ABN 42 100 504 883

NATA & RCPA ACCREDITED LABORATORY Number 2465

To: PETER MAC CANCER CENTRE
ST ANDREWS PL
EAST MELBOURNE
VIC 3002

Patient: No Patient
URN:
DOB: 01-Jan-2000
SEX: U
Location:
Requester:

Sample: 16K1023
Ext Ref:
Collected: 01-Jan-2000
Received: 01-Jan-2000
Specimen:
Block ID:

NEXTGEN SEQUENCING AMPLICON PANEL REPORT

Specimen Details

Tumour site: Histology/Morphology: , Tumour cell content: % tumour estimated in marked area.

Results

Gene	Reference	Nucleotide Change	Inferred Protein Change	Read Depth¶	Classification
EGFR	NM_005228.3	c.2126A>C	NP_005219.2:p.(Glu709Ala)	2092/8925 23.4%	C5: Pathogenic
EGFR	NM_005228.3	c.2573T>G	NP_005219.2:p.(Leu858Arg)	2374/12002 19.8%	C5: Pathogenic

¶ variant reads / total reads

Interpretation

EGFR: CurVariant chr7:g.55241678A>C not yet curated.

EGFR: The L858R mutation results in an amino acid substitution at position 858 in EGFR, from a leucine (L) to an arginine (R). This mutation occurs within exon 21, which encodes part of the kinase domain, and occurs with a frequency of approximately 43% in EGFR mutant lung tumors. In the metastatic setting, EGFR mutations are strong predictors of efficacy for the EGFR tyrosine kinase inhibitors (TKIs), including the "first-generation" drugs erlotinib (Tarceva) and gefitinib (Iressa).

Methods

Tumour DNA is analysed using the Illumina TruSeq Custom Amplicon Cancer Panel, which targets the mutation hotspots of 48 cancer genes in 212 targeted amplicons. Samples are uniquely indexed, pooled and sequenced on the Illumina MiSeq using MiSeq v2 chemistry at 2x151bp reads. Alignment, variant calling and annotation are performed using Peter Mac's amplicon-optimised pipeline v1.0. Only plausible pathogenic variants passing multiple functional and quality filters are reported above. Amplicons with less than 100 aligned reads are not analysed. These are listed below.

This mutation panel is designed to detect single nucleotide variants and indels in the target regions only. Mutations in the 48 cancer genes that lie outside the target regions will not be detected. The technology employed here is not suitable for detecting loss of heterozygosity, copy number variations, gross structural rearrangements, or aneuploidies. At 1000x coverage, this assay has a detection limit of approximately 5%. The variants detected by this assay should be confirmed by a second method before being used to guide clinical decisions.

Comments

DNA extraction of this tissue sample produced sufficient good quality material for TruSeq Amplicon testing. Sample processing passed all expected QC metrics and high quality sequence with high coverage (0 mean aligned reads/amplicon) and uniformity (0 % amplicons >0.2 mean aligned reads) was obtained.

The Epidermal Growth Factor Receptor (EGFR) belongs to a family of receptor tyrosine kinases (RTKs) that include EGFR/ERBB1, HER2/ERBB2/NEU, HER3/ERBB3, and HER4/ERBB4. The binding of ligands, such as epidermal growth factor (EGF), induces a conformational change that facilitates receptor homo- or heterodimer formation, thereby resulting in activation of EGFR tyrosine kinase activity. Activated EGFR then phosphorylates its substrates, resulting in activation of multiple downstream pathways within the cell, including the PI3K-AKT-mTOR pathway, which is involved in cell survival, and the RAS-RAF-MEK-ERK pathway, which is involved in cell proliferation. Approximately 10% of patients with NSCLC in the US and 35% in East Asia have tumor associated EGFR mutations. These mutations occur within EGFR exons 18-21, which encodes a portion of the EGFR kinase domain. EGFR mutations are usually heterozygous, with the mutant allele also showing gene amplification. Approximately 90% of these mutations are exon 19 deletions or exon 21 L858R point mutations. These mutations increase the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways. Regardless of ethnicity, EGFR mutations are more often found in tumors from female never smokers (defined as less than 100 cigarettes in a patient's lifetime) with adenocarcinoma histology. However, EGFR mutations can also be found in other subsets of NSCLC, including in former and current smokers as well as in other histologies. In the vast majority of cases, EGFR mutations are non-overlapping with other oncogenic mutations found in NSCLC (e.g., KRAS mutations, ALK rearrangements, etc.).

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Please contact the laboratory on 03 9734 4565 if you wish to discuss this report further.

This test has not yet been fully validated to the current NPAAC requirements for an in-house IVD and results should be interpreted accordingly. All findings should be confirmed by an independent clinical assay. For further information, please contact the laboratory.

Reported by: Dr A Asimov, Scientist in Charge Molecular Pathology Diagnostic Development
Authorised by: Prof S Pathohead, Director of Pathology
Reported: 11-Jul-2016 7:31 PM
Low quality amplicons:

Regions of interest coverage:

References: