鄭憶媚
Project ID: C23-M001-02053
Report No.: AA-23-04367_ONC
Date Reported: Jul 13, 2023

ACT Onco®+ Report

PATIENT		
Identifier: 鄭憶媚		Patient ID: 33157141
Date of Birth: Jun 10, 1966		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: S11230179A, S11230179B	Collection site: Lung	Type: FFPE tissue
Date received: Jul 04, 2023	Lab ID: AA-23-04366, AA-23-04367	D/ID: NA

ABOUT ACTOnco®+

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) (≤ 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	
ERBB2 Y772_A775dup (Exon 20 insertion)	Ado-trastuzumab emtansine, Fam-trastuzumab deruxtecan-nxki	-	-

VARIANTS/BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Genomic Alterations/Biomarkers	Possibly Sensitive	Possibly Resistant
ERBB2 Y772_A775dup (Exon 20 insertion)	Mobocertinib, Trastuzumab	Erlotinib, Gefitinib, Lapatinib, Osimertinib
SMAD4 R361H	-	Cetuximab

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 criterial 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
ERBB2	Y772_A775dup (Exon 20 insertion)	34.9%
SMAD4	R361H	7.2%

- 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)	0.7 muts/Mb
Microsatellite Instability (MSI)	Microsatellite stable (MSS)

Note:
- Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on 40% tumor purity.
- TMB was calculated by using the sequenced regions of ACTOnco®+ 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 ≥ 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
Level 1		
ERBB2 Y772_A775dup (Exon 20 insertion)	Fam-trastuzumab deruxtecan-nxki	**sensitive**
Level 2		
ERBB2 Y772_A775dup (Exon 20 insertion)	Ado-trastuzumab emtansine	**sensitive**
Level 3B		
ERBB2 Y772_A775dup (Exon 20 insertion)	Trastuzumab	**sensitive**
Level 4		
ERBB2 Y772_A775dup (Exon 20 insertion)	Mobocertinib	**sensitive**
ERBB2 Y772_A775dup (Exon 20 insertion)	Erlotinib, Gefitinib, Lapatinib, Osimertinib	**resistant**
SMAD4 R361H	Cetuximab	**resistant**

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
1	FDA-recognized biomarkers predictive of response or resistance to FDA approved drugs in this indication
2	Standard care biomarkers (recommended by the NCCN guideline) predictive of response or resistance to FDA approved drugs in this indication
3A	Biomarkers predictive of response or resistance to therapies approved by the FDA or NCCN guideline in a different cancer type
3B	Biomarkers that serve as inclusion criteria for clinical trials (minimal supportive data required)
4	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
Not detected	

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

Genomic Alterations	Therapies	Effect	Level of Evidence	Cancer Type
SMAD4 R361H	Fluorouracil	**Resistant**	Clinical	Colorectal cancer

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

ERBB2 Y772_A775dup (Exon 20 insertion)

Biological Impact

The epidermal growth factor receptor 2 (HER2, or ERBB2) gene encodes a transmembrane receptor tyrosine kinase that belongs to the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases[1]. Amplification or activating mutations of ERBB2 can lead to aberrant activation of downstream pathways, such as phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascades, which are involved in cell survival and proliferation, respectively[1][2]. ERBB2 mutations are mostly observed in HER2-negative (non-overexpressed or non-amplified) samples[3].

ERBB2 Y772_A775dup mutation (also referred to as A775_G776insYVMA) results in the insertion of four amino acids in the protein kinase domain of the ERBB2 protein. This is a gain-of-function mutation that could result in constitutive phosphorylation of ERBB2[4]. Y772_A775dup is the most frequently appearing subtype of ERBB2 exon 20 insertions[5][6].

Therapeutic and prognostic relevance

Clinical trials of afatinib, dacomitinib and neratinib indicated that different ERBB2 variant subtypes responded differently to individual HER2-targeted agents. Some studies suggested that afatinib, dacomitinib, and neratinib was not effective in lung cancer patients harboring ERBB2 Y772_A775dup. In a retrospective study, afatinib treatment resulted in an ORR of 15.6% (5/32) and a DCR of 68.8% (22/32) in patients with lung adenocarcinoma harboring ERBB2 mutations; however, ORR (0%, 0/14), DCR (35.7%, 5/14), and median PFS of 1.2 months were significantly lower in patients with Y772_A775dup than those with other exon 20 insertions[6]. In addition, dacomitinib treatment also showed no partial response in patients with metastatic lung adenocarcinomas harboring ERBB2 Y772_A775dup according to the result of phase II trial (NCT00818441)[7]. In another phase II trial (SUMMIT; NCT01953926), neratinib treatment showed clinical response in breast cancer patients harboring ERBB2 Y772_A775dup or G788_P780dup; however, the response was not observed in NSCLC[8].

In a phase II trial (NCT03066206), poziotinib treatment resulted in a confirmed ORR of 42% and DCR of 83% in patients with NSCLC harboring ERBB2 Y772_A775dup (n=9) or ERBB2 G778_P780dup (n=3), with a median PFS of 5.6 months[9]. Besides, the combination of low-dose poziotinib and T-DM1 treatment showed antitumor efficacy in vivo[9].

There are case reports and cohort studies of NSCLC patients with HER2 exon 20 mutations, including Y772_A775dup, experiencing benefit from trastuzumab-based therapies[10][11], or ado-trastuzumab emtansine (T-DM1)[11]. In a phase II basket trial, three out of five lung cancer patients harboring Y772_A775dup showed partial response to T-DM1 treatment (NCT02675829)[12].

In preclinical studies, transformed cells expressing ERBB2 Y772_A775dup were sensitive to mobocertinib in vitro and in vivo, but resistant to lapatinib, osimertinib, and trastuzumab treatment in vitro[13][14].

Clinical trials and case reports have demonstrated that afatinib and dacomitinib treatment offer clinical benefits to NSCLC patients with ERBB2 exon 20 insertion[15][16][6][7]. Case reports have also shown that trastuzumab-based therapies or trastuzumab emtansine may benefit NSCLC patients with ERBB2 exon 20 insertion[17][11][11][18].

Preclinical studies have shown that neratinib and poziotinib can be effective against NSCLC cells with ERBB2 exon 20 insertion[19][9], which result in constitutive phosphorylation and activation of ERBB2, leading to resistance to EGFR tyrosine kinase inhibitors erlotinib and gefitinib[20][16].

Fam-trastuzumab deruxtecan-nxki is FDA-approved for treating adult patients with unresectable or metastatic NSCLC harboring ERBB2 activating mutations.

In NCCN guidelines for NSCLC, ERBB2 (HER2) mutations have been suggested as an emerging biomarker for ado-trastuzumab emtansine and fam-trastuzumab deruxtecan-nxki (T-DXd; DS-8201) in patients with NSCLC.

ERBB2 mutations have been determined as an inclusion criterion for the trials evaluating neratinib, lapatinib, afatinib, dacomitinib, and trastuzumab efficacies in multiple types of solid tumors (NCT01670877, NCT01953926, NCT01306045, NCT02780687, NCT00818441, and NCT02693535).

SMAD4 R361H

Biological Impact

The SMAD family member 4 (SMAD4) gene encodes a transcription factor that acts as a downstream effector in the TGF-β signaling pathway. Upon phosphorylated and activated by serine-threonine receptor kinase, Smad4 is the Co-Smad which recruits other activated R-Smad proteins to the Smad transcriptional complex and regulate TGF-β-targeted genes[21]. Smad4 has been identified as a haploinsufficient gene with one copy loss may lead to a weak protein expression and is insufficient to execute its original physiological function[22]. SMAD4 germline mutations are associated with juvenile polyposis syndrome (JPS)[23][24][25][26]. Somatic mutations of SMAD4 are commonly observed in pancreatic cancer[27], colorectal cancer (CRC)[25][28][29], and less frequently seen in other cancers such as lung adenocarcinoma[30], head and neck cancer[31][32], and cutaneous squamous cell carcinoma[33].

R361H is a hotspot mutation occurred within the MH2 domain of the SMAD4 protein (UniProtKB). This mutation was predicted to confer a loss of function on SMAD4 due to loss of heterocomplex formation[34].

Therapeutic and prognostic relevance

In Chinese patients with metastatic colorectal cancer, SMAD4 or NF1 mutations are suggested as a potential biomarker for poor prognosis to cetuximab-based therapy[35]. Preclinical data demonstrated that depletion of SMAD4 by shRNA knockdown increased clonogenic survival and cetuximab resistance in HPV-negative head and neck squamous cell carcinoma cells[36].

SMAD4 is also suggested as a predictive marker for 5-fluorouracil-based chemotherapy in colorectal cancer (CRC)[37][38]. CRC patients with normal SMAD4 diploidy exhibited three-fold higher benefit of 5-FU/mitomycin-based adjuvant therapy when compared with those with SMAD4 deletion[39].

Results from clinical and meta-analyses showed that loss of SMAD4 in CRC, pancreatic cancer was correlated with poor prognosis[40][41][42][43][44][45][46][47]. In cervical cancer patients, weak cytoplasmic SMAD4 expression and absent nuclear SMAD4 expression were shown to be significantly associated with poor disease-free and overall 5-year survival[48].

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US FDA-APPROVED DRUG(S)

Ado-trastuzumab emtansine (KADCYLA)

Ado-trastuzumab emtansine, also known as T-DM1, is a HER2-targeting monoclonal antibody conjugated with a microtubule inhibitor, emtansine (DM1). Ado-trastuzumab emtansine is developed and marketed by Genentech under the trade name KADCYLA.

- FDA Approval Summary of Ado-trastuzumab emtansine (KADCYLA)

EMILIA[49] NCT00829166	**Her2-receptor positive breast cancer** (Approved on 2013/02/22)
	HER2+
	Ado-trastuzumab emtansine vs. Lapatinib + capecitabine [PFS(M): 9.6 vs. 6.4]

Fam-trastuzumab deruxtecan-nxki (ENHERTU)

Fam-trastuzumab deruxtecan-nxki is a HER2-directed antibody and topoisomerase inhibitor conjugate. Fam-trastuzumab deruxtecan-nxki is manufactured by Daiichi Sankyo and marketed by Daiichi Sankyo and AstraZeneca under the trade name ENHERTU.

- FDA Approval Summary of Fam-trastuzumab deruxtecan-nxki (ENHERTU)

DESTINY-Lung02 NCT04644237	**Her2-mutated non-small cell lung cancer** (Approved on 2022/08/11)
	HER2 mutation
	Fam-trastuzumab deruxtecan-nxki [ORR(%): 58.0]
DESTINY-Breast04 NCT03734029	**Her2-low breast cancer** (Approved on 2022/08/05)
	HER2-low
	Fam-trastuzumab deruxtecan-nxki [PFS(M): 9.9 vs. 5.1]
DESTINY-Gastric01 NCT03329690	**Gastroesophageal adenocarcinomas, Gastric adenocarcinoma** (Approved on 2021/01/15)
	HER2+
	Fam-trastuzumab deruxtecan-nxki vs. Physician's choice of either irinotecan or paclitaxel monotherapy [ORR(%): 40.5 vs. 11.3, OS(M): 12.5 vs. 8.4]
DESTINY-Breast01[50] NCT03248492	**Her2-receptor positive breast cancer** (Approved on 2019/12/20)
	HER2+
	Fam-trastuzumab deruxtecan-nxki [ORR(%): 60.3]

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[51] 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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Trastuzumab (HERCEPTIN)

Trastuzumab is a monoclonal antibody that that targets the extracellular domain of the HER2 receptor. Trastuzumab is marketed in the United States by Genentech, in Japan by Chugai, and internationally by Roche, under the trade name HERCEPTIN.

- FDA Approval Summary of Trastuzumab (HERCEPTIN)

MOUNTAINEER NCT03043313	**Colorectal cancer** (Approved on 2023/01/19)
	HER2+ & RAS wild-type
	Tucatinib [ORR(%): 38.0, DOR(M): 12.4]
ToGA[52] NCT01041404	**Gastric adenocarcinoma** (Approved on 2010/10/20)
	HER2+
	Trastuzumab + cisplatin + fluoropyrimidine (fc) vs. Cisplatin + fluoropyrimidine (fc)+ placebo [OS(M): 13.1 vs. 11.7]
NSABP B-31[53] NCT00004067	**Her2-receptor positive breast cancer** (Approved on 2006/11/16)
	HER2+
	Trastuzumab + doxorubicin + cyclophosphamide followed by paclitaxel (ac→paclitaxel) vs. Placebo + doxorubicin + cyclophosphamide follow ed by paclitaxel (ac→paclitaxel) [DFS(%): 92.9 vs. 86.1]
NCCTG N9831[53] NCT00005970	**Her2-receptor positive breast cancer** (Approved on 2006/11/16)
	HER2+
	Trastuzumab + doxorubicin + cyclophosphamide followed by paclitaxel (ac→paclitaxel) vs. Placebo + doxorubicin + cyclophosphamide follow ed by paclitaxel (ac→paclitaxel) [DFS(%): 92.9 vs. 86.1]
[54]	**Her2-receptor positive breast cancer** (Approved on 1998/09/25)
	HER2+
	Paclitaxel or anthracycline + cyclophosphamide + trastuzumab vs. Paclitaxel or anthracycline + cyclophosphamide + placebo [TTP(M): 7.2 vs. 4.5]

D=day; W=w eek; 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

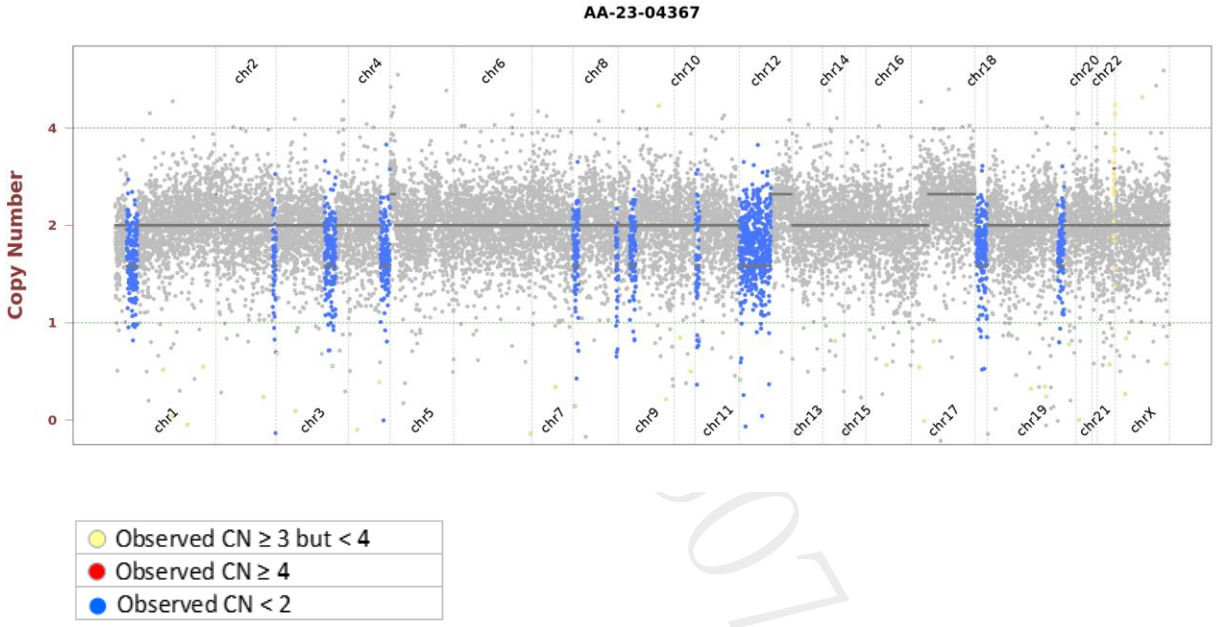
DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

- Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
ERBB2	Y772_A775dup (Exon 20 insertion)	20	c.2313_2324dup	NM_004448	COSM20959	34.9%	962
SMAD4	R361H	9	c.1082G>A	NM_005359	COSM14122	7.2%	889

- 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
ADAMTSL1	P1330L	22	c.3989C>T	NM_001040272	-	55.9%	1515
AMER1	R646W	2	c.1936C>T	NM_152424	-	51.3%	879
ATRX	Splice region	-	c.189+7A>G	NM_000489	-	43.1%	1099
BARD1	R529Q	7	c.1586G>A	NM_000465	-	49.0%	553
CARD11	S694L	16	c.2081C>T	NM_032415	COSM5505215	47.3%	560
EPHB1	R905C	15	c.2713C>T	NM_004441	-	42.6%	1441
HSPA4	T175S	5	c.524C>G	NM_002154	-	9.3%	894
IL7R	R140W	4	c.418C>T	NM_002185	COSM1695578	51.4%	834
NBN	V346M	9	c.1036G>A	NM_002485	COSM1258764	48.3%	963
SYNE1	S289T	10	c.866G>C	NM_182961	-	49.3%	708

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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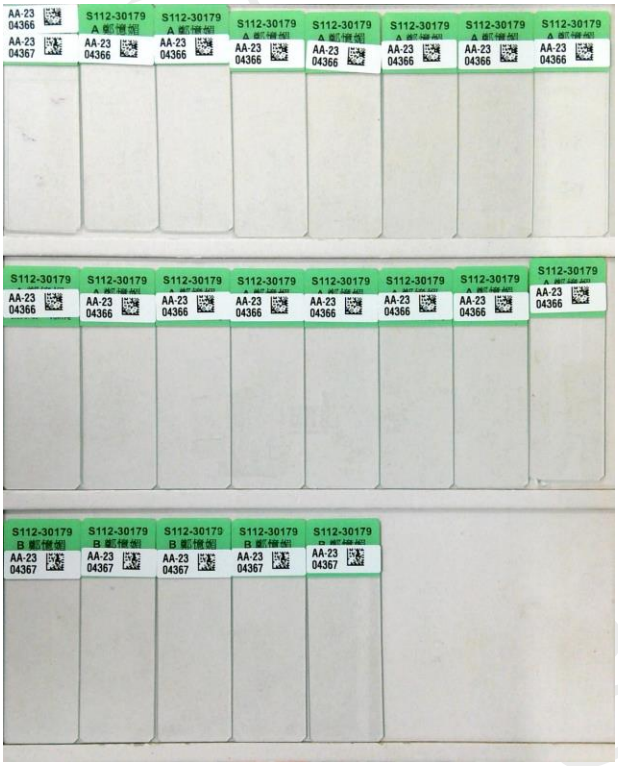
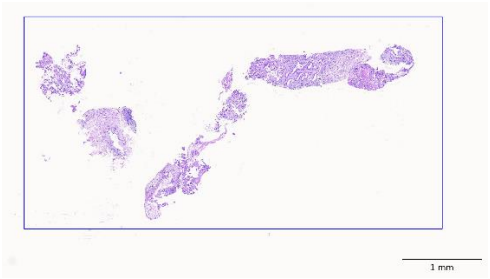
TEST DETAILS

SPECIMEN RECEIVED AND PATHOLOGY REVIEW





- Collection date: Jun 27, 2023
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11230179A, S11230179B
- Collection site: Lung
- Examined by: Dr. Yun-An Chen
  1. The percentage of viable tumor cells in total cells in the whole slide (%): 30%/40%
  2. The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 30%/40%
  3. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%/0%
  4. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%/0%
  5. Additional comment: NA
- Manual macrodissection: Not performed
- The outline highlights the area of malignant neoplasm annotated by a pathologist.

RUN QC

- Panel: ACTOnco®+

DNA test

- Mean Depth: 840x
- Target Base Coverage at 100x: 95%

RNA test

- Average unique RNA Start Sites per control GSP2: 111

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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 ≥ 20, allele frequency ≥ 5% and actionable variants with allele frequency ≥ 2% were retained. This test provides uniform coverage of the targeted regions, enabling target base coverage at 100x ≥ 85% with a mean coverage ≥ 500x.

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 ≥ 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®+ 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 ≥ 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 ≥ 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 ≥ 3; (2) Number of supporting reads spanning the fusion junction ≥ 5; (3) Percentage of supporting reads spanning the fusion junction ≥ 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:
解剖病理專科醫師朱盈霞
Ying-Hsia Chu, M.D.
病解字第 000653 號

Sign Off
解剖病理專科醫師朱盈霞
Ying-Hsia Chu, M.D.
病解字第 000653 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*	BTK	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*	SLCO1B1*
SLCO1B3*	SMAD2	SMAD3	SMAD4	SMARCA4	SMARCB1	SMO	SOCS1*	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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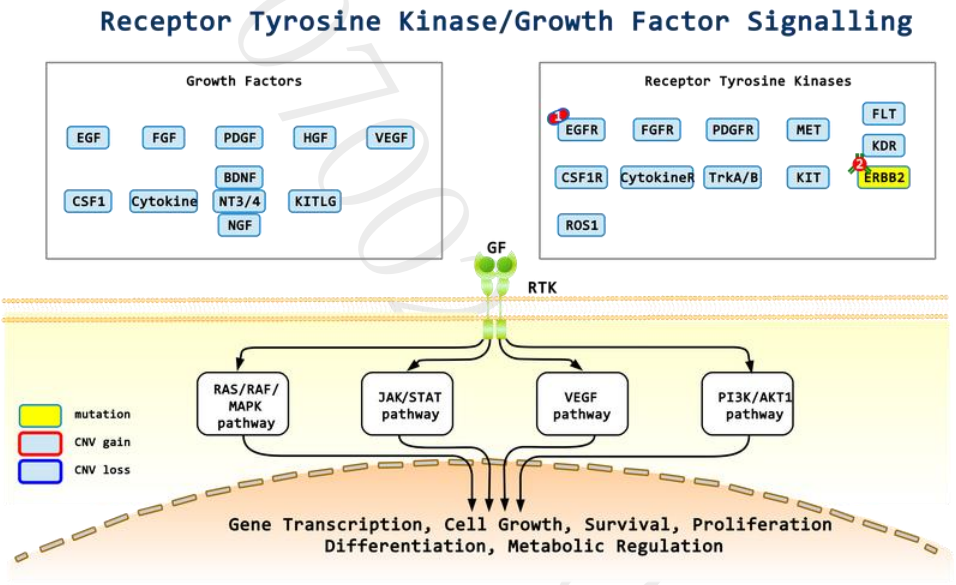
APPENDIX

POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

Not Applicable.

SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



1: Mobocertinib; 2: Trastuzumab, Ado-trastuzumab emtansine, Fam-trastuzumab deruxtecan-nxki

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DISCLAIMER

法律聲明

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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醫療決策需由醫師決定

任何治療與用藥需經由醫師在考慮病患所有健康狀況相關資訊包含健檢、其他檢測報告和病患意願後，依照該地區醫療照護標準由醫師獨立判斷。醫師不應僅依據單一報告結果(例如本檢測或本報告書內容)做決策。

基因突變與用藥資訊並非依照有效性排序

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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