRAF p.Val600 mutation is of therapeutic importance for a variety of human cancers, including anaplastic thyroid cancer, biliary tract cancer not-otherwise-specified (NOS), colorectal cancer, diffuse glioma, encapsulated glioma, Erdheim-Chester disease, ganglioglioma, hairy cell leukemia, histocytosis, Langerhan cell histiocytosis, low-grade glioma NOS, melanoma, non-small cell lung cancer, pilocytic astrocytoma, and pleomorphic xanthoastrocytoma (OncoKB, data version v4.23, last accessed: December 12th, 2024).

Based on the clinical indication of this patient, Dabrafenib + Trametinib or Encorafenib + Binimetinib can be of therapeutic importance (OncoKB, data version v4.23, last accessed: December 12th, 2024; PMID: 28919011, 27283860, 37270692). This result should be interpreted in the context of clinical and other laboratory findings.

METHODOLOGY

The Idylla™ BRAF Mutation Test, performed on the Biocartis Idylla™ System, is an in vitro diagnostic test (IVD) that uses ready-to-use cartridges for the qualitative detection of V600E/E2/D and V600K/R/M mutations in codon 600 of the BRAF gene. The test was conducted following the manufacturer's recommended protocol. In brief, a tumor-rich section of tissue was macrodissected from glass slides, or an FFPE curl/scroll was loaded into the Idylla cartridge. Inside the cartridge, a combination of chemical reagents, enzymes, heat, and high-intensity focused ultrasound facilitated deparaffinization, tissue disruption, and cell lysis. This process released nucleic acids, making them available for subsequent PCR amplification.

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Real-time PCR was then carried out using allele-specific primers, with simultaneous detection of an endogenous sample processing control. Specific targets were identified using fluorescent-labeled probes. The entire process within the cartridge was automated. This method has been previously described in the literature (PMID: 28114309).

Vendor-provided software automatically analyzed fluorescent signals and displayed results on the Idylla Console. The software evaluated PCR curve validity and calculated a cycle of quantification (Cq) value for each valid curve.
The presence of a mutant genotype was determined by comparing the Cq of the sample processing control to the Cq of the mutant signal, producing a ΔCq value. Mutant signals were considered valid if the ΔCq fell within a vendor-defined range, and any detected variants were reported. At the end of the run, a final report confirmed the presence or absence of specific codon mutations in the targeted gene.

LIMITATIONS

The assay effectively covers ~95% of all oncogenic BRAF mutations listed in Table 1, as it includes the dominant codon 600 mutations and their key variants. This assay does not identify rare non-codon 600 mutations, which account for approximately 5% of all BRAF mutations. BRAF V600 mutation below the 1% detection limit may not be detected. Rare polymorphisms in the primer-binding site could lead to false-negative or false-positive results.

Table 1: BRAF p.Val600 Mutations Detectable by this Assay

No.	DNA Change	Protein Change	No.	DNA Change	Protein Change
1	c.1799T>A	p.Val600Glu	5	c.1798_1799delinsAA	p.Val600Lys
2	c.1799_1800delinsAA*	p.Val600Glu	6	c.1798_1799delinsAG	p.Val600Arg
3	c.1799_1800delinsAT	p.Val600Asp	7	c.1798G>A	p.Val600Met
4	c.1799_1800delinsAC	p.Val600Asp			

*Also known as V600E2.

DISCLAIMER

This test was developed, and its performance characteristics were determined by the Molecular Diagnostics & Genomics Laboratory of NRL. This test is marked with In-Vitro Diagnostics (IVD) CE approval. "CE" stands for "Conformité Européenne" [European Conformity]. The IVD CE mark assures that the product meets quality and purity requirements according to the European IVD Regulation [2017/746 EU IVDR) and this guarantees maximum reproducibility for analysis. At NRL, this test is used for clinical purposes and should not be regarded as investigational or for research. The test is currently not accredited by the Emirates International Accreditation Centre (EIAC) but will be added to the scope of accreditation in the next assessment cycle.

REFERENCES

1. OncoKB (https://www.oncokb.org/gene/BRAF/)
2. MyCancerGenome (https://www.mycancergenome.org/)

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