

# ACT Onco<sup>®</sup> + Report

PATIENT		
Name: 周玉萍		Patient ID: 24765819
Date of Birth: Dec 27, 1973		Gender: Female
Diagnosis: Pancreatic cancer		
ORDERING PHYSICIAN		
Name: 姜乃榕醫師		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: S11171314A	Collection site: Pancreas	Type: FFPE tissue
Date received: Oct 03, 2022	Lab ID: AA-22-05889	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	
Not detected			

### VARIANTS/BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Genomic Alterations/Biomarkers	Possibly Sensitive	Possibly Resistant
FBXW7 Q98*	Everolimus, Temsirolimus	Gefitinib, Regorafenib
KRAS G12R	-	Afatinib, Cetuximab, Dacomitinib, Erlotinib, Gefitinib, Osimertinib, Panitumumab
AKT2 Amplification	-	Erlotinib

#### 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
<i>FBXW7</i>	Q98*	29.9%
<i>KRAS</i>	G12R	56.6%
<i>TP53</i>	R248W	39.4%

#### - Copy Number Alterations

Chromosome	Gene	Variation	Copy Number
Chr17	<i>TP53</i>	Heterozygous deletion	1
Chr9	<i>CDKN2A</i>	Heterozygous deletion	1
Chr19	<i>AKT2</i>	Amplification	9

#### - 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)	1.3 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 60% tumor purity.
- For more therapeutic agents which are possibly respond to heterozygous deletion of genes listed above, please refer to APPENDIX for more information.
- 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 3A</b>		
<b>KRAS</b> G12R	Afatinib, Cetuximab, Dacomitinib, Erlotinib, Gefitinib, Osimertinib, Panitumumab	<b>resistant</b>
<b>Level 4</b>		
<b>FBXW7</b> Q98*	Everolimus, Temsirolimus	<b>sensitive</b>
<b>AKT2</b> Amplification	Erlotinib	<b>resistant</b>
<b>FBXW7</b> Q98*	Gefitinib, Regorafenib	<b>resistant</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
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
<b>TP53</b> R248W	Platinum- and taxane-based regimens	<b>Less sensitive</b>	Clinical	Ovarian 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

### FBXW7 Q98\*

#### Biological Impact

The F-box/WD repeat-containing protein 7 (FBXW7) gene encodes a protein that belongs to the SCF (SKP1-CUL1-F-box protein) E3 ligase complex. FBXW7 is recognized as a tumor suppressor which is involved in the negative regulation of oncogenes such as c-Myc<sup>[1][2]</sup>, c-Jun<sup>[3]</sup>, cyclin E<sup>[4]</sup>, Notch family members<sup>[5][6]</sup>, Aurora-A<sup>[7]</sup>, mTOR<sup>[8]</sup>, KLF5<sup>[9]</sup>, and MCL-1<sup>[10]</sup>. Inactivating FBXW7 mutation or copy number loss may result in the accumulation of oncoproteins and therefore lead to malignant transformation<sup>[11]</sup>. FBXW7 is a haploinsufficient tumor suppressor gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[9][10][12]</sup>.

Q98\* mutation results in a premature truncation of the FBXW7 protein at amino acid 98 (UniProtKB). This mutation is predicted to lead to a loss of FBXW7 function, despite not having characterized in the literature.

#### Therapeutic and prognostic relevance

Clinical efficacy of mTOR inhibitors was seen in patients harboring aberrations in the FBXW7 gene (one patient with refractory fibrolamellar hepatocellular carcinoma, and one patient with lung adenocarcinoma)<sup>[13][14]</sup>. Moreover, in vitro assay also suggested that loss or inactivation of FBXW7 may confer sensitivity to mTOR inhibitor<sup>[8]</sup>.

Preclinical studies suggested that mutations or loss of FBXW7 were associated with regorafenib and oxaliplatin resistance in CRC cell lines and gefitinib resistance in lung cancer cells<sup>[15][16][17][18]</sup>.

Retrospective studies have indicated that a relatively low expression level of FBXW7 is an independent prognostic marker of poor survival for patients with hepatocellular carcinoma, lung adenocarcinoma and squamous cell carcinoma<sup>[19][17]</sup>.

### KRAS G12R

#### Biological Impact

The V-Ki-Ras2 Kirsten Rat Sarcoma 2 Viral Oncogene Homolog (KRAS) gene encodes a small GTPase protein, a member of the RAS family of small GTPases, which catalyze the hydrolysis of GTP to GDP. RAS proteins cycle between an active (GTP-bound) and an inactive (GDP-bound) state, to activate the downstream oncogenic pathways, including the PI3K/AKT/mTOR and MAPK pathways<sup>[20]</sup>. KRAS mutations occur primarily in three hotspots G12, G13 and Q61, and less frequently in codon A146<sup>[20][21]</sup>. These are activating mutations that lead to constitutive activation and persistent stimulation of the downstream signaling pathways<sup>[22][23]</sup>. Mutations in KRAS have been reported in a diverse spectrum of human malignancies, including pancreatic carcinomas (>80%)<sup>[20][24]</sup>, colon carcinomas (40-50%)<sup>[25][26]</sup>, and lung carcinomas (30-50%)<sup>[27][28]</sup>, but are also present in biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, myeloid leukemia and breast cancer<sup>[21]</sup>.

KRAS G12R is a hotspot mutation that lies within a GTP binding region of the KRAS protein (UniProtKB). G12R results in decreased KRAS GTPase activity and increased activation of downstream signaling in vitro<sup>[29][30]</sup>.

#### Therapeutic and prognostic relevance

Except for KRAS G12C, other KRAS mutants are not currently targetable, but the downstream MEK serves as a potential target<sup>[31]</sup>. MEK inhibitors trametinib, cobimetinib, and binimetinib were approved by the U.S. FDA for patients with advanced metastatic melanoma whose tumors harbor BRAF V600 mutations<sup>[32][33][34][35]</sup>.

There are case reports indicated that patients harboring a KRAS mutation may benefit from MEK inhibitor treatment. A patient with small cell neuroendocrine carcinoma (SCNEC) of the cervix harboring a KRAS G12D mutation showed significant response with trametinib<sup>[36]</sup>. Another low-grade serous carcinoma case with KRAS G12D also has sustained

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response to trametinib (Am J Clin Exp Obstet Gynecol 2015;2(3):140-143). In addition, a low-grade serous ovarian cancer patient harboring KRAS G12V mutation showed stable disease after 8 weeks of binimetinib treatment, and demonstrated a partial response after another 26 weeks of treatment<sup>[37]</sup>. However, trametinib did not demonstrate superiority to docetaxel in KRAS-mutant non-small cell lung cancer (NSCLC) patients, based on results from a randomized Phase II study<sup>[38]</sup>.

Both clinical and preclinical studies demonstrated a limited response to monotherapy using MEK inhibitors<sup>[39]</sup>. Moreover, several clinical trials are in progress to evaluate the combination of MEK and mTOR inhibition as a new potential therapeutic strategy in CRC<sup>[40]</sup>, and in patient-derived xenografts of RAS-mutant CRC, inhibition of MEK and mTOR suppressed tumor growth, but not tumor regression<sup>[41]</sup>. A study using the CRC patient-derived xenograft (PDX) model showed that the combination of trametinib, a MEK inhibitor, and palbociclib, a CDK4/6 inhibitor, was well tolerated and resulted in objective responses in all KRAS mutant models<sup>[42]</sup>.

KRAS mutation has been determined as an inclusion criterion for the trials evaluating MEK inhibitors efficacies in various types of solid tumors (NCT03704688, NCT02399943, NCT02285439, NCT03637491, NCT04214418).

Cetuximab and panitumumab are two EGFR-specific antibodies approved by the U.S. FDA for patients with KRAS wild-type metastatic colorectal cancer (NCT00154102, NCT00079066, NCT01412957, NCT00364013). Results from the PRIME and FIRE-3 trials indicated that panitumumab and cetuximab did not benefit patients with KRAS or NRAS mutations and may even have a detrimental effect in these patients<sup>[43]</sup>. Taken together, the National Comprehensive Cancer Network (NCCN) recommended that, cetuximab and panitumumab should only be used if both KRAS and NRAS genes are normal (NCCN guidelines)<sup>[44][45]</sup>. Numerous studies have demonstrated the presence of KRAS or NRAS mutations at exon 2, 3 or 4 as a predictor of resistance to anti-EGFR therapies<sup>[46][47][48][49][50][51][52]</sup>.

Sorafenib, a multi-kinase inhibitor, has been shown to be beneficial in KRAS-mutant CRC<sup>[53]</sup>, KRAS-mutant NSCLC<sup>[54]</sup>, and KRAS-amplified melanoma<sup>[55]</sup>.

There has been conflicting data on the effect of KRAS mutation on the efficacy of bevacizumab in metastatic CRC patients (J Clin Oncol 34, 2016 (suppl; abstr 3525))<sup>[56][57]</sup>.

In NCCN guidelines for NSCLC, KRAS mutations have been suggested as an emerging biomarker for EGFR TKIs in NSCLC patients. KRAS mutations are associated with a lack of efficacy of EGFR TKIs, including erlotinib, gefitinib, afatinib, and osimertinib, in NSCLC patients<sup>[58][59][60]</sup>.

Studies have shown that KRAS mutation, especially those occurs in exon 2 (codon 12 or 13) and codon 61 indicated a poor prognosis for patients with CRC<sup>[61]</sup>.

In low-grade serous carcinoma of the ovary or peritoneum, patients with KRAS or BRAF mutations (n=21) had a significantly better OS than those with wild-type KRAS or BRAF (n=58) (106.7 months vs 66.8 months), respectively<sup>[62]</sup>. In ovarian serous borderline tumor with recurrent low-grade serous carcinoma, patient harboring KRAS G12V mutation appeared to have shorter survival time<sup>[63]</sup>.

In 242 patients with unresectable pancreatic cancer, the presence of KRAS G12R (N=17) or G12D (N=92) was associated with poorer prognosis (overall survival HR 1.6; 95% confidence interval 1.11-2.28)<sup>[64]</sup>.

In a Phase II trial, a patient with Erdheim-Chester disease harboring KRAS G12R and ARAF P216A achieved a complete response to cobimetinib treatment<sup>[65]</sup>. An exploratory study demonstrated that pancreatic cancer patients with KRAS G12R mutation had better response to the combination of gemcitabine and cobimetinib than patients with KRAS G12D and G12V. One of six patients with KRAS G12R had partial response and five had stable disease and median PFS was 6 months<sup>[66]</sup>.



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## TP53 R248W, Heterozygous deletion

### Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis<sup>[67]</sup>. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation<sup>[68]</sup>.

R248W is a missense mutation located in the DNA-binding domain (DBD) of the p53 protein<sup>[69]</sup>. This mutation results in abrogation of the p53 tumor suppressor activity and a gain-of-function in ATM inactivation, resulting in increased genetic instability and increased tumorigenesis in mouse models<sup>[70]</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>[71]</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>[72]</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>[73]</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>[74][75][76]</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>[77]</sup>. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy<sup>[78][79]</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>[80]</sup>.

TP53 oncomorphic mutations, including P151S, Y163C, R175H, L194R, Y220C, R248Q, R248W, R273C, R273H, R273L, and R282W have been shown to predict resistance to platinum- and taxane-based chemotherapy in advanced serous ovarian carcinoma patients<sup>[81]</sup>.

## AKT2 Amplification

### Biological Impact

The v-akt murine thymoma viral oncogene homolog 2 (AKT2, also known as HIHGH, PRKBB, PKBBETA, RAC-BETA, PKBB) gene encodes an AKT family of serine/threonine protein kinases, including AKT1 and AKT3 isoforms, that act as a downstream effector of the pro-oncogenic PI3-kinase signaling pathway<sup>[82][83][84][85][86]</sup>. Whereas somatic AKT2 mutations have been described rarely in cancer, germline autosomal dominant mutations in AKT2 are associated with familial diabetes mellitus in humans<sup>[87]</sup>.

### Therapeutic and prognostic relevance

A preclinical study demonstrated that an AKT2-amplified pancreatic cancer cell line exhibited resistance to erlotinib. Besides, amplification of AKT2 was predominantly expressed across pancreatic cancer patients in TCGA datasets and correlated with high mRNA expression. Patients with a high AKT2 expression tended to have poor response to erlotinib plus gemcitabine<sup>[88]</sup>.

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## 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 whereas p14 (ARF) blocks the oncogenic activity of MDM2 by inhibiting MDM2-induced degradation of p53<sup>[89][90][91]</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>[92]</sup>. Loss of CDKN2A has been frequently found in human tumors that result in uncontrolled cell proliferation<sup>[93][94]</sup>.

### Therapeutic and prognostic relevance

Intact p16-Cdk4-Rb axis is known to be associated with sensitivity to cyclin-dependent kinase inhibitors<sup>[95][96]</sup>. Several case reports also revealed that patients with CDKN2A-deleted tumors respond to the CDK4/6-specific inhibitor treatments<sup>[97][98][99]</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>[100][101][102]</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).

The phase II TAPUR trial demonstrated clinical benefits to palbociclib monotherapy in advanced NSCLC or head and neck cancer harboring a CDKN2A mutation or copy number loss. However, pancreatic and biliary cancer patients harboring a CDKN2A mutation or copy number loss did not demonstrate an objective response or stable disease when treated with palbociclib monotherapy for 16 weeks (DOI: 10.1200/JCO.2021.39.15\_suppl.6043)<sup>[103][104]</sup>.

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>[96][105][106]</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>[98]</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>[107]</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>[108]</sup>.



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## US FDA-APPROVED DRUG(S)

### 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</b> <sup>[109]</sup> 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</b> <sup>[110]</sup> NCT00863655	<b>Breast cancer</b> (Approved on 2012/07/20)
	<b>ER+/HER2-</b> Everolimus + exemestane vs. Placebo + exemestane [PFS(M): 7.8 vs. 3.2]
<b>EXIST-2</b> NCT00790400	<b>Tuberous sclerosis complex (tsc)-associated renal angiomyolipoma</b> (Approved on 2012/04/26)
	- Everolimus vs. Placebo [ORR(%): 41.8 vs. 0]
<b>RADIANT-3</b> <sup>[111]</sup> NCT00510068	<b>Pancreatic neuroendocrine tumor</b> (Approved on 2011/05/05)
	- Everolimus vs. Placebo [PFS(M): 11 vs. 4.6]
<b>EXIST-1</b> <sup>[112]</sup> NCT00789828	<b>Subependymal giant cell astrocytoma</b> (Approved on 2010/10/29)
	- Everolimus vs. Placebo [ORR(%): 35.0]
<b>RECORD-1</b> <sup>[113]</sup> NCT00410124	<b>Renal cell carcinoma</b> (Approved on 2009/05/30)
	- Everolimus vs. Placebo [PFS(M): 4.9 vs. 1.9]

### 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)

[114] 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

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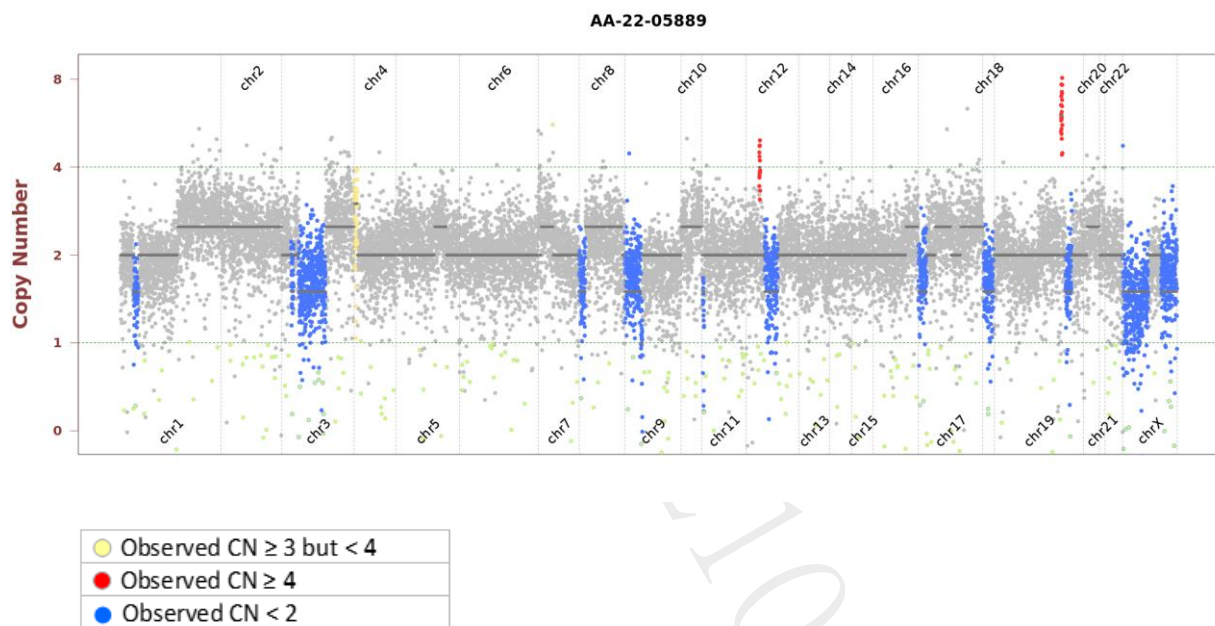
## SUPPLEMENTARY INFORMATION OF TESTING RESULTS DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

### - Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
FBXW7	Q98*	2	c.292C>T	NM_033632	COSM33085	29.9%	1743
KRAS	G12R	2	c.34G>C	NM_004985	COSM518	56.6%	3540
TP53	R248W	7	c.742C>T	NM_000546	COSM10656	39.4%	1644

### - 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
AMER1	S280R	2	c.840C>A	NM_152424	-	29.9%	405
CIC	Splice region	-	c.4460-8A>G	NM_015125	-	55.9%	68
KAT6A	G1590V	17	c.4769G>T	NM_006766	-	76.1%	243
MUC4	E5075K	19	c.15223G>A	NM_018406	-	40.1%	895
PRKDC	V3716M	79	c.11146G>A	NM_006904	-	51.9%	755
RUNX1T1	Splice region	10	c.1281C>T	NM_175634	COSM2790695	55.2%	945
SYNE1	L1575F	36	c.4723C>T	NM_182961	COSM3726493	52.2%	910
SYNE1	M3369V	63	c.10105A>G	NM_182961	-	39.0%	899
ZNF217	T634I	3	c.1901C>T	NM_006526	COSM5858130	44.3%	515

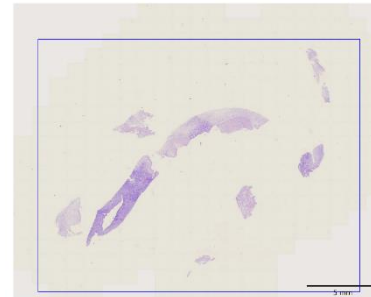
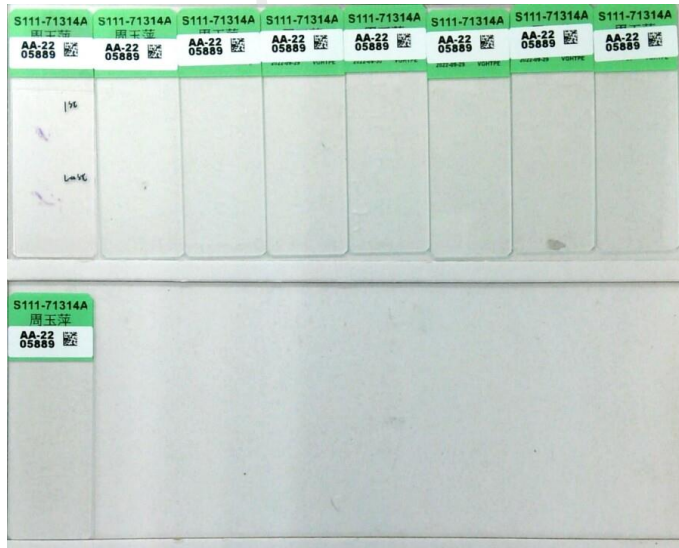
### Note:

- 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: Sep 14, 2022
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11171314A
- Collection site: Pancreas
- Examined by: Yeh-Han Wang
- 1. The percentage of viable tumor cells in total cells in the whole slide (%): 60%
- 2. The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 60%
- 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: Not performed
- The outline highlights the area of malignant neoplasm annotated by a pathologist.

## RUN QC

- Panel: ACTOnco<sup>®</sup>+

### DNA test

- Mean Depth: 841x
- Target Base Coverage at 100x: 94%

### RNA test

- Average unique RNA Start Sites per control GSP2: 123

## 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

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- 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  $\geq 10$ .

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



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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:

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### Sign Off

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Yeh

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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	BMP1R1A	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTG2*	BTX	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*	SLC01B1*
SLC01B3*	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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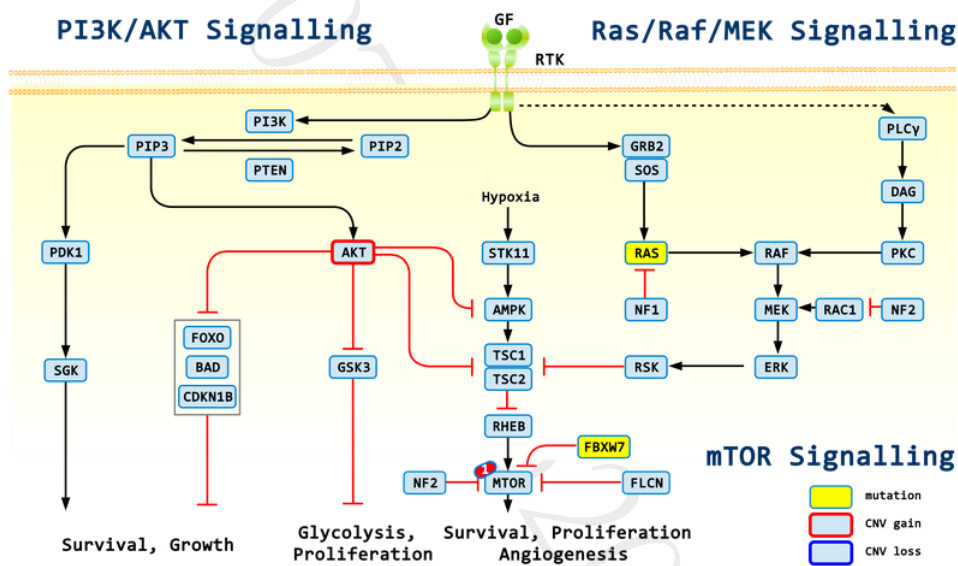
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## APPENDIX

### POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

Not Applicable.

### SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



1: Everolimus, Temsirolimus

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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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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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