

# ACT Onco<sup>®</sup> + Report

PATIENT		
Identifier: 柯青姣		Patient ID: 48000982
Date of Birth: Jun 11, 1975		Gender: Female
Diagnosis: Lung adenocarcinoma		
ORDERING PHYSICIAN		
Name: 陳育民醫師		Tel: 886-228712121
Facility: 臺北榮總		
Address: 臺北市北投區石牌路二段 201 號		
SPECIMEN		
Specimen ID: S11205180B		Type: FFPE tissue
Collection site: Lung		
Date received: Mar 10, 2023	Lab ID: AA-23-01453	D/ID: NA

## ABOUT ACT Onco<sup>®</sup>+

The test is a next-generation sequencing (NGS)-based assay developed for efficient and comprehensive genomic profiling of cancers. This test interrogates coding regions of 440 genes associated with cancer treatment, prognosis and diagnosis. Genetic mutations detected by this test include small-scale mutations like single nucleotide variants (SNVs), small insertions and deletions (InDels) ( $\leq 15$  nucleotides) and large-scale genomic alterations like copy number alterations (CNAs). The test also includes an RNA test, detecting fusion transcripts of 13 genes.

## SUMMARY FOR ACTIONABLE VARIANTS

### VARIANTS/BIOMARKERS WITH EVIDENCE OF CLINICAL SIGNIFICANCE

Genomic Alterations/Biomarkers	Probable Effects in Patient's Cancer Type		Probable Sensitive in Other Cancer Types
	Sensitive	Resistant	
EGFR N771_H773dup (Exon 20 insertion)	Amivantamab-vmjw, Mobocertinib	Erlotinib, Gefitinib	-

### VARIANTS/BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Genomic Alterations/Biomarkers	Possibly Sensitive	Possibly Resistant
EGFR N771_H773dup (Exon 20 insertion)	Afatinib, Dacomitinib, Neratinib, Osimertinib	-

#### Note:

- The above summary tables present genomic variants and biomarkers based on the three-tiered approach proposed by US FDA for reporting tumor profiling NGS testing. "Variants/biomarkers with evidence of clinical significance" refers to mutations that are widely recognized as standard-of-care biomarkers (FDA level 2/AMP tier 1). "Variants/biomarkers with potential clinical significance" refers to mutations that are not included in the standard of care but are informational for clinicians, which are commonly biomarkers used as inclusion criteria for clinical trials (FDA level 3/AMP tier 2).
- The therapeutic agents and possible effects to a given drug are based on mapping the variants/biomarkers with ACT Genomics clinical knowledge database. The mapping results only provide information for reference, but not medical recommendation.
- Please refer to corresponding sections for more detailed information about genomic alteration and clinical relevance listed above.

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## TESTING RESULTS

### VARIANT(S) WITH CLINICAL RELEVANCE

#### - Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Allele Frequency
EGFR	N771_H773dup (Exon 20 insertion)	26.3%
TP53	T211P	27.4%

#### - Copy Number Alterations

Chromosome	Gene	Variation	Copy Number
Not detected			

#### - Fusions

Fusion Gene & Exon	Transcript ID
No fusion gene detected in this sample	

#### - Immune Checkpoint Inhibitor (ICI) Related Biomarkers

Biomarker	Results
Tumor Mutational Burden (TMB)	2.6 muts/Mb
Microsatellite Instability (MSI)	Microsatellite stable (MSS)

#### Note:

- Variant(s) enlisted in the SNV table may currently exhibit no relevance to treatment response prediction. Please refer to INTERPRETATION for more biological information and/or potential clinical impacts of the variants.
- Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on 45% tumor purity.
- TMB was calculated by using the sequenced regions of ACTOnco<sup>®</sup> to estimate the number of somatic nonsynonymous mutations per megabase of all protein-coding genes (whole exome). The threshold for high mutation load is set at  $\geq 7.5$  mutations per megabase. TMB, microsatellite status and gene copy number deletion cannot be determined if calculated tumor purity is  $< 30\%$ .

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## THERAPEUTIC IMPLICATIONS TARGETED THERAPIES

Genomic Alterations	Therapies	Effect
<b>Level 1</b>		
<b>EGFR</b> N771_H773dup (Exon 20 insertion)	Amivantamab-vmjw, Mobocertinib	<b>sensitive</b>
<b>Level 2</b>		
<b>EGFR</b> N771_H773dup (Exon 20 insertion)	Erlotinib, Gefitinib	<b>resistant</b>
<b>Level 3B</b>		
<b>EGFR</b> N771_H773dup (Exon 20 insertion)	Osimertinib	<b>sensitive</b>
<b>Level 4</b>		
<b>EGFR</b> N771_H773dup (Exon 20 insertion)	Afatinib, Dacomitinib, Neratinib	<b>sensitive</b>

Therapies associated with benefit or lack of benefit are based on biomarkers detected in this tumor and published evidence in professional guidelines or peer-reviewed journals.

Level	Description
<b>1</b>	FDA-recognized biomarkers predictive of response or resistance to FDA approved drugs in this indication
<b>2</b>	Standard care biomarkers (recommended by the NCCN guideline) predictive of response or resistance to FDA approved drugs in this indication
<b>3A</b>	Biomarkers predictive of response or resistance to therapies approved by the FDA or NCCN guideline in a different cancer type
<b>3B</b>	Biomarkers that serve as inclusion criteria for clinical trials (minimal supportive data required)
<b>4</b>	Biomarkers that show plausible therapeutic significance based on small studies, few case reports, or preclinical studies

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## IMMUNE CHECKPOINT INHIBITORS (ICIs)

No genomic alterations detected to confer sensitivity or lack of benefit to immune checkpoint therapies.

### - Other Biomarkers with Potential Clinical Effects for ICIs

Genomic Alterations	Potential Clinical Effects
EGFR aberration	Likely associated with WORSE response to ICIs

Note: Tumor non-genomic factors, such as patient germline genetics, PDL1 expression, tumor microenvironment, epigenetic alterations or other factors not provided by this test may affect ICI response.

## CHEMOTHERAPIES

No genomic alterations detected in this tumor predicted to confer sensitivity or lack of benefit to chemotherapies.

## HORMONAL THERAPIES

No genomic alterations detected in this tumor predicted to confer sensitivity or lack of benefit to hormonal therapies.

## OTHERS

No genomic alterations detected in this tumor predicted to confer sensitivity or lack of benefit to other therapies.

### Note:

Therapeutic implications provided in the test are based solely on the panel of 440 genes sequenced. Therefore, alterations in genes not covered in this panel, epigenetic and post-transcriptional and post-translational factors may also determine a patient's response to therapies. In addition, several other patient-associated clinical factors, including but not limited to, prior lines of therapies received, dosage and combinations with other therapeutic agents, patient's cancer types, sub-types, and/or stages, may also determine the patient's clinical response to therapies.

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## VARIANT INTERPRETATION

### EGFR N771\_H773dup (Exon 20 insertion)

#### Biological Impact

The EGFR gene encodes for the Epidermal Growth Factor Receptor, a receptor tyrosine kinase which binds to its ligands, including Epidermal Growth Factor (EGF) and Transforming Growth Factor-alpha (TGF-alpha), activates downstream signaling pathways, including the canonical oncogenic MAPK and PI3K/AKT/mTOR signaling cascades<sup>[1]</sup>. Increased EGFR activity by mutations and/or amplification of the EGFR gene has been described in a wide range of cancers, such as lung, brain, colorectal and head and neck cancer<sup>[2]</sup>. Mutations in the kinase domain of EGFR are commonly observed in non-small cell lung cancer (NSCLC), resulting in a constitutively activated form of the receptor<sup>[3]</sup>. On the other hand, in the brain and colorectal cancers, the most prevalent EGFR alteration is copy number amplification that results in receptor overexpression<sup>[4]</sup>.

EGFR N771\_H773dup results in the insertion of 3 duplicate amino acids, asparagine (N)-771 through histidine (H)-773, in the protein kinase domain of the EGFR protein(UniProtKB). N771\_H773dup confers a gain of function to the EGFR protein as demonstrated by increased cell proliferation and cell viability, promoting cell transformation in vitro<sup>[5][6][7]</sup>.

#### Therapeutic and prognostic relevance

There is accumulated clinical evidence suggested that patients with MDM2/MDM4 amplification or EGFR aberrations exhibited poor clinical outcome and demonstrated a significantly increased rate of tumor growth (hyper-progression) after receiving immune checkpoint (PD-1/PD-L1) inhibitors therapies<sup>[8]</sup>(Annals of Oncology (2017) 28 (suppl\_5): v403-v427. 10.1093/annonc/mdx376).

A retrospective study demonstrated that 5 lung cancer patients harboring EGFR N771\_H773dup led to different responses to first-generation TKIs, afatinib, and osimertinib<sup>[9]</sup>. Two patients showed progressive disease and one patient was stable disease after first-generation TKIs treatment. A patient who was treated by afatinib and showed stable disease. Of note, a patient using osimertinib as second-line TKI treatment demonstrated effective disease control (> 3 months stable disease). In a case report, a patient with EGFR N771\_H773dup-positive lung adenocarcinoma showed stable disease for 4 months after osimertinib treatment (AACR annual meeting 2017, #2718). Preclinical studies demonstrated that lung cancer patient-derived cells harboring EGFR N771\_H773dup were sensitive to amivantamab, mobocertinib, and dacomitinib, as indicated by inhibited cell viability, EGFR phosphorylation, and downstream signaling<sup>[10][11][12]</sup>. In another preclinical study, head and neck carcinoma cells harboring EGFR N771\_H773dup were shown to be sensitive to tyrosine kinase inhibitor neratinib but resistant to gefitinib and erlotinib<sup>[13]</sup>.

Amivantamab-vmjw and mobocertinib are FDA-approved for treating adult patients with locally advanced or metastatic NSCLC harboring EGFR exon 20 insertion mutations.

NCCN guidelines for NSCLC has suggested that EGFR exon 20 alternations are generally associated with lack of sensitivity to TKI therapy, except for A763\_Y764insFQEA.

EGFR exon 20 insertion has been selected as an inclusion criteria for the trial examining osimertinib efficacy in NSCLC (NCT03414814).

In other clinical studies, afatinib showed lower clinical benefit in patients with EGFR exon 20 insertion mutations<sup>[14][15][16]</sup>. A case study showed that a combination therapy with afatinib plus cetuximab could overcome primary EGFR TKI resistance in EGFR exon 20 insertion positive NSCLC patient<sup>[17]</sup>. Tumor inhibitory effect of osimertinib was observed in cells harboring EGFR exon 20 insertion in vitro and in vivo<sup>[18]</sup>.

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## TP53 T211P

### Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis<sup>[19]</sup>. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation<sup>[20]</sup>.

### Therapeutic and prognostic relevance

Despite having a high mutation rate in cancers, there are currently no approved targeted therapies for TP53 mutations. A phase II trial demonstrated that Wee1 inhibitor (AZD1775) in combination with carboplatin was well tolerated and showed promising anti-tumor activity in TP53-mutated ovarian cancer refractory or resistant (< 3 months) to standard first-line therapy (NCT01164995)<sup>[21]</sup>.

In a retrospective study (n=19), advanced sarcoma patients with TP53 loss-of-function mutations displayed improved progression-free survival (208 days versus 136 days) relative to patients with wild-type TP53 when treated with pazopanib<sup>[22]</sup>. Results from another Phase I trial of advanced solid tumors (n=78) demonstrated that TP53 hotspot mutations are associated with better clinical response to the combination of pazopanib and vorinostat<sup>[23]</sup>.

Advanced solid tumor and colorectal cancer patients harboring a TP53 mutation have been shown to be more sensitive to bevacizumab when compared with patients harboring wild-type TP53<sup>[24][25][26]</sup>. In a pilot trial (n=21), TP53-negative breast cancer patients demonstrated increased survival following treatment with bevacizumab in combination with chemotherapy agents, Adriamycin (doxorubicin) and Taxotere (docetaxel)<sup>[27]</sup>. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy<sup>[28][29]</sup>. In a retrospective study of non-small cell lung cancer (NSCLC), TP53 mutations were associated with high expression of VEGF-A, the primary target of bevacizumab, offering a mechanistic explanation for why patients exhibit improved outcomes after bevacizumab treatment when their tumors harbor mutant TP53 versus wild-type TP53<sup>[30]</sup>.

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## US FDA-APPROVED DRUG(S)

### Afatinib (GILOTRIF)

Afatinib acts as an irreversible covalent inhibitor of the ErbB family of receptor tyrosine kinases, including epidermal growth factor receptor (EGFR) and erbB-2 (HER2). Afatinib is developed and marketed by Boehringer Ingelheim under the trade name GILOTRIF (United States) and GIOTRIF (Europe).

#### - FDA Approval Summary of Afatinib (GILOTRIF)

LUX-Lung 8 <sup>[31]</sup> NCT01523587	Non-small cell lung carcinoma (Approved on 2016/04/15)
	EGFR ex19del or L858R
	Afatinib vs. Erlotinib [PFS(M): 2.4 vs. 1.9]
LUX-Lung 3 <sup>[32]</sup> NCT00949650	Non-small cell lung carcinoma (Approved on 2013/07/13)
	EGFR ex19del or L858R
	Afatinib vs. Pemetrexed + cisplatin [PFS(M): 11.1 vs. 6.9]

### Amivantamab-vmjw (RYBREVANT)

Amivantamab-vmjw is a bispecific antibody directed against epidermal growth factor (EGF) and MET receptors. Amivantamab-vmjw is developed and marketed by Janssen Biotech, Inc. under the trade name RYBREVANT.

#### - FDA Approval Summary of Amivantamab-vmjw (RYBREVANT)

CHRYSLIS NCT02609776	Non-small cell lung carcinoma (Approved on 2021/05/21)
	EGFR ex20ins
	Amivantamab-vmjw [ORR(%): 40, DOR(M): 11.1]

### Dacomitinib (VIZIMPRO)

Dacomitinib is an oral kinase inhibitor that targets EGFR. Dacomitinib is developed and marketed by Pfizer under the trade name VIZIMPRO.

#### - FDA Approval Summary of Dacomitinib (VIZIMPRO)

ARCHER 1050 <sup>[33]</sup> NCT01774721	Non-small cell lung carcinoma (Approved on 2018/09/27)
	EGFR ex19del or L858R
	Dacomitinib vs. Gefitinib [PFS(M): 14.7 vs. 9.2]

### Mobocertinib (EXKIVITY)

Mobocertinib is a first-in-class, oral tyrosine kinase inhibitor (TKI) specifically designed to selectively target epidermal growth factor receptor (EGFR) Exon 20 insertion mutations. Mobocertinib is developed and marketed by Takeda under the trade name EXKIVITY.

#### - FDA Approval Summary of Mobocertinib (EXKIVITY)

Study 101 <sup>[34]</sup> NCT02716116	Non-small cell lung carcinoma (Approved on 2021/09/15)
	EGFR ex20ins
	Mobocertinib [ORR(%): 28.0, DOR(M): 17.5]



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## Neratinib (NERLYNX)

Neratinib is an oral, irreversible, tyrosine-kinase inhibitor of HER1, HER2, and HER4. Neratinib is developed and marketed by Puma Biotechnology under the trade name NERLYNX.

### - FDA Approval Summary of Neratinib (NERLYNX)

<b>ExteNET<sup>[35]</sup></b> NCT00878709	<b>Her2-receptor positive breast cancer</b> (Approved on 2017/07/17)
	<b>HER2+</b>
	Neratinib vs. Placebo [DFS(%): 94.2 vs. 91.9]

## Osimertinib (TAGRISSO)

Osimertinib is a third-generation tyrosine kinase inhibitor (TKI) for patients with tumors harboring EGFR T790M mutation. Osimertinib is developed and marketed by AstraZeneca under the trade name TAGRISSO.

### - FDA Approval Summary of Osimertinib (TAGRISSO)

<b>ADAURA</b> NCT02511106	<b>Non-small cell lung carcinoma</b> (Approved on 2020/12/18)
	<b>EGFR ex19del or L858R</b>
	Osimertinib vs. Placebo + adjuvant chemotherapy [DFS(M): NR vs. 19.6]
<b>FLAURA<sup>[36]</sup></b> NCT02296125	<b>Non-small cell lung carcinoma</b> (Approved on 2018/04/18)
	<b>EGFR ex19del or L858R</b>
	Osimertinib vs. Gefitinib or erlotinib [PFS(M): 18.9 vs. 10.2]
<b>AURA3<sup>[37]</sup></b> NCT02151981	<b>Non-small cell lung carcinoma</b> (Approved on 2017/03/30)
	<b>EGFR T790M</b>
	Osimertinib vs. Chemotherapy [PFS(M): 10.1 vs. 4.4]
<b>AURA<sup>[38]</sup></b> NCT01802632	<b>Non-small cell lung carcinoma</b> (Approved on 2015/11/13)
	<b>EGFR T790M</b>
	Osimertinib [ORR(%): 59.0]

D=day; W=week; M=month



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## ONGOING CLINICAL TRIALS

Trials were searched by applying filters: study status, patient's diagnosis, intervention, location and/or biomarker(s). Please visit <https://clinicaltrials.gov> to search and view for a complete list of open available and updated matched trials.

No trial has been found.

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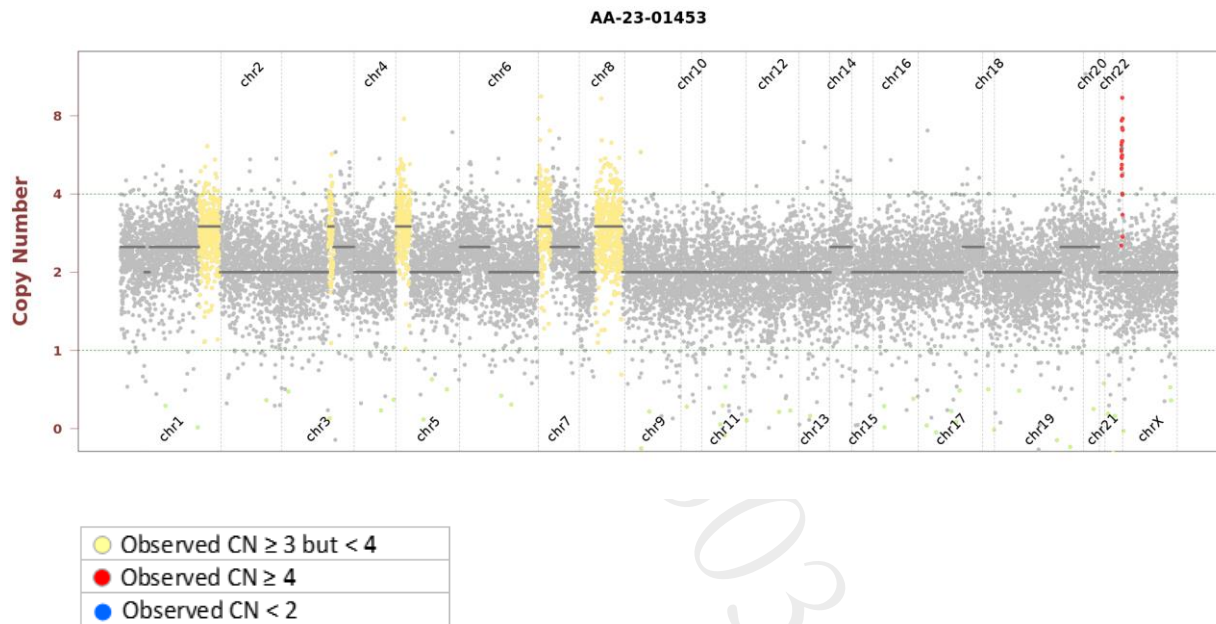
## SUPPLEMENTARY INFORMATION OF TESTING RESULTS DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

### - Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
EGFR	N771_H773dup (Exon 20 insertion)	20	c.2311_2319dup	NM_005228	COSM12381	26.3%	422
TP53	T211P	6	c.631A>C	NM_000546	COSM46038	27.4%	993

### - Copy Number Alterations

Observed copy number (CN) for each evaluated position is shown on the y-axis. Regions referred to as amplification or deletion are shown in color. Regions without significant changes are represented in gray.



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## OTHER DETECTED VARIANTS

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
ALK	A371T	4	c.1111G>A	NM_004304	COSM5949450	66.8%	918
BRD4	V1110del	16	c.3328_3330del	NM_058243	-	42.0%	207
CSF1R	T621M	14	c.1862C>T	NM_005211	COSM4970976	57.3%	818
DCUN1D1	N197S	5	c.590A>G	NM_020640	-	44.0%	580
DDR2	T681I	15	c.2042C>T	NM_006182	COSM2088236	54.2%	1973
ERBB4	R979Q	24	c.2936G>A	NM_005235	COSM4090859	36.5%	1839
FAT1	T789P	2	c.2365A>C	NM_005245	-	59.4%	1725
HGF	R393C	10	c.1177C>T	NM_000601	COSM1092111	11.8%	576
MDM2	W335R	11	c.1003T>C	NM_002392	-	61.0%	974
MET	A48G	2	c.143C>G	NM_001127500	-	51.8%	2096
MUC16	P13777Q	63	c.41330C>A	NM_024690	-	5.5%	649
PTPRD	Splice region	10	c.66A>G	NM_002839	-	35.1%	1254
REL	N551S	11	c.1652A>G	NM_002908	-	62.2%	439
RUNX1T1	Splice region	-	c.227-4A>T	NM_175634	-	10.2%	1672
SMO	S590T	10	c.1769G>C	NM_005631	-	50.1%	1468
SYNE1	E7706Q	127	c.23116G>C	NM_182961	-	38.7%	1310
USH2A	N2356K	37	c.7068T>G	NM_206933	-	53.5%	2158

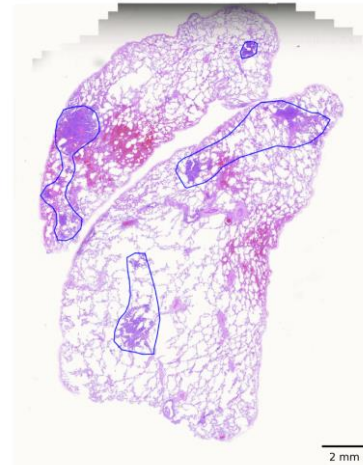
### Note:

- This table enlists variants detected by the panel other than those with clinical relevance (reported in Testing Result section). The clinical impact of a genetic variant is determined according to ACT Genomics in-house clinical knowledge database. A negative result does not necessarily indicate absence of biological effect on the tumor. Some variants listed here may possibly have preclinical data or may show potential clinical relevance in the future.

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## TEST DETAILS

### SPECIMEN RECEIVED AND PATHOLOGY REVIEW



- Collection date: Feb 10, 2023
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11205180B
- Collection site: Lung
- Examined by: Dr. Chien-Ta Chiang
  1. The percentage of viable tumor cells in total cells in the whole slide (%): 10%
  2. The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 45%
  3. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
  4. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
  5. Additional comment: NA
- Manual macrodissection: Performed on the highlighted region
- The outline highlights the area of malignant neoplasm annotated by a pathologist.

## RUN QC

- Panel: ACTOnco<sup>®</sup>+

### DNA test

- Mean Depth: 934x
- Target Base Coverage at 100x: 94%

### RNA test

- Average unique RNA Start Sites per control GSP2: 162

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## LIMITATIONS

1. This test does not provide information of variant causality and does not detect variants in non-coding regions that could affect gene expression. This report does not report polymorphisms and we do not classify whether a mutation is germline or somatic. Variants identified by this assay were not subject to validation by Sanger or other technologies.
2. The possibility cannot be excluded that certain pathogenic variants detected by other sequencing tools may not be reported in the test because of technical limitation of bioinformatics algorithm or the NGS sequencing platform, e.g. low coverage.
3. This test has been designed to detect fusions in 13 genes sequenced. Therefore, fusion in genes not covered by this test would not be reported. For novel fusions detected in this test, Sanger sequencing confirmation is recommended if residue specimen is available.

## NEXT-GENERATION SEQUENCING (NGS) METHODS

### DNA test

Extracted genomic DNA was amplified using primers targeting coding exons of analyzed genes and subjected to library construction. Barcoded libraries were subsequently conjugated with sequencing beads by emulsion PCR and enriched using Ion Chef system. Sequencing was performed according to Ion Proton or Ion S5 sequencer protocol (Thermo Fisher Scientific).

Raw reads generated by the sequencer were mapped to the hg19 reference genome using the Ion Torrent Suite. Coverage depth was calculated using Torrent Coverage Analysis plug-in. Single nucleotide variants (SNVs) and short insertions/deletions (InDels) were identified using the Torrent Variant Caller plug-in. VEP (Variant Effect Predictor) was used to annotate every variant using databases from Clinvar, COSMIC and Genome Aggregation database. Variants with coverage  $\geq 20$ , allele frequency  $\geq 5\%$  and actionable variants with allele frequency  $\geq 2\%$  were retained. This test provides uniform coverage of the targeted regions, enabling target base coverage at  $100\times \geq 85\%$  with a mean coverage  $\geq 500\times$ .

Variants reported in Genome Aggregation database with  $> 1\%$  minor allele frequency (MAF) were considered as polymorphisms. ACT Genomics in-house database was used to determine technical errors. Clinically actionable and biologically significant variants were determined based on the published medical literature.

The copy number alterations (CNAs) were predicted as described below:

Amplicons with read counts in the lowest 5th percentile of all detectable amplicons and amplicons with a coefficient of variation  $\geq 0.3$  were removed. The remaining amplicons were normalized to correct the pool design bias. ONCOCNV (an established method for calculating copy number aberrations in amplicon sequencing data by Boeva et al., 2014) was applied for the normalization of total amplicon number, amplicon GC content, amplicon length, and technology-related biases, followed by segmenting the sample with a gene-aware model. The method was used as well for establishing the baseline of copy number variations.

Tumor mutational burden (TMB) was calculated by using the sequenced regions of ACTOnco<sup>®</sup> to estimate the number of somatic nonsynonymous mutations per megabase of all protein-coding genes (whole exome). The TMB calculation predicted somatic variants and applied a machine learning model with a cancer hotspot correction. TMB may be reported as "TMB-High", "TMB-Low" or "Cannot Be Determined". TMB-High corresponds to  $\geq 7.5$  mutations per megabase (Muts/Mb); TMB-Low corresponds to  $< 7.5$  Muts/Mb. TMB is reported as "Cannot Be Determined" if the tumor purity of the sample is  $< 30\%$ .

Classification of microsatellite instability (MSI) status is determined by a machine learning prediction algorithm. The change of a number of repeats of different lengths from a pooled microsatellite stable (MSS) baseline in  $> 400$  genomic loci are used as the features for the algorithm. The final output of the results is either microsatellite Stable (MSS) or microsatellite instability high (MSI-H).

### RNA test

Extracted RNA was reverse-transcribed and subjected to library construction. Sequencing was performed according to Ion Proton or Ion S5 sequencer protocol (Thermo Fisher Scientific). To ensure sequencing quality for fusion variant analysis, the average unique RNA Start Sites (SS) per control Gene Specific Primer 2 (GSP 2) should be  $\geq 10$ .

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The fusion analysis pipeline aligned sequenced reads to the human reference genome, identified regions that map to noncontiguous regions of the genome, applied filters to exclude probable false-positive events and, annotated previously characterized fusion events according to Quiver Gene Fusion Database, a curated database owned and maintained by ArcherDX. In general, samples with detectable fusions need to meet the following criteria: (1) Number of unique start sites (SS) for the GSP2  $\geq 3$ ; (2) Number of supporting reads spanning the fusion junction  $\geq 5$ ; (3) Percentage of supporting reads spanning the fusion junction  $\geq 10\%$ ; (4) Fusions annotated in Quiver Gene Fusion Database.

## DATABASE USED

- Reference genome: Human genome sequence hg19
- COSMIC v.92
- Genome Aggregation database r2.1.1
- ClinVar (version 20210404)
- ACT Genomics in-house database
- Quiver Gene Fusion Database version 5.1.18

## Variant Analysis:

醫藥資訊研究員  
楊杭哲 博士  
Hang-Che Yang Ph.D.



## Sign Off

解剖病理專科醫師王業翰  
Yeh-Han Wang M.D.  
病解字第 000545 號





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## GENE LIST SNV & CNV

ABCB1*	ABCC2*	ABCG2*	ABL1	ABL2	ADAMTS1	ADAMTS13	ADAMTS15	ADAMTS16	ADAMTS18	ADAMTS6	ADAMTS9
ADAMTSL1	ADGRA2	ADH1C*	AKT1	AKT2	AKT3	ALDH1A1*	ALK	AMER1	APC	AR	ARAF
ARID1A	ARID1B	ARID2	ASXL1	ATM	ATR	ATRX	AURKA	AURKB	AXIN1	AXIN2	AXL
B2M	BAP1	BARD1	BCL10	BCL2*	BCL2L1	BCL2L2*	BCL6	BCL9	BCOR	BIRC2	BIRC3
BLM	BMPR1A	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTG2*	BTB	BUB1B	CALR
CANX	CARD11	CASP8	CBFB	CBL	CCNA1	CCNA	CCNB1	CCNB2	CCNB3	CCND1	CCND2
CCND3	CCNE1	CCNE2	CCNH	CD19	CD274	CD58	CD70*	CD79A	CD79B	CDC73	CDH1
CDK1	CDK12	CDK2	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9	CDKN1A	CDKN1B	CDKN2A
CDKN2B	CDKN2C	CEBPA*	CHEK1	CHEK2	CIC	CREBBP	CRKL	CRLF2	CSF1R	CTCF	CTLA4
CTNNA1	CTNNB1	CUL3	CYLD	CYP1A1*	CYP2B6*	CYP2C19*	CYP2C8*	CYP2D6	CYP2E1*	CYP3A4*	CYP3A5*
DAXX	DCUN1D1	DDR2	DICER1	DNMT3A	DOT1L	DPYD	DTX1	E2F3	EGFR	EP300	EPCAM
EPHA2	EPHA3	EPHA5	EPHA7	EPHB1	ERBB2	ERBB3	ERBB4	ERCC1	ERCC2	ERCC3	ERCC4
ERCC5	ERG	ESR1	ESR2	ETV1	ETV4	EZH2	FAM46C	FANCA	FANCC	FANCD2	FANCE
FANCF	FANCG	FANCL	FAS	FAT1	FBXW7	FCGR2B	FGF1*	FGF10	FGF14	FGF19*	FGF23
FGF3	FGF4*	FGF6	FGFR1	FGFR2	FGFR3	FGFR4	FH	FLCN	FLT1	FLT3	FLT4
FOXL2*	FOXP1	FRG1	FUBP1	GATA1	GATA2	GATA3	GNA11	GNA13	GNAQ	GNAS	GREM1
GRIN2A	GSK3B	GSTP1*	GSTT1*	HGF	HIF1A	HIST1H1C*	HIST1H1E*	HNF1A	HR	HRAS*	HSP90AA1
HSP90AB1	HSPA4	HSPA5	IDH1	IDH2	IFNL3*	IGF1	IGF1R	IGF2	IKBKB	IKBKE	IKZF1
IL6	IL7R	INPP4B	INSR	IRF4	IRS1	IRS2*	JAK1	JAK2	JAK3	JUN*	KAT6A
KDM5A	KDM5C	KDM6A	KDR	KEAP1	KIT	KMT2A	KMT2C	KMT2D	KRAS	LCK	LIG1
LIG3	LMO1	LRP1B	LYN	MALT1	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAP3K7	MAPK1	MAPK3
MAX	MCL1	MDM2	MDM4	MED12	MEF2B	MEN1	MET	MITF	MLH1	MPL	MRE11
MSH2	MSH6	MTHFR*	MTOR	MUC16	MUC4	MUC6	MUTYH	MYC	MYCL	MYCN	MYD88
NAT2*	NBN	NEFH	NF1	NF2	NFE2L2	NFKB1	NFKBIA	NKX2-1*	NOTCH1	NOTCH2	NOTCH3
NOTCH4	NPM1	NQO1*	NRAS	NSD1	NTRK1	NTRK2	NTRK3	PAK3	PALB2	PARP1	PAX5
PAX8	PBRM1	PDCD1	PDCD1LG2	PDGFRA	PDGFRB	PDIA3	PGF	PHOX2B*	PIK3C2B	PIK3C2G	PIK3C3
PIK3CA	PIK3CB	PIK3CD	PIK3CG	PIK3R1	PIK3R2	PIK3R3	PIM1	PMS1	PMS2	POLB	POLD1
POLE	PPARG	PPP2R1A	PRDM1	PRKAR1A	PRKCA	PRKCB	PRKCG	PRKCI	PRKCQ	PRKDC	PRKN
PSMB8	PSMB9	PSME1	PSME2	PSME3	PTCH1	PTEN	PTGS2	PTPN11	PTPRD	PTPRT	RAC1
RAD50	RAD51	RAD51B	RAD51C	RAD51D	RAD52	RAD54L	RAF1	RARA	RB1	RBM10	RECQL4
REL	RET	RHOA	RICTOR	RNF43	ROS1	RPPH1	RPTOR	RUNX1	RUNX1T1	RXRA	SDHA
SDHB	SDHC	SDHD	SERPINB3	SERPINB4	SETD2	SF3B1	SGK1	SH2D1A*	SLC19A1*	SLC22A2*	SLC18A1*
SLC18B3*	SMAD2	SMAD3	SMAD4	SMARCA4	SMARCB1	SMO	SOC1*	SOX2*	SOX9	SPEN	SPOP
SRC	STAG2	STAT3	STK11	SUFU	SYK	SYNE1	TAF1	TAP1	TAP2	TAPBP	TBX3
TEK	TERT	TET1	TET2	TGFBR2	TMSB4X*	TNF	TNFAIP3	TNFRSF14	TNFSF11	TOP1	TP53
TPMT*	TSC1	TSC2	TSHR	TYMS	U2AF1	UBE2A*	UBE2K	UBR5	UGT1A1*	USH2A	VDR*
VEGFA	VEGFB	VHL	WT1	XIAP	XPO1	XRCC2	ZNF217				

\*Analysis of copy number alterations NOT available.

## FUSION

ALK	BRAF	EGFR	FGFR1	FGFR2	FGFR3	MET	NRG1	NTRK1	NTRK2	NTRK3	RET	ROS1
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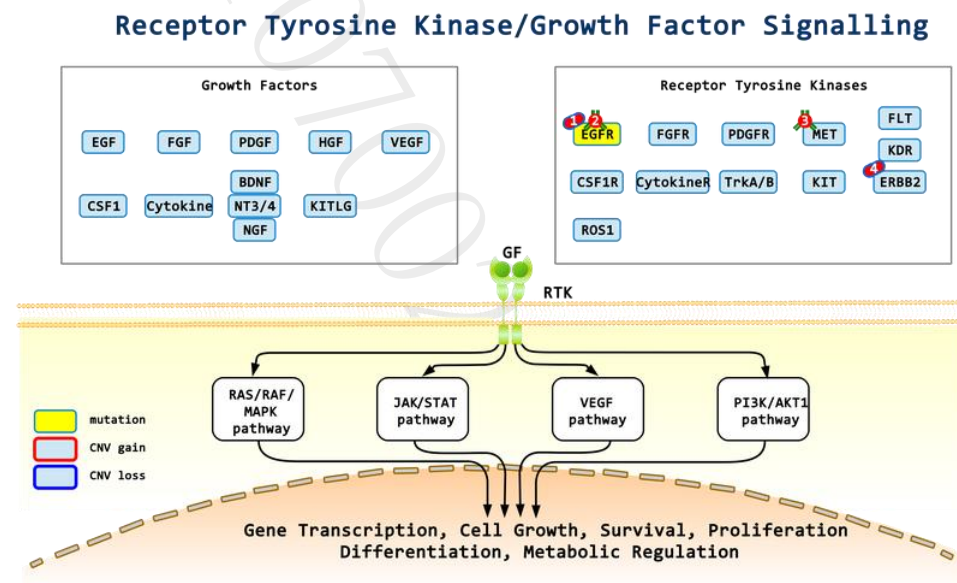
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## APPENDIX

### POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

Not Applicable.

### SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



1: Osimertinib, Dacomitinib, Afatinib, Neratinib, Mobocertinib; 2: Amivantamab-vmjw; 3: Amivantamab-vmjw; 4: Afatinib, Neratinib

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本檢驗報告僅提供專業醫療參考，結果需經專業醫師解釋及判讀。基因突變資訊非必具備藥物或治療有效性指標，反之亦然。本檢驗報告提供之用藥指引不聲明或保證其臨床有效性，反之亦然。本基因檢測方法係由本公司研究開發，已經過有效性測試。

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本報告中列出之生物標記變異與藥物資訊並非依照潛在治療有效性排序。

### 證據等級

藥物潛在臨床效益(或缺乏潛在臨床效益)的實證證據是依據至少一篇臨床療效個案報告或臨床前試驗做為評估。本公司盡力提供適時及準確之資料，但由於醫學科技之發展日新月異，本公司不就本報告提供的資料是否為準確、適宜或最新作保證。

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