

REPORT SUMMARY

PATIENT AND SAMPLE INFORMATION .....2

VARIANT(S) WITH CLINICAL RELEVANCE .....2

THERAPEUTIC IMPLICATIONS.....3

REPORT DETAILS

VARIANT INTERPRETATION .....5

US FDA-APPROVED DRUG(S) .....7

ONGOING CLINICAL TRIALS .....10

DETAILED TEST RESULTS .....11

HOTSPOT GENOTYPES.....13

TEST DETAILS .....15

ACTOnco<sup>®</sup>+ GENE LIST .....19

DISCLAIMER .....20

APPENDIX

SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS.....22

REFERENCES.....24

## PATIENT AND SAMPLE INFORMATION

### PATIENT

Name: 李正伸  
Gender: Male  
Date of Birth: Apr 28, 1962  
Patient ID: 1128581  
Diagnosis: Metastatic adenocarcinoma

### SPECIMEN

Type: FFPE tissue  
Date received: Dec 10, 2021  
Collection site: Soft tissue  
Specimen ID: S10938629A  
Lab ID: AA-21-06136  
D/ID: NA

### ORDERING PHYSICIAN

Name: 陳明晃醫師  
Facility: 臺北榮總  
Tel: 886-228712121  
Address: 臺北市北投區石牌路二段 201 號

## VARIANT(S) WITH CLINICAL RELEVANCE

Only variant(s) with clinical significance are listed. See the "DETAILED TEST RESULTS" section for full details.

### SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Not detected.

### COPY NUMBER VARIANTS (CNVS)

Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on **45%** tumor purity.

#### Amplification (Copy number ≥ 8)

Chr	Gene	Copy Number
ND	ND	ND

#### Homozygous deletion (Copy number=0)

Chr	Gene
ND	ND

#### Heterozygous deletion (Copy number=1)

Chr	Gene
chr9	CDKN2A, TSC1
chr17	FLCN

ND, Not Detected

### TUMOR MUTATIONAL BURDEN (TMB)

3.2 muts/Mb

Muts/Mb, mutations per megabase

### MICROSATELLITE INSTABILITY (MSI)

Microsatellite stable (MSS)

#### Note:

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%.

#### Variant Analysis:

醫檢師張筑芃 博士  
Chu-Yuan Chang Ph.D.  
檢字第 020115 號



#### Sign Off

醫檢師張筑芃 博士  
Chu-Yuan Chang Ph.D.  
檢字第 020115 號



## THERAPEUTIC IMPLICATIONS

### TARGETED THERAPIES

Genomic Alterations	Therapies	Effect
<b>Level 3B</b>		
<b>CDKN2A</b> Heterozygous deletion	Abemaciclib, Palbociclib, Ribociclib	<b>sensitive</b>
<b>Level 4</b>		
<b>FLCN</b> Heterozygous deletion	Everolimus	<b>sensitive</b>
<b>TSC1</b> Heterozygous deletion	Everolimus, Temsirolimus	<b>sensitive</b>

† Refer to "ONGOING CLINICAL TRIALS" section for detailed trial information.

**Note:** Therapies associated with benefit or lack of benefit are based on biomarkers detected in this tumor and published evidence.

Level	Description
1	FDA-recognized biomarker predictive of response to an FDA approved drug in this indication
2	Standard care biomarker (recommended as standard care by the NCCN or other expert panels) predictive of response to an FDA approved drug in this indication
3	A Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor
	B Biomarkers that serve as inclusion criteria for clinical trials
4	Biomarkers that show plausible therapeutic significance based on small studies, few case reports or preclinical studies

## IMMUNE CHECKPOINT INHIBITORS (ICI) THERAPIES

### Genomic markers and alterations that are associated with response to ICI therapies

Positive Biomarker	Negative Biomarker
TMB-H: ND	EGFR aberration: ND
MSI-H: ND	MDM2/MDM4 amplification: ND
MMR biallelic inactivation: ND	STK11 biallelic inactivation: ND
PBRM1 biallelic inactivation: ND	PTEN biallelic inactivation: ND
SERPINB3/SERPINB4 mutation: ND	B2M biallelic inactivation: ND
	JAK1/2 biallelic inactivation: ND

MMR, mismatch repair; ND, 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

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.

## VARIANT INTERPRETATION

### **CDKN2A Heterozygous deletion**

#### **Biological Impact**

The Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A) gene encodes the p16 (p16INK4a) and p14 (ARF) proteins. p16INK4a binds to CDK4 and CDK6, inhibiting these CDKs from binding D-type cyclins and phosphorylating the retinoblastoma (RB) protein<sup>[1][2]</sup> whereas p14 (ARF) blocks the oncogenic activity of MDM2 by inhibiting MDM2-induced degradation of p53<sup>[3]</sup>. CDKN2A has been reported as a haploinsufficient tumor suppressor with one copy loss that may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[4]</sup>. Loss of CDKN2A has been frequently found in human tumors that result in uncontrolled cell proliferation<sup>[5][6]</sup>.

#### **Therapeutic and prognostic relevance**

Intact p16-Cdk4-Rb axis is known to be associated with sensitivity to cyclin-dependent kinase inhibitors<sup>[7][8]</sup>. Several case reports also revealed that patients with CDKN2A-deleted tumors respond to the CDK4/6-specific inhibitor treatments<sup>[9][10][11]</sup>. However, there are clinical studies that demonstrated CDKN2A nuclear expression, CDKN2A/CDKN2B co-deletion, or CDKN2A inactivating mutation was not associated with clinical benefit from CDK4/6 inhibitors, such as palbociclib and ribociclib, in RB-positive patients<sup>[12][13][14]</sup>. However, CDKN2A loss or mutation has been determined as an inclusion criterion for the trial evaluating CDK4/6 inhibitors efficacy in different types of solid tumors (NCT02693535, NCT02187783).

Notably, the addition of several CDK4/6 inhibitors to hormone therapies, including palbociclib in combination with letrozole, ribociclib plus letrozole, and abemaciclib combines with fulvestrant, have been approved by the U.S. FDA for the treatment of ER+ and HER2- breast cancer<sup>[8][15][16]</sup>.

In a Phase I trial, a KRAS wild-type squamous non-small cell lung cancer (NSCLC) patient with CDKN2A loss had a partial response when treated with CDK4/6 inhibitor abemaciclib<sup>[10]</sup>. Administration of combined palbociclib and MEK inhibitor PD-0325901 yield promising progression-free survival among patients with KRAS mutant non-small cell lung cancer (NSCLC) (AACR 2017, Abstract CT046). Moreover, MEK inhibitor in combination with CDK4/6 inhibitor demonstrates significant anti-KRAS-mutant NSCLC activity and radiosensitizing effect in preclinical models<sup>[17]</sup>.

A retrospective analysis demonstrated that concurrent deletion of CDKN2A with EGFR mutation in patients with non-small cell lung cancer (NSCLC), predicts worse overall survival after EGFR-TKI treatment<sup>[18]</sup>.

## **FLCN Heterozygous deletion**

### **Biological Impact**

The FLCN gene encodes the tumor suppressor, Folliculin, a GTPase activating protein (GAP) for RagC/D GTPase proteins involved in amino acid sensing and signaling to mTORC1<sup>[19]</sup>. FLCN has been implicated as a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[20][21]</sup>. Inactivation of the FLCN gene by mutation or deletion results in the activation of the mTOR pathway and AKT signaling<sup>[22][23]</sup>. Germline mutation of the FLCN gene causes the Birt-Hogg-Dubé syndrome, a rare disorder that is characterized by benign hamartomatous skin lesions and an increased risk of pneumothorax and renal tumors<sup>[24]</sup>.

### **Therapeutic and prognostic relevance**

In a prospective Phase 2 study, four anaplastic thyroid cancer (ATC)/ poorly differentiated thyroid cancer (PDTC) patients who had PI3K/mTOR/AKT alterations, including TSC2, FLCN or NF1, showed impressive progression-free survival (PFS) of 15.2 months after receiving everolimus<sup>[25]</sup>. mTOR inhibition via rapamycin also demonstrated potential in inhibiting the growth of renal cells deficient in FLCN in the preclinical setting<sup>[26]</sup>.

## **TSC1 Heterozygous deletion**

### **Biological Impact**

The tuberous sclerosis complex 1 (TSC1) gene encodes a tumor suppressor, hamartin, a key negative regulator of the mammalian target of rapamycin (mTOR) pathway<sup>[27][28]</sup>. Mutations in TSC1/TSC2 tumor suppressor genes that result in inactivation of the complex are commonly found in patients with tuberous sclerosis<sup>[29][30][31]</sup>, while LOH in TSC1/TSC2 has been identified in head and neck squamous cell carcinoma (HNSCC)<sup>[32]</sup> and endometrial cancer<sup>[33]</sup>. Loss of single TSC1 allele (haploinsufficiency) may provide a growth advantage to bladder epithelial cells, contributing to bladder cancer development<sup>[34]</sup>. Both TSC1 and TSC2 mutations cause the autosomal dominant genetic disorder tuberous sclerosis complex (TSC), in which individuals develop a variety of benign but often progressive neoplasms<sup>[35]</sup>.

### **Therapeutic and prognostic relevance**

Genomic alterations with activating effects of the mTOR signaling pathway (including deletion/inactivation of TSC1/TSC2) have been shown to confer sensitivity to everolimus across multiple neoplasms, such as bladder tumors<sup>[36]</sup>, gastric, sarcoma, thyroid cancer, and HNSCC<sup>[37]</sup>. There were case reports demonstrated the efficacy of sirolimus in malignant uterine perivascular epithelioid cell tumors (PEComa) patients harboring mutations/deletions in TSC1 and TSC2 genes, and temsirolimus in PEComa patients with hyperactivated mTOR pathway. Genomic profiling analysis of GOG248, a Phase II study of temsirolimus or temsirolimus and alternating megestrol acetate and tamoxifen for advanced endometrial cancer showed that mutations in AKT1, TSC1 and TSC2 may predict clinical benefit from temsirolimus<sup>[38]</sup>. Recent studies indicate that there are mTORC1-independent signaling pathways downstream of hamartin-tuberin, which may represent new therapeutic targets<sup>[39]</sup>.

## US FDA-APPROVED DRUG(S)

### Abemaciclib (VERZENIO)

Abemaciclib is a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor. Abemaciclib is developed and marketed by Eli Lilly under the trade name VERZENIO.

#### FDA Approval Summary of Abemaciclib (VERZENIO)

<b>monarchE</b> NCT03155997	<b>Breast cancer</b> (Approved on 2021/10/12)
	<b>HR-positive, HER2-negative</b> Abemaciclib + tamoxifen/aromatase inhibitor vs. Tamoxifen/aromatase inhibitor [IDFS at 36 months(%): 86.1 vs. 79.0]
<b>MONARCH 3<sup>[40]</sup></b> NCT00246621	<b>Breast cancer</b> (Approved on 2018/02/26)
	<b>HR-positive, HER2-negative</b> Abemaciclib + anastrozole/letrozole vs. Placebo + anastrozole/letrozole [PFS(M): 28.2 vs. 14.8]
<b>MONARCH 1<sup>[41]</sup></b> NCT02102490	<b>Breast cancer</b> (Approved on 2017/09/28)
	<b>HR-positive, HER2-negative</b> Abemaciclib [ORR(%): 19.7 vs. 17.4]
<b>MONARCH 2<sup>[16]</sup></b> NCT02107703	<b>Breast cancer</b> (Approved on 2017/09/28)
	<b>HR-positive, HER2-negative</b> Abemaciclib + fulvestrant vs. Placebo + fulvestrant [PFS(M): 16.4 vs. 9.3]

### Everolimus (AFINITOR)

Everolimus, a derivative of sirolimus, works as an inhibitor of mammalian target of rapamycin complex 1 (mTORC1) and blocks mTORC1-mediated downstream signals for cell growth, proliferation, and survival. Everolimus is developed and marketed by Novartis under the trade name AFINITOR.

#### FDA Approval Summary of Everolimus (AFINITOR)

<b>RADIANT-4<sup>[42]</sup></b> NCT01524783	<b>Lung or gastrointestinal neuroendocrine tumor</b> (Approved on 2016/02/26)
	-
	Everolimus vs. Placebo [PFS(M): 11 vs. 3.9]



<b>BOLERO-2<sup>[43]</sup></b> NCT00863655	<b>Breast cancer</b> (Approved on 2012/07/20)
	ER+/HER2- Everolimus + exemestane vs. Placebo + exemestane [PFS(M): 7.8 vs. 3.2]
<b>RADIANT-3<sup>[44]</sup></b> NCT00510068	<b>Pancreatic neuroendocrine tumor</b> (Approved on 2011/05/05)
	- Everolimus vs. Placebo [PFS(M): 11 vs. 4.6]
<b>EXIST-1<sup>[45]</sup></b> NCT00789828	<b>Subependymal giant cell astrocytoma</b> (Approved on 2010/10/29)
	- Everolimus vs. Placebo [ORR(%): 35.0]
<b>RECORD-1<sup>[46]</sup></b> NCT00410124	<b>Renal cell carcinoma</b> (Approved on 2009/05/30)
	- Everolimus vs. Placebo [PFS(M): 4.9 vs. 1.9]

### Palbociclib (IBRANCE)

Palbociclib is an oral, cyclin-dependent kinase (CDK) inhibitor specifically targeting CDK4 and CDK6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Palbociclib is developed and marketed by Pfizer under the trade name IBRANCE.

### FDA Approval Summary of Palbociclib (IBRANCE)

<b>PALOMA-2<sup>[47]</sup></b> NCT01740427	<b>Breast cancer</b> (Approved on 2017/03/31)
	ER+, HER2- Palbociclib + letrozole vs. Placebo + letrozole [PFS(M): 24.8 vs. 14.5]
<b>PALOMA-3<sup>[48]</sup></b> NCT01942135	<b>Breast cancer</b> (Approved on 2016/02/19)
	ER+, HER2- Palbociclib + fulvestrant vs. Placebo + fulvestrant [PFS(M): 9.5 vs. 4.6]



### Ribociclib (KISQALI)

Ribociclib is a cyclin-dependent kinase (CDK) inhibitor specifically targeting cyclin D1/CDK4 and cyclin D3/CDK6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Ribociclib is developed by Novartis and Astex Pharmaceuticals and marketed by Novartis under the trade name KISQALI.

### FDA Approval Summary of Ribociclib (KISQALI)

<b>MONALEESA-2<sup>[15]</sup></b>  NCT01958021	<b>Breast cancer</b> (Approved on 2017/03/13)
	<b>HR+, HER2-</b>
	Ribociclib vs. Letrozole [PFS(M): NR vs. 14.7]

### Temsirolimus (TORISEL)

Temsirolimus is a soluble ester of sirolimus (rapamycin, brand-name drug Rapamune) and functions as an inhibitor of mammalian target of rapamycin complex (mTORC). The inhibitory molecular mechanism is similar to Everolimus. Temsirolimus is developed by Wyeth Pharmaceuticals and marketed by Pfizer under the trade name TORISEL.

### FDA Approval Summary of Temsirolimus (TORISEL)

<b>[49]</b>  NCT00065468	<b>Renal cell carcinoma</b> (Approved on 2007/05/30)
	-
	Temsirolimus vs. Ifn- $\alpha$ [OS(M): 10.9 vs. 7.3]

d=day; w=week; m=month

## ONGOING CLINICAL TRIALS

Clinical trials shown below were selected 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.

## DETAILED TEST RESULTS

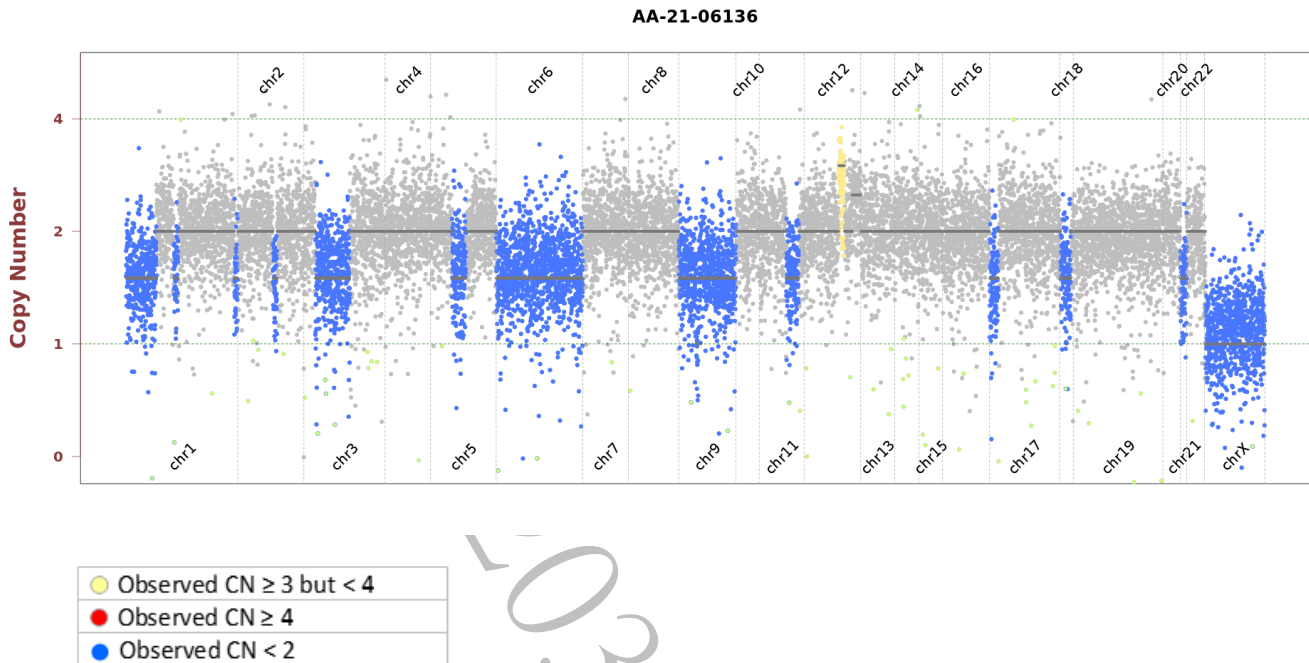
### SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Gene	Chr	Exon	Accession Number	cDNA Change	Amino Acid Change	Coverage	Allele Frequency	COSMIC ID
ADAMTS15	11	1	NM_139055	c.287C>T	S96L	260	23.8%	-
ATRX	X	9	NM_000489	c.1007C>T	S336F	657	45.5%	-
CANX	5	-	NM_001746	c.447-4T>C	Splice region	942	48.9%	-
EGFR	7	28	NM_005228	c.3353C>T	A1118V	1879	56.4%	COSM7293955
FAT1	4	10	NM_005245	c.5881G>A	G1961S	2635	50.5%	COSM6476268
LRP1B	2	37	NM_018557	c.5968A>G	I1990V	953	21.9%	-
MUC16	19	1	NM_024690	c.7201G>T	A2401S	1318	51.7%	-
MUC16	19	3	NM_024690	c.22216C>T	P7406S	1172	20.8%	COSM3542645

Mutations with clinical relevance are highlighted in red.

## COPY NUMBER VARIANTS (CNVs)

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.



## HOTSPOT GENOTYPES

Listed variants are biomarkers or hotspots that are recommended as standard care by the NCCN or other expert panels and not necessarily FDA-recognized for a particular indication. The genotypes have been manually checked to ensure sufficient coverage for each hotspot of the target gene.

Gene	Variant	Genotype Detected
<i>BRAF</i>	V600X	Not detected
<i>EGFR</i>	A763_Y764insFQEA, E709K, E709_T710delinsD, Exon 19 deletion, Exon 19 insertion, Exon 20 insertion, G719A/C/D/S, L747P, L833V, L858R, L861Q/R, S768I, T790M	Not detected
<i>IDH2</i>	R140Q, R172G/K/M/S	Not detected
<i>KIT</i>	A502_Y503dup, D419del, D579del, D816F/V/Y, D820A/E/G/Y, E554_I571del, E554_K558del, E554_V559del, Exon 11 mutation, F522C, H697Y, I563_L576del, I653T, K550_W557del, K558N, K558_E562del, K558_V559del, K558delinsNP, K642E, M552_W557del, N505I, N564_Y578del, N822H/I/K/Y, P551_M552del, P573_D579del, P577_D579del, P577_W582delinsPYD, P838L, Q556_K558del, T417_D419delinsI, T417_D419delinsRG, T574_Q575insTQLPYD, V530I, V555_L576del, V555_V559del, V559A/C/D/G, V559_V560del, V559del, V560D/G, V560del, V569_L576del, V654A, W557G/R, W557_K558del, Y553N, Y553_K558del, Y570H, Y578C	Not detected
<i>KRAS</i>	A146T/V/P, G12X, G13X, Q61X	Not detected
<i>MET</i>	D1028H/N/Y	Not detected
<i>NRAS</i>	G12X, G13X, Q61X	Not detected
<i>PDGFRA</i>	A633T, C450_K451insMIEWMI, C456_N468del, C456_R481del, D568N, D842I/V, D842_H845del, D842_M844del, D846Y, E311_K312del, G853D, H650Q, H845Y, H845_N848delinsP, I843del, N659K/R/S, N848K, P577S, Q579R, R560_V561insER, R748G, R841K, S566_E571delinsR, S584L, V469A, V536E, V544_L545insAVLVLLVIVISLI, V561A/D, V561_I562insER, V658A, W559_R560del, Y375_K455del, Y555C, Y849C/S	Not detected
<i>PIK3CA</i>	C420R, E542K/V, E545A/D/G/K, H1047X, Q546E/R	Not detected

V600X= any mutation in the valine (V) at amino acid 600 being replaced by a different amino acid.

G12X= any mutation in the glycine (G) at amino acid 12 being replaced by a different amino acid.

G13X= any mutation in the glycine (G) at amino acid 13 being replaced by a different amino acid.

Q61X= any mutation in the glutamine (Q) at amino acid 61 being replaced by a different amino acid.

H1047X= any mutation in the histidine (H) at amino acid 1047 being replaced by a different amino acid.

Gene	Copy Number Detected
<i>CDK4</i>	2
<i>EGFR</i>	2
<i>ERBB2</i>	2
<i>MET</i>	2

Copy number  $\geq 8$  is considered amplification

Other known alterations that are associated with sensitivity, resistance, and toxicity to therapies.

Gene	Variant	Genotype Detected
AKT1	E17K	Not detected
ALK	C1156Y, D1203N, G1202R, L1152R, S1206Y, T1151_L1152insT	Not detected
BRAF	K601E, L597V/Q/R/S	Not detected
DPYD	D949V, I560S, splice-site mutation	Not detected
EGFR	A750P, C797S/Y, S492R	Not detected
ERBB2	V659E	Not detected
ESR1	D538G, E380Q, L469V, L536H/P/Q/R, S432L, S463P, V422del, V534E, Y537C/N/S	Not detected
FGFR3	G370C, G380R, K650E/N/R/M/T/Q, R248C, S249C, S371C, Y373C	Not detected
IDH1	R132C/G/H/L/Q/S	Not detected
MAP2K1	D67N, E203K, F53L, K57E/N, P124S, Q56P, Q56_V60del, R47Q, R49L, S222D	Not detected
PTEN	R130*/fs/G/L/P/Q	Not detected
TPMT	A154T, Y240C	Not detected

Gene	Copy Number Detected
FGFR1	2
MDM2	2
MDM4	2

Copy number  $\geq 8$  is considered amplification

## TEST DETAILS

### 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) ( $\leq 15$  nucleotides) and large-scale genomic alterations like copy number variations (CNVs).

See ACTOnco®+ Gene List' Section for details of gene sequenced.

### DATABASE USED

- Reference genome: human genome sequence hg19
- COSMIC v.92
- Genome Aggregation database r2.1.1
- ClinVar (version 20210208)
- ACT Genomics in-house database

### NEXT-GENERATION SEQUENCING (NGS) METHODS

Extracted genomic DNA was amplified using four pools of primer pairs targeting coding exons of analyzed genes. Amplicons were ligated with barcoded adaptors. Quality and quantity of amplified library were determined using the fragment analyzer (AATI) and Qubit (Invitrogen). Barcoded libraries were subsequently conjugated with sequencing beads by emulsion PCR and enriched using Ion Chef system (Thermo Fisher Scientific) according to the Ion PI Hi-Q Chef Kit protocol (Thermo Fisher Scientific) or Ion 540 Kit-Chef protocol (Thermo Fisher Scientific). Sequencing was performed on the Ion Proton or Ion S5 sequencer (Thermo Fisher Scientific).

Raw reads generated by the sequencer were mapped to the hg19 reference genome using the Ion Torrent Suite (version 5.10). 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 (version 5.10). The coverage was down-sampled to 4000. VEP (Variant Effect Predictor) (version 100) was used to annotate every variant using databases from Clinvar (version 20210208), COSMIC v.92 and Genome Aggregation database r2.1.1. Variants with coverage  $\geq 25$ , 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  $100x \geq 85\%$  with a mean coverage  $\geq 500x$ .

Variants reported in Genome Aggregation database r2.1.1 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 variations (CNVs) 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 from samples in ACT Genomics in-house database.

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  $\geq 7.5$  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).

## STANDARD OPERATING PROCEDURES (SOPS)

Standard operating procedures (SOPs) are shown below:

- AG2-QP-15 Specimen Management Procedure
- AG3-QP16-03 SOP of Cancer Cell DNA and RNA Extraction
- AG3-QP16-07 SOP of Nucleic Acid Extraction with QIAasympyphony SP
- AG3-QP16-08 SOP of FFPE Nucleic Acid Extraction
- AG3-QP16-10 SOP of HE Staining
- AG3-QP16-13 SOP of Library Construction and Preparation
- AG3-QP16-17 SOP of DNA Quantification with Qubit Fluorometer
- AG3-QP16-20 SOP of CE-Fragment Analysis
- AG3-QP16-22 SOP of Variant Calling
- AG3-QP16-24 SOP of Ion Torrent System Sequencing Reaction
- AG3-QP16-26 SOP of Ion Chef Preparation

行動基因僅提供技術檢測服務及檢測報告，檢測結果之臨床解釋及相關醫療處置，請諮詢專業醫師。報告結果僅對此試驗件有效。

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AG4-QP4001-02(05)

Page 16 of 27

- AG3-QP16-35 SOP of Variant Annotation
- AG3-QP16-96 SOP of Manual Inspection for SNV/Indel Variant
- AG3-QP16-95 SOP of Manual Inspection for Copy Number Variant
- AG3-QP40-08 (02) Standard protocol for variant interpretation, curation and classification
- AG3-QP16-41 SOP of The user manual for clinical report system (CRS)

## LIMITATIONS

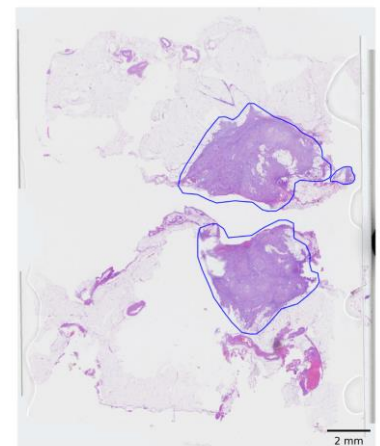
This test does not provide information of variant causality and does not detect variants in non-coding regions that could affect gene expression. This report does not report polymorphisms and we do not classify whether a mutation is germline or somatic. Variants identified by this assay were not subject to validation by Sanger or other technologies.

## NOTES

We do not exclude the possibility that pathogenic variants may not be reported by one or more of the tools and the parameters used.

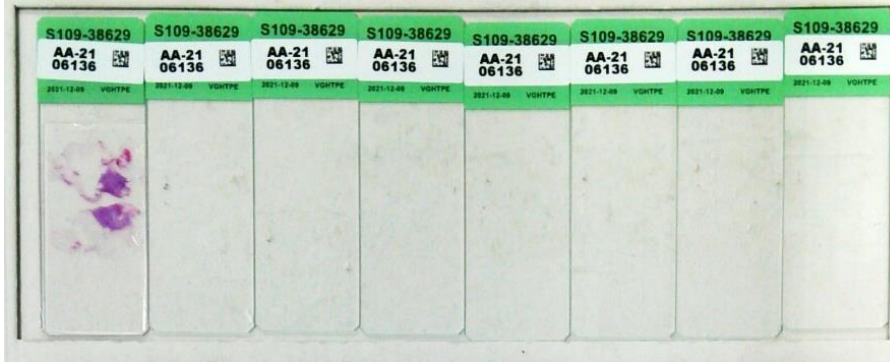
## PATHOLOGY EVALUATION

- H&E-stained section No.: S10938629A
- Collection site: Soft tissue
- Examined by: Dr. Pei-Yi Chu
- Estimated neoplastic nuclei (whole sample): The percentage of viable tumor cells in total cells in the whole slide (%): 40%  
The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 45%  
The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%  
The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%  
Additional comment: NA
- Manual macrodissection: Performed on the highlighted region



The outline highlights the area of malignant neoplasm annotated by a pathologist.

## SPECIMEN PHOTO(S)



- Collection date: Nov 2020
- Facility retrieved: 臺北榮總

## RUN QC

- Panel: ACTOnco®+
- Mean Depth: 857x
- Target Base Coverage at 100x: 94%

## ACTOnco® + GENE LIST

ABCB1*	AURKB	CBL	CDKN2B	E2F3	FAT1	GRIN2A	JAK2	MED12	NOTCH4	PMS1	RAD51D	SLCO1B3*	TNFRSF14
ABCC2*	AXIN1	CCNA1	CDKN2C	EGFR	FBXW7	GSK3B	JAK3	MEF2B	NPM1	PMS2	RAD52	SMAD2	TNFSF11
ABCG2*	AXIN2	CCNA2	CEBPA*	EP300	FCGR2B	GSTP1*	JUN*	MEN1	NQO1*	POLB	RAD54L	SMAD3	TOP1
ABL1	AXL	CCNB1	CHEK1	EPCAM	FGF1*	GSTT1*	KAT6A	MET	NRAS	POLD1	RAF1	SMAD4	TP53
ABL2	B2M	CCNB2	CHEK2	EPHA2	FGF10	HGF	KDM5A	MITF	NSD1	POLE	RARA	SMARCA4	TPMT*
ADAMTS1	BAP1	CCNB3	CIC	EPHA3	FGF14	HIF1A	KDM5C	MLH1	NTRK1	PPARG	RB1	SMARCB1	TSC1
ADAMTS13	BARD1	CCND1	CREBBP	EPHA5	FGF19*	HIST1H1C*	KDM6A	MPL	NTRK2	PPP2R1A	RBM10	SMO	TSC2
ADAMTS15	BCL10	CCND2	CRKL	EPHA7	FGF23	HIST1H1E*	KDR	MRE11	NTRK3	PRDM1	RECQL4	SOC1*	TSHR
ADAMTS16	BCL2*	CCND3	CRLF2	EPHB1	FGF3	HNF1A	KEAP1	MSH2	PAK3	PRKAR1A	REL	SOX2*	TYMS
ADAMTS18	BCL2L1	CCNE1	CSF1R	ERBB2	FGF4*	HR	KIT	MSH6	PALB2	PRKCA	RET	SOX9	U2AF1
ADAMTS6	BCL2L2*	CCNE2	CTCF	ERBB3	FGF6	HRAS*	KMT2A	MTHFR*	PARP1	PRKCB	RHOA	SPEN	UBE2A*
ADAMTS9	BCL6	CCNH	CTLA4	ERBB4	FGFR1	HSP90AA1	KMT2C	MTOR	PAX5	PRKCG	RICTOR	SPOP	UBE2K
ADAMTSL1	BCL9	CD19	CTNNA1	ERCC1	FGFR2	HSP90AB1	KMT2D	MUC16	PAX8	PRKCI	RNF43	SRC	UBR5
ADGRA2	BCOR	CD274	CTNNB1	ERCC2	FGFR3	HSPA4	KRAS	MUC4	PBRM1	PRKCQ	ROS1	STAG2	UGT1A1*
ADH1C*	BIRC2	CD58	CUL3	ERCC3	FGFR4	HSPA5	LCK	MUC6	PDCD1	PRKDC	RPPH1	STAT3	USH2A
AKT1	BIRC3	CD70*	CYLD	ERCC4	FH	IDH1	LIG1	MUTYH	PDCD1LG2	PRKN	RPTOR	STK11	VDR*
AKT2	BLM	CD79A	CYP11A1*	ERCC5	FLCN	IDH2	LIG3	MYC	PDGFRA	PSMB8	RUNX1	SUFU	VEGFA
AKT3	BMPR1A	CD79B	CYP2B6*	ERG	FLT1	IFNL3*	LMO1	MYCL	PDGFRB	PSMB9	RUNX1T1	SYK	VEGFB
ALDH1A1*	BRAF	CDC73	CYP2C19*	ESR1	FLT3	IGF1	LRP1B	MYCN	PDIA3	PSME1	RXRA	SYNE1	VHL
ALK	BRCA1	CDH1	CYP2C8*	ESR2	FLT4	IGF1R	LYN	MYD88	PGF	PSME2	SDHA	TAF1	WT1
AMER1	BRCA2	CDK1	CYP2D6	ETV1	FOXL2*	IGF2	MALT1	NAT2*	PHOX2B*	PSME3	SDHB	TAP1	XIAP
APC	BRD4	CDK12	CYP2E1*	ETV4	FOXP1	IKKB	MAP2K1	NBN	PIK3C2B	PTCH1	SDHC	TAP2	XPO1
AR	BRIP1	CDK2	CYP3A4*	EZH2	FRG1	IKBKE	MAP2K2	NEFH	PIK3C2G	PTEN	SDHD	TAPBP	XRCC2
ARAF	BTG1*	CDK4	CYP3A5*	FAM46C	FUBP1	IKZF1	MAP2K4	NF1	PIK3C3	PTGS2	SERPINB3	TBX3	ZNF217
ARID1A	BTG2*	CDK5	DAXX	FANCA	GATA1	IL6	MAP3K1	NF2	PIK3CA	PTPN11	SERPINB4	TEK	
ARID1B	BTK	CDK6	DCUN1D1	FANCC	GATA2	IL7R	MAP3K7	NFE2L2	PIK3CB	PTPRD	SETD2	TERT	
ARID2	BUB1B	CDK7	DDR2	FANCD2	GATA3	INPP4B	MAPK1	NFKB1	PIK3CD	PTPRT	SF3B1	TET1	
ASXL1	CALR	CDK8	DICER1	FANCE	GNA11	INSR	MAPK3	NFKBIA	PIK3CG	RAC1	SGK1	TET2	
ATM	CANX	CDK9	DNMT3A	FANCF	GNA13	IRF4	MAX	NKX2-1*	PIK3R1	RAD50	SH2D1A*	TGFBR2	
ATR	CARD11	CDKN1A	DOT1L	FANCG	GNAQ	IRS1	MCL1	NOTCH1	PIK3R2	RAD51	SLC19A1*	TMSB4X*	
ATRX	CASP8	CDKN1B	DPYD	FANCL	GNAS	IRS2*	MDM2	NOTCH2	PIK3R3	RAD51B	SLC22A2*	TNF	
AURKA	CBFB	CDKN2A	DTX1	FAS	GREM1	JAK1	MDM4	NOTCH3	PIM1	RAD51C	SLCO1B1*	TNFAIP3	

\*Analysis of copy number alteration not available.

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行動基因臨床分子醫學實驗室 台北市內湖區新湖二路 345 號 3F

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AG4-QP4001-02(05)

## DISCLAIMER

### Legal Statement

This test was developed by ACT Genomics and its performing characteristics were determined by ACT Genomics. This test result is to be used for clinical consultative purposes only and is not intended as a substitute for a clinical guidance of your doctor or another qualified medical practitioner. It should not be regarded as investigational or used for research.

The detection of genomic alterations does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; the detection of no genomic alteration does not necessarily indicate lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment regimen.

### Treatment Decisions are the Responsibility of the Physician

Decisions on clinical care and treatment should be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, including physical examinations, information from other diagnostics tests and patient preferences, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test, such as this test, or the information contained in this report.

In terms of consulting a different treating physician, the patient must file an application and fulfill the listed criteria for ACT Genomics to provide the patient's report to the assigned physician. The report may not be copied or reproduced except in its totality.

### Genetic Alterations and Drugs Not Presented in Ranked Order

In this report, neither any biomarker alteration nor any drug associated with a potential clinical benefit (or potential lack of clinical benefit), are ranked in order of potential or predicted efficacy.

### Level of Evidence Provided

Drugs with a potential clinical benefit (or potential lack of clinical benefit) are evaluated for level of published evidence with at least one clinical efficacy case report or preclinical study. We endeavor to keep the information in the report up to date. However, customers must be aware that scientific understanding and technologies change over time, and we make no warranty as to the accuracy, suitability or currency of information provided in this report at any time.

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### Liability

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### 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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本公司於提供檢驗報告後，即已完成本次契約義務，後續之報告解釋、判讀及用藥、治療，應自行尋求相關專業醫師協助，若需將報告移件其他醫師，本人應取得該醫師同意並填寫移件申請書，主動告知行動基因，行動基因僅能配合該醫師意願與時間提供醫師解說。

### 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

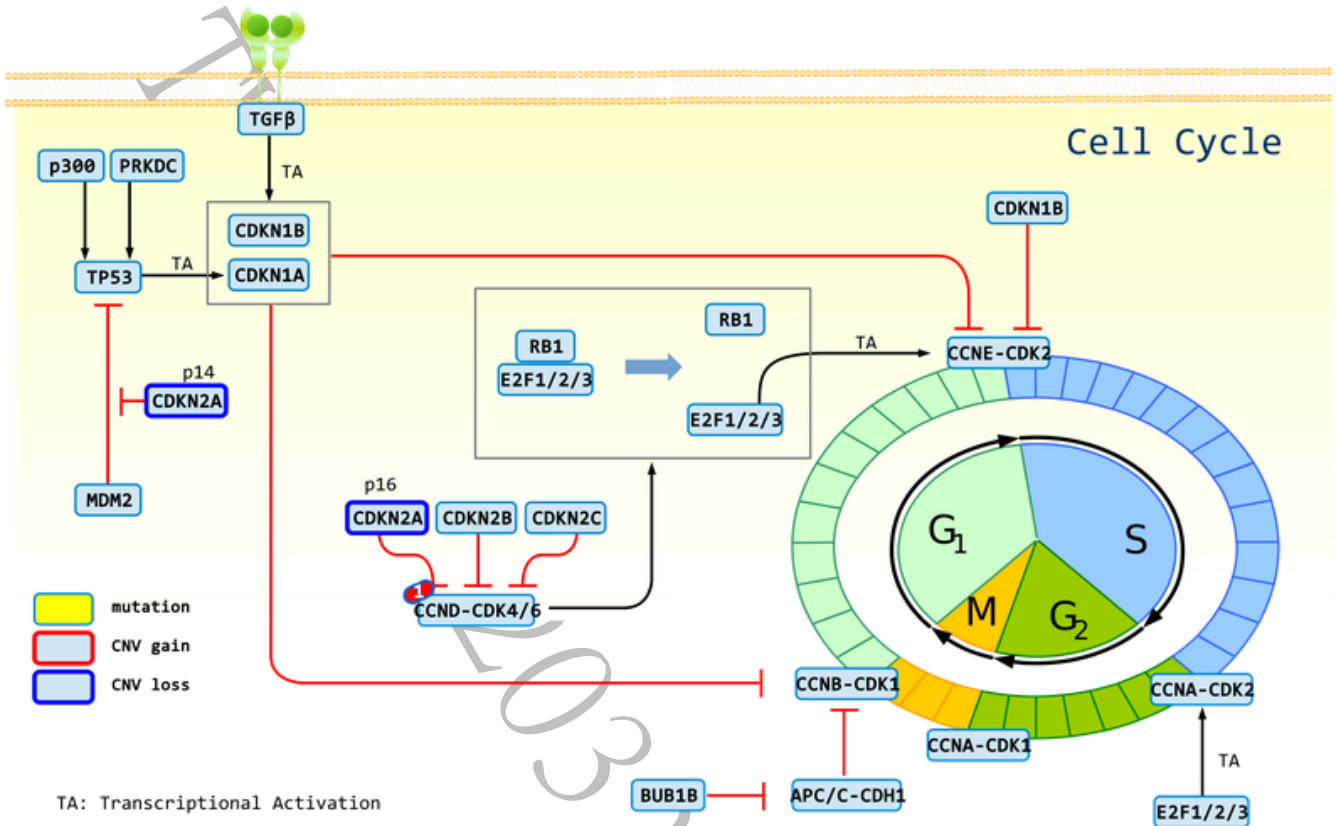
藥物潛在臨床效益(或缺乏潛在臨床效益)的實證證據是依據至少一篇臨床療效個案報告或臨床前試驗做為評估。本公司盡力提供適時及準確之資料，但由於醫學科技之發展日新月異，本公司不就本報告提供的資料是否為準確、適宜或最新作保證。

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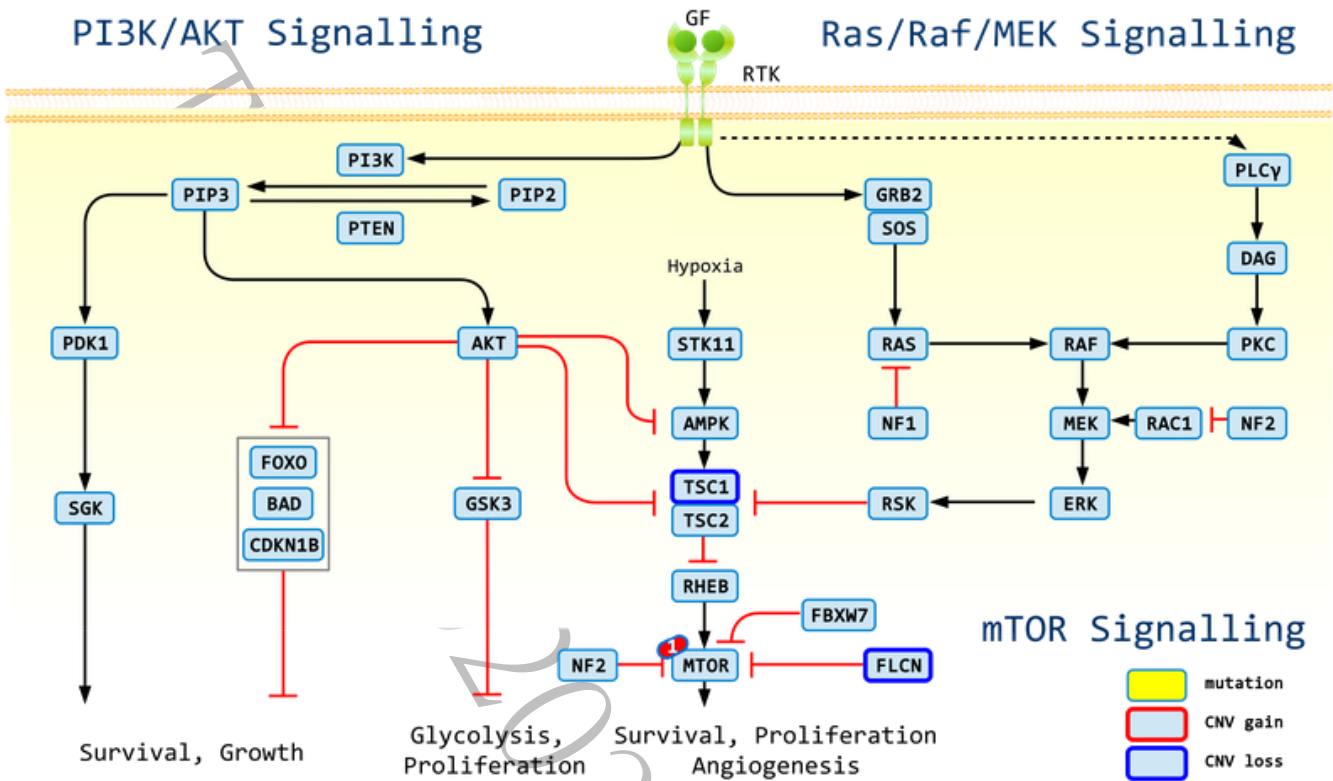


## SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



1: Abemaciclib, Palbociclib, Ribociclib





1: Everolimus, Temsirolimus

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Temsirrolimus, interferon alfa, or both for advanced renal-cell carcinoma.

PMID: 17538086; 2007, N Engl J Med;356(22):2271-81

# ACTFusion™ Report

PATIENT		
Name: 李正伸		Patient ID: 1128581
Date of Birth: Apr 28, 1962		Gender: Male
Diagnosis: Metastatic adenocarcinoma		
ORDERING PHYSICIAN		
Name: 陳明晃醫師		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: S10938629A	Collection site: Soft tissue	Date received: Dec 10, 2021
Lab ID: AA-21-06136	Type: FFPE tissue	D/ID: NA

## ABOUT ACTFusion™

The test is a next-generation sequencing (NGS) based in vitro diagnostic assay to detect fusion transcripts of 13 genes, including *ALK*, *BRAF*, *EGFR*, *FGFR1*, *FGFR2*, *FGFR3*, *MET*, *NRG1*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, and *ROS1*.

## TESTING RESULTS

### VARIANT(S) WITH CLINICAL RELEVANCE

#### - Fusions

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

# ACTFusion™ Report

## THERAPEUTIC IMPLICATION

Not Applicable.

## VARIANT INTERPRETATION

Not Applicable.

## US FDA-APPROVED DRUG(S)

Not Applicable.



# ACTFusion™ Report

## ONGOING CLINICAL TRIALS

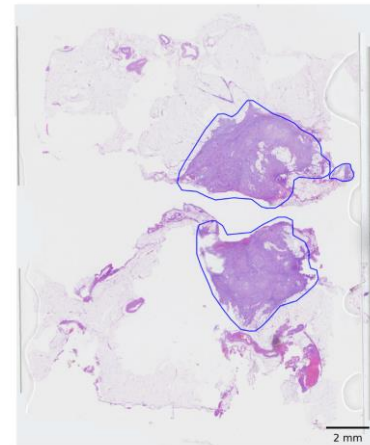
Trials were searched by applying filters: study status, patient's diagnosis, intervention, location and/or biomarker(s). Please visit <https://clinicaltrials.gov> to search and view for a complete list of open available and updated matched trials.

No trial has been found.

# ACTFusion™ Report

## TEST DETAILS

### SPECIMEN RECEIVED AND PATHOLOGY REVIEW



- Collection date: Nov 2020
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S10938629A
- Collection site: Soft tissue
- Examined by: Dr. Pei-Yi Chu
  1. The percentage of viable tumor cells in total cells in the whole slide (%): 40%
  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: ACTFusion™
- Total reads: 796568
- Average unique RNA Start Sites per control GSP2: 94

## LIMITATIONS

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.

# ACTFusion™ Report

## NEXT-GENERATION SEQUENCING (NGS) METHODS

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  $\geq 10$ .

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.

## STANDARD OPERATING PROCEDURES (SOPs)

- AG2-QP-15 Specimen Management Procedure
- AG3-QP16-08 SOP of FFPE Nucleic Acid Extraction
- AG3-QP16-10 SOP of HE Staining
- AG3-QP16-17 SOP of DNA Quantification with Qubit Fluorometer
- AG3-QP16-20 SOP of CE-Fragment Analysis
- AG3-QP16-24 SOP of Ion Torrent System Sequencing Reaction
- AG3-QP16-26 SOP of Ion Chef Preparation
- AG3-QP40-08 (02) Standard protocol for variant interpretation, curation and classification
- AG3-QP16-94 (01) SOP of ACTFusion v3 Library Construction and Preparation
- AG3-QP16-36(02) SOP of Fusion Gene Detection
- AG3-QP16-41 SOP of The user manual for clinical report system (CRS)

## DATABASES USED

- Quiver Gene Fusion Database version 5.1.18

## GENE LIST

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

### Variant Analysis:

醫檢師張筑芃 博士  
Chu-Yuan Chang Ph.D.  
檢字第 020115 號



### Sign Off

醫檢師張筑芃 博士  
Chu-Yuan Chang Ph.D.  
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# ACTFusion™ Report

## 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。

本檢驗報告非經本公司許可，不得私自變造、塗改，或以任何方式作為廣告及其他宣傳之用途。

本公司於提供檢驗報告後，即已完成本次契約義務，後續之報告解釋、判讀及用藥、治療，應自行尋求相關專業醫師協助，若需將報告移件其他醫師，本人應取得該醫師同意並填寫移件申請書，主動告知行動基因，行動基因僅能配合該醫師意願與時間提供醫師解說。

### 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。

### 責任

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# ACTFusion™ Report

## REFERENCE

Not Applicable.