

# ACT Onco<sup>®</sup> + Report

PATIENT		
Name: 吳春桃		Patient ID: 24555622
Date of Birth: Sep 03, 1955		Gender: Female
Diagnosis: Pancreatic cancer		
ORDERING PHYSICIAN		
Name: 姜乃榕醫師		Tel: 886-228712121
Facility: 臺北榮總		
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SPECIMEN		
Specimen ID: S11170477N	Collection site: Pancreas	Type: FFPE tissue
Date received: May 09, 2022	Lab ID: AA-22-02437	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
KRAS G12D	-	Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib, Cetuximab, Panitumumab
SMAD4 E377fs	-	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 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
KRAS	G12D	22.2%
SETD2	Q1794*	13.2%
SMAD4	E377fs	13.0%
TP53	W146*	41.9%

#### - Copy Number Alterations

Chromosome	Gene	Variation	Copy Number
Chr22	CHEK2	Heterozygous deletion	1

#### - 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:

- Variant(s) enlisted in the SNV table may currently exhibit no relevance to treatment response prediction. Please refer to INTERPRETATION for more biological information and/or potential clinical impacts of the variants.
- Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on 40% 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> G12D	Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib, Cetuximab, Panitumumab	<b>resistant</b>
<b>Level 4</b>		
<b>SMAD4</b> E377fs	Cetuximab	<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>SMAD4</b> E377fs	Fluorouracil	<b>Resistant</b>	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

### KRAS G12D

#### 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>[1]</sup>. KRAS mutations occur primarily in three hotspots G12, G13 and Q61, and less frequently in codon A146<sup>[1][2]</sup>. These are activating mutations that lead to constitutive activation and persistent stimulation of the downstream signaling pathways<sup>[3][4]</sup>. Mutations in KRAS have been reported in a diverse spectrum of human malignancies, including pancreatic carcinomas (>80%)<sup>[1][5]</sup>, colon carcinomas (40-50%)<sup>[6][7]</sup>, and lung carcinomas (30-50%)<sup>[8][9]</sup>, but are also present in biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, myeloid leukemia and breast cancer<sup>[2]</sup>.

G12D is a hotspot mutation located in the GTP binding region of the KRAS protein (UniProtKB). This mutation results in decreased KRAS GTPase activity, increased activation of downstream signaling, and promotes tumor formation in preclinical studies<sup>[10][11][12]</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>[13]</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>[14][15][16][17]</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>[18]</sup>. Another low-grade serous carcinoma case with KRAS G12D also has sustained 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>[19]</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>[20]</sup>.

Both clinical and preclinical studies demonstrated a limited response to monotherapy using MEK inhibitors<sup>[21]</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>[22]</sup>, and in patient-derived xenografts of RAS-mutant CRC, inhibition of MEK and mTOR suppressed tumor growth, but not tumor regression<sup>[23]</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>[24]</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>[25]</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>[26][27]</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>[28][29][30][31][32][33][34]</sup>.

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Sorafenib, a multi-kinase inhibitor, has been shown to be beneficial in KRAS-mutant CRC<sup>[35]</sup>, KRAS-mutant NSCLC<sup>[36]</sup>, and KRAS-amplified melanoma<sup>[37]</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>[38][39]</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>[40][41][42]</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>[43]</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>[44]</sup>. In ovarian serous borderline tumor with recurrent low-grade serous carcinoma, patient harboring KRAS G12V mutation appeared to have shorter survival time<sup>[45]</sup>.

## **SETD2 Q1794\***

### **Biological Impact**

SET Domain Containing 2 (SETD2) gene encodes a chromatin modulating enzyme that functions by site specific trimethylation of histone H3K36 and plays essential roles in gene regulation<sup>[46][47]</sup> and DNA mismatch repair. Inactivation of SETD2 leads to genetic instability, enrichment of nonsense and frameshift mutations and ultimately tumorigenesis<sup>[48][49][50]</sup>. Importantly, SETD2-mutant renal tumors failed to activate the p53 tumor suppressor, thus providing an alternative pathway for the inactivation of p53 that leads to defects in DNA damage repair<sup>[51]</sup>. Loss-of-function mutations of SETD2 has been reported in leukemia<sup>[52]</sup>, renal carcinomas<sup>[49]</sup>, and high-grade gliomas<sup>[53]</sup>.

Q1794\* mutation results in a premature truncation of the SETD2 protein at amino acid 1794 (UniProtKB). This mutation is predicted to lead to a loss of SETD2 function, despite not having characterized in the literature.

### **Therapeutic and prognostic relevance**

A study of metastatic renal cell carcinoma patients (n=111) treated with sunitinib or sorafenib indicated that Low SETD2 expression was associated with poorer overall survival and progression-free survival<sup>[54]</sup>. In chronic lymphocytic leukemia, patients harboring SETD2 abnormalities along with wild-type of TP53 and ATM genes from clinical trials employing chemotherapy or chemoimmunotherapy had shorter progression-free survival and overall survival compared with cases harboring wild-type for all three genes<sup>[55]</sup>.

Low expression of SETD2 was associated with large tumor size, advanced pT stage, poor overall survival and recurrence-free survival in non-metastatic clear-cell renal cell carcinoma<sup>[56]</sup>.

## **SMAD4 E377fs**

### **Biological Impact**

The SMAD family member 4 (SMAD4) gene encodes a transcription factor that acts as a downstream effector in the TGF- $\beta$  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- $\beta$ -targeted genes<sup>[57]</sup>. 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<sup>[58]</sup>. SMAD4 germline mutations are associated with juvenile polyposis syndrome (JPS)<sup>[59][60][61][62]</sup>. Somatic mutations of SMAD4 are commonly observed in pancreatic



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cancer<sup>[63]</sup>, colorectal cancer (CRC)<sup>[61][64][65]</sup>, and less frequently seen in other cancers such as lung adenocarcinoma<sup>[66]</sup>, head and neck cancer<sup>[67][68]</sup>, and cutaneous squamous cell carcinoma<sup>[69]</sup>.

E377fs mutation results in a change in the amino acid sequence beginning at 377, likely to cause premature truncation of the functional SMAD4 protein (UniProtKB). This mutation is predicted to lead to a loss of SMAD4 protein function, despite not being characterized in the literature.

## 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<sup>[70]</sup>. 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<sup>[71]</sup>.

SMAD4 is also suggested as a predictive marker for 5-fluorouracil-based chemotherapy in colorectal cancer (CRC)<sup>[72][73]</sup>. 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<sup>[74]</sup>.

Results from clinical and meta-analyses showed that loss of SMAD4 in CRC, pancreatic cancer was correlated with poor prognosis<sup>[75][76][77][78][79][80][81][82]</sup>. 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<sup>[83]</sup>.

## TP53 W146\*

### Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis<sup>[84]</sup>. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation<sup>[85]</sup>.

W146\* mutation results in a premature truncation of the p53 protein at amino acid 146 (UniProtKB). This mutation is predicted to lead to a loss of p53 function, despite not having characterized in the literature.

## 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>[86]</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>[87]</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>[88]</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>[89][90][91]</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>[92]</sup>. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy<sup>[93][94]</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

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bevacizumab treatment when their tumors harbor mutant TP53 versus wild-type TP53<sup>[95]</sup>.

## CHEK2 Heterozygous deletion

### Biological Impact

The checkpoint kinase 2 (CHEK2 or CHK2) gene encodes a serine/threonine protein kinase involved in transducing DNA damage signals that are required for both the intra-S phase and G2/M checkpoints<sup>[96]</sup>. CHEK2 heterozygosity has been shown to cause haploinsufficient phenotypes that can contribute to tumorigenesis through inappropriate S phase entry, accumulation of DNA damage during replication, and failure to restrain mitotic entry<sup>[97][98]</sup>. CHEK2 aberrations are associated with glioblastoma, breast, ovarian, prostate, colorectal, gastric, thyroid, and lung cancers<sup>[99][100][101][102][103]</sup>.

### Therapeutic and prognostic relevance

In May 2020, the U.S. FDA approved olaparib for the treatment of adult patients with metastatic castration-resistant prostate cancer (mCRPC) who carry mutations in homologous recombination repair (HRR) genes, including BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L, and progressed following prior treatment with enzalutamide or abiraterone acetate<sup>[104]</sup>.

In addition, CHEK2 has been determined as an inclusion criterion for the trials evaluating rucaparib efficacy in ovarian cancer or prostate cancer (NCT03533946)<sup>[105][106]</sup>, niraparib efficacy in melanoma (NCT03925350), pancreatic cancer (NCT03553004), prostate cancer (NCT02854436), and any malignancy, except prostate (NCT03207347), and talazoparib efficacy in HER2-negative breast cancer (NCT02401347), prostate cancer (NCT03148795), and lung cancer (NCT03377556), respectively.

In a phase 2 trial, two prostate cancer patients harboring CHEK2 homozygous deletion was enrolled. One of the two patients had a response to olaparib<sup>[107]</sup>.



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## US FDA-APPROVED DRUG(S)

### Niraparib (ZEJULA)

Niraparib is an oral, small molecule inhibitor of the DNA repair enzyme poly (ADP-ribose) polymerase-1 and -2 (PARP-1, -2). Niraparib is developed and marketed by Tesaro under the trade name ZEJULA.

### - FDA Approval Summary of Niraparib (ZEJULA)

<b>PRIMA</b> NCT02655016	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2020/04/29)
	- Niraparib vs. Placebo [PFS (overall population)(M): 13.8 vs. 8.2]
<b>QUADRA<sup>[108]</sup></b> NCT02354586	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2019/10/23)
	<b>HRD-positive (defined by either a deleterious or suspected deleterious BRCA mutation, and/or genomic instability)</b> Niraparib [ORR(%): 24.0, DOR(M): 8.3]
<b>NOVA<sup>[109]</sup></b> NCT01847274	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2017/03/27)
	- Niraparib vs. Placebo [PFS (overall population)(M): 11.3 vs. 4.7]

### Olaparib (LYNPARZA)

Olaparib is an oral, small molecule inhibitor of poly (ADP-ribose) polymerase-1, -2, and -3 (PARP-1, -2, -3). Olaparib is developed by KuDOS Pharmaceuticals and marketed by AstraZeneca under the trade name LYNPARZA.

### - FDA Approval Summary of Olaparib (LYNPARZA)

<b>OlympiA</b> NCT02032823	<b>Her2-negative high-risk early breast cancer</b> (Approved on 2022/03/11)
	<b>gBRCA</b> Olaparib vs. Placebo [invasive disease-free survival (IDFS)(M): ]
<b>PROfound<sup>[104]</sup></b> NCT02987543	<b>Prostate cancer</b> (Approved on 2020/05/19)
	<b>ATMm, BRCA1m, BRCA2m, BARD1m, BRIP1m, CDK12m, CHEK1m, CHEK2m, FANCLm, PALB2m, RAD51Bm, RAD51Cm, RAD51Dm, RAD54Lm</b> Olaparib vs. Enzalutamide or abiraterone acetate [PFS(M): 5.8 vs. 3.5]
<b>PAOLA-1<sup>[110]</sup></b> NCT02477644	<b>Ovarian cancer</b> (Approved on 2020/05/08)
	<b>HRD-positive (defined by either a deleterious or suspected deleterious BRCA mutation, and/or genomic instability)</b> Olaparib + bevacizumab vs. Placebo + bevacizumab [PFS(M): 37.2 vs. 17.7]
<b>POLO<sup>[111]</sup></b> NCT02184195	<b>Pancreatic adenocarcinoma</b> (Approved on 2019/12/27)
	<b>Germline BRCA mutation (deleterious/suspected deleterious)</b> Olaparib vs. Placebo [ORR(%): 23.0 vs. 12.0, PFS(M): 7.4 vs. 3.8]
<b>SOLO-1<sup>[112]</sup></b> NCT01844986	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2018/12/19)
	<b>Germline or somatic BRCA-mutated (gBRCAm or sBRCAm)</b> Olaparib vs. Placebo [PFS(M): NR vs. 13.8]
<b>OlympiAD<sup>[113]</sup></b> NCT02000622	<b>Breast cancer</b> (Approved on 2018/02/06)
	<b>Germline BRCA mutation (deleterious/suspected deleterious) HER2-negative</b> Olaparib vs. Chemotherapy [PFS(M): 7 vs. 4.2]
<b>SOLO-2/ENGOT-Ov21<sup>[114]</sup></b> NCT01874353	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2017/08/17)
	<b>gBRCA+</b> Olaparib vs. Placebo [PFS(M): 19.1 vs. 5.5]

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<b>Study19</b> <sup>[115]</sup> NCT00753545	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2017/08/17)
	-
	Olaparib vs. Placebo [PFS(M): 8.4 vs. 4.8]
<b>Study 42</b> <sup>[116]</sup> NCT01078662	<b>Ovarian cancer</b> (Approved on 2014/12/19)
	<b>Germline BRCA mutation (deleterious/suspected deleterious)</b>
	Olaparib [ORR(%): 34.0, DOR(M): 7.9]

## Rucaparib (RUBRACA)

Rucaparib is an inhibitor of the DNA repair enzyme poly (ADP-ribose) polymerase-1, -2 and -3 (PARP-1, -2, -3). Rucaparib is developed and marketed by Clovis Oncology under the trade name RUBRACA.

### - FDA Approval Summary of Rucaparib (RUBRACA)

<b>TRITON2</b> NCT02952534	<b>Prostate cancer</b> (Approved on 2020/05/15)
	<b>gBRCA+, sBRCA</b>
	Rucaparib [ORR(%): 44.0, DOR(M): NE]
<b>ARIEL3</b> <sup>[105]</sup> NCT01968213	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2018/04/06)
	<b>All HRD tBRCA</b>
	Rucaparib vs. Placebo [PFS (All)(M): 10.8 vs. 5.4, PFS (HRD)(M): 13.6 vs. 5.4, PFS (tBRCA)(M): 16.6 vs. 5.4]
<b>ARIEL2</b> <sup>[117]</sup> NCT01482715, NCT01891344	<b>Ovarian cancer</b> (Approved on 2016/12/19)
	<b>Germline and/or somatic BRCA mutation</b>
	Rucaparib [ORR(%): 54.0]

## Talazoparib (TALZENNA)

Talazoparib is an inhibitor of poly (ADP-ribose) polymerase (PARP) enzymes, including PARP1 and PARP2. Talazoparib is developed and marketed by Pfizer under the trade name TALZENNA.

### - FDA Approval Summary of Talazoparib (TALZENNA)

<b>EMBRACA</b> <sup>[118]</sup> NCT01945775	<b>Breast cancer</b> (Approved on 2018/10/16)
	<b>Germline BRCA mutation (deleterious/suspected deleterious) HER2-negative</b>
	Talazoparib vs. Chemotherapy [PFS(M): 8.6 vs. 5.6]

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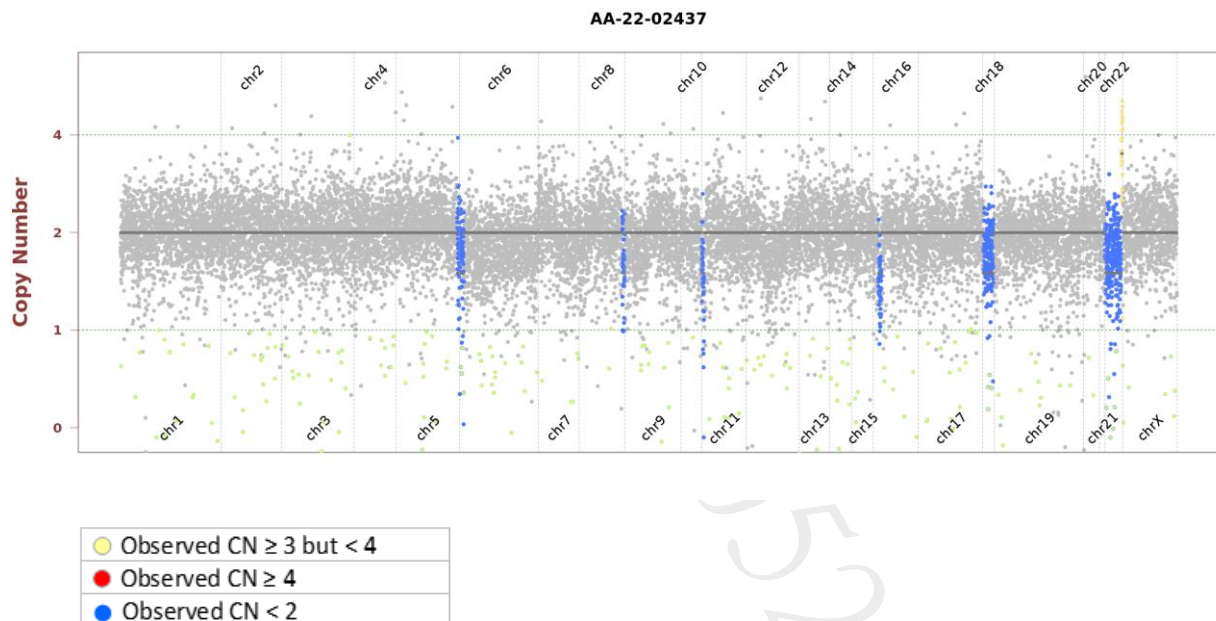
## SUPPLEMENTARY INFORMATION OF TESTING RESULTS DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

### - Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
KRAS	G12D	2	c.35G>A	NM_004985	COSM521	22.2%	2075
SETD2	Q1794*	11	c.5380C>T	NM_014159	-	13.2%	1310
SMAD4	E377fs	9	c.1130_1131insTA	NM_005359	-	13.0%	355
TP53	W146*	5	c.438G>A	NM_000546	COSM10727	41.9%	499

### - 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
BRCA1	E1586G	15	c.4757A>G	NM_007294	-	51.4%	951
BRD4	V1110del	16	c.3328_3330del	NM_058243	-	53.6%	181
CCND3	Splice region	-	c.712-4C>T	NM_001760	-	58.0%	514
CREBBP	Q507R	6	c.1520A>G	NM_004380	-	49.2%	539
IDH2	P23R	1	c.68C>G	NM_002168	-	47.8%	67
IKZF1	N93D	4	c.277A>G	NM_006060	-	14.4%	958
NEFH	Splice region	-	c.1208+8G>A	NM_021076	-	58.1%	618
PALB2	D446G	4	c.1337A>G	NM_024675	-	46.7%	809
PIK3R3	R255Q	6	c.764G>A	NM_003629	COSM5418556	50.4%	1260
ROS1	H1999N	37	c.5995C>A	NM_002944	-	40.1%	724
SYNE1	S4678F	78	c.14033C>T	NM_182961	-	43.5%	1521

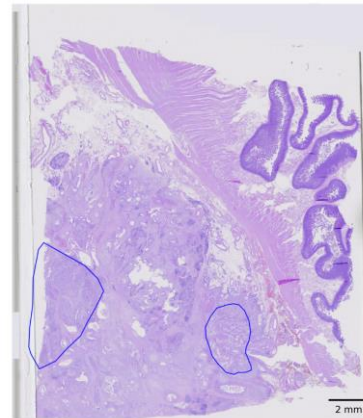
### Note:

- 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: Apr 2022
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11170477N
- Collection site: Pancreas
- Examined by: Dr. Yeh-Han Wang
  1. The percentage of viable tumor cells in total cells in the whole slide (%): 20%
  2. The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 40%
  3. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
  4. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
  5. Additional comment: NA
- Manual macrodissection: Performed on the highlighted region
- The outline highlights the area of malignant neoplasm annotated by a pathologist.

## RUN QC

- Panel: ACTOnco<sup>®</sup>+

### DNA test

- Mean Depth: 794x
- Target Base Coverage at 100x: 94%

### RNA test

- Average unique RNA Start Sites per control GSP2: 159



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## LIMITATIONS

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

## NEXT-GENERATION SEQUENCING (NGS) METHODS

### DNA test

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

Raw reads generated by the sequencer were mapped to the hg19 reference genome using the Ion Torrent Suite. Coverage depth was calculated using Torrent Coverage Analysis plug-in. Single nucleotide variants (SNVs) and short insertions/deletions (InDels) were identified using the Torrent Variant Caller plug-in. VEP (Variant Effect Predictor) was used to annotate every variant using databases from Clinvar, COSMIC and Genome Aggregation database. Variants with coverage  $\geq 25$ , 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  $100\times \geq 85\%$  with a mean coverage  $\geq 500\times$ .

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

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

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

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

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

### RNA test

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

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

## DATABASE USED

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

## Variant Analysis:

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



## Sign Off

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



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## GENE LIST SNV & CNV

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

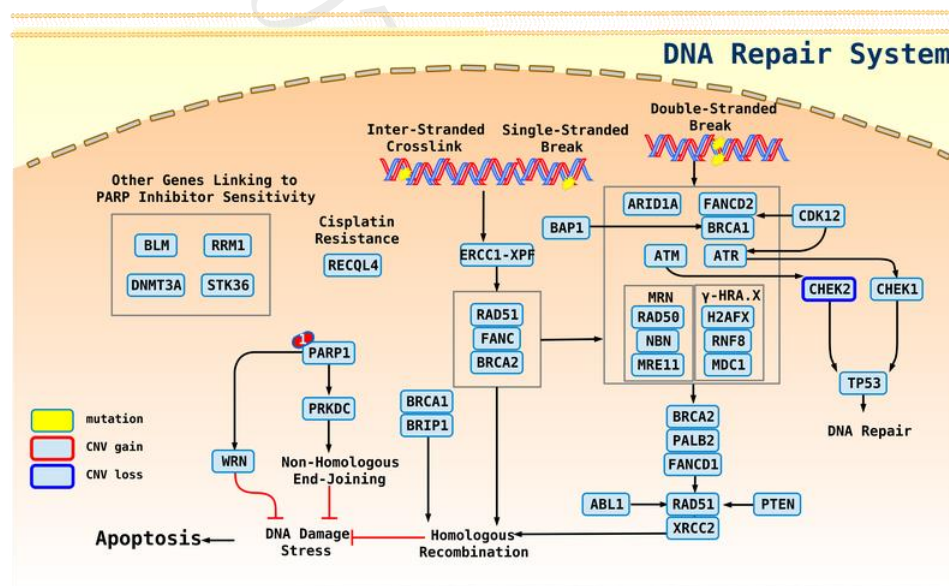
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## APPENDIX

### POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

Gene	Therapies	Possible effect
CHEK2	Niraparib, Olaparib, Rucaparib, Talazoparib	sensitive

### SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



1: Olaparib, Niraparib, Rucaparib, Talazoparib

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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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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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## REFERENCE

1. PMID: 2453289; 1988, Cell;53(4):549-54  
Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes.
2. PMID: 2114981; 1990, Eur J Clin Invest;20(3):225-35  
ras oncogenes: their role in neoplasia.
3. PMID: 20617134; 2010, J Biomed Biotechnol;2010():150960  
Clinical relevance of KRAS in human cancers.
4. PMID: 21993244; 2011, Nat Rev Cancer;11(11):761-74  
RAS oncogenes: weaving a tumorigenic web.
5. PMID: 3047672; 1988, Nucleic Acids Res;16(16):7773-82  
KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas.
6. PMID: 3587348; 1987, Nature;327(6120):293-7  
Prevalence of ras gene mutations in human colorectal cancers.
7. PMID: 1942608; 1991, Nihon Shokakibyo Gakkai Zasshi;88(8):1539-44  
[Prevalence of K-ras gene mutations in human colorectal cancers].
8. PMID: 2252272; 1990, Am Rev Respir Dis;142(6 Pt 2):S27-30  
The ras oncogenes in human lung cancer.
9. PMID: 1486840; 1992, Environ Health Perspect;98():13-24  
Role of proto-oncogene activation in carcinogenesis.
10. PMID: 16474405; 2006, Nat Genet;38(3):331-6  
Germline KRAS mutations cause Noonan syndrome.
11. PMID: 26037647; 2015, Mol Cancer Res;13(9):1325-35  
Biochemical and Structural Analysis of Common Cancer-Associated KRAS Mutations.
12. PMID: 22871572; 2012, Mol Cancer Res;10(9):1228-39  
KRAS(G12D)- and BRAF(V600E)-induced transformation of murine pancreatic epithelial cells requires MEK/ERK-stimulated IGF1R signaling.
13. PMID: 25414119; 2014, Drugs;74(18):2111-28  
The biology and clinical development of MEK inhibitors for cancer.
14. PMID: 25265492; 2014, N Engl J Med;371(20):1877-88  
Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma.
15. PMID: 22663011; 2012, N Engl J Med;367(2):107-14  
Improved survival with MEK inhibition in BRAF-mutated melanoma.
16. PMID: 25265494; 2014, N Engl J Med;371(20):1867-76  
Combined vemurafenib and cobimetinib in BRAF-mutated melanoma.
17. PMID: 29573941; 2018, Lancet Oncol;19(5):603-615  
Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial.
18. PMID: 26075998; 2014, Gynecol Oncol Rep;10():28-9  
Response to MEK inhibitor in small cell neuroendocrine carcinoma of the cervix with a KRAS mutation.
19. PMID: 29946554; 2018, Gynecol Oncol Rep;25():41-44  
Binimetinib (MEK162) in recurrent low-grade serous ovarian cancer resistant to chemotherapy and hormonal treatment.



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20. PMID: 25722381; 2015, Ann Oncol;26(5):894-901  
A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC)†.
21. PMID: 24947927; 2014, Clin Cancer Res;20(16):4251-61  
Phase I expansion and pharmacodynamic study of the oral MEK inhibitor RO4987655 (CH4987655) in selected patients with advanced cancer with RAS-RAF mutations.
22. PMID: 27340376; 2016, Curr Colorectal Cancer Rep;12():141-150  
Molecular Subtypes and Personalized Therapy in Metastatic Colorectal Cancer.
23. PMID: 22392911; 2012, Clin Cancer Res;18(9):2515-25  
Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas.
24. PMID: 26369631; 2016, Clin Cancer Res;22(2):405-14  
Sensitivity of KRAS-Mutant Colorectal Cancers to Combination Therapy That Cotargets MEK and CDK4/6.
25. PMID: 25937522; 2015, Eur J Cancer;51(10):1243-52  
FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer.
26. PMID: 19188670; 2009, J Clin Oncol;27(12):2091-6  
American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy.
27. PMID: 18802721; 2008, Virchows Arch;453(5):417-31  
KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program.
28. PMID: 25605843; 2015, J Clin Oncol;33(7):692-700  
Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer.
29. PMID: 27422777; 2016, Tumour Biol;37(9):11645-11655  
Potential biomarkers for anti-EGFR therapy in metastatic colorectal cancer.
30. PMID: 24024839; 2013, N Engl J Med;369(11):1023-34  
Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer.
31. PMID: 24666267; 2014, Acta Oncol;53(7):852-64  
The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis.
32. PMID: 27722750; 2017, JAMA Oncol;3(2):194-201  
Prognostic and Predictive Relevance of Primary Tumor Location in Patients With RAS Wild-Type Metastatic Colorectal Cancer: Retrospective Analyses of the CRYSTAL and FIRE-3 Trials.
33. PMID: 27736842; 2016, Br J Cancer;115(10):1206-1214  
A phase 3 trial evaluating panitumumab plus best supportive care vs best supportive care in chemorefractory wild-type KRAS or RAS metastatic colorectal cancer.
34. PMID: 20921465; 2010, J Clin Oncol;28(31):4697-705  
Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study.
35. PMID: 24407191; 2014, Br J Cancer;110(5):1148-54  
Sorafenib and irinotecan (NEXIRI) as second- or later-line treatment for patients with metastatic colorectal cancer and KRAS-mutated tumours: a multicentre Phase I/II trial.
36. PMID: 23224737; 2013, Clin Cancer Res;19(3):743-51

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A phase II study of sorafenib in patients with platinum-pretreated, advanced (Stage IIIB or IV) non-small cell lung cancer with a KRAS mutation.

37. PMID: 26307133; 2016, Clin Cancer Res;22(2):374-82  
Copy Number Changes Are Associated with Response to Treatment with Carboplatin, Paclitaxel, and Sorafenib in Melanoma.
38. PMID: 23828442; 2013, Med Oncol;30(3):650  
KRAS as prognostic biomarker in metastatic colorectal cancer patients treated with bevacizumab: a pooled analysis of 12 published trials.
39. PMID: 28632865; 2017, JAMA;317(23):2392-2401  
Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial.
40. PMID: 18349398; 2008, J Clin Oncol;26(9):1472-8  
Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib.
41. PMID: 23401440; 2013, J Clin Oncol;31(8):1112-21  
KRAS mutation: should we test for it, and does it matter?
42. PMID: 18024870; 2007, J Clin Oncol;25(33):5240-7  
Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer.
43. PMID: 15923428; 2005, Ann Oncol;16 Suppl 4():iv44-49  
Prognostic and predictive factors in colorectal cancer: Kirsten Ras in CRC (RASCAL) and TP53CRC collaborative studies.
44. PMID: 26484411; 2015, Br J Cancer;113(9):1254-8  
Impact of mutational status on survival in low-grade serous carcinoma of the ovary or peritoneum.
45. PMID: 24549645; 2013, J Pathol;231(4):449-56  
KRAS (but not BRAF) mutations in ovarian serous borderline tumour are associated with recurrent low-grade serous carcinoma.
46. PMID: 16118227; 2005, J Biol Chem;280(42):35261-71  
Identification and characterization of a novel human histone H3 lysine 36-specific methyltransferase.
47. PMID: 25123655; 2014, Biochim Biophys Acta;1846(2):366-79  
Role of somatic cancer mutations in human protein lysine methyltransferases.
48. PMID: 23622243; 2013, Cell;153(3):590-600  
The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutSα.
49. PMID: 25728682; 2015, Oncogene;34(46):5699-708  
SETD2 loss-of-function promotes renal cancer branched evolution through replication stress and impaired DNA repair.
50. PMID: 24931610; 2014, Cell Rep;7(6):2006-18  
SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability.
51. PMID: 24843002; 2014, Elife;3():e02482  
SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint.
52. PMID: 24509477; 2014, Nat Genet;46(3):287-93  
Identification of functional cooperative mutations of SETD2 in human acute leukemia.
53. PMID: 23417712; 2013, Acta Neuropathol;125(5):659-69  
Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas.
54. PMID: 27288695; 2016, J Urol;196(5):1363-1370  
Prognostic Value of SETD2 Expression in Patients with Metastatic Renal Cell Carcinoma Treated with Tyrosine Kinase Inhibitors.
55. PMID: 27282254; 2016, Leukemia;30(11):2179-2186  
Genomic disruption of the histone methyltransferase SETD2 in chronic lymphocytic leukaemia.

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56. PMID: 26559293; 2015, Medicine (Baltimore);94(45):e2004  
Decreased Expression of SETD2 Predicts Unfavorable Prognosis in Patients With Nonmetastatic Clear-Cell Renal Cell Carcinoma.
57. PMID: 25935112; 2015, Trends Biochem Sci;40(6):296-308  
Structural determinants of Smad function in TGF- $\beta$  signaling.
58. PMID: 19014666; 2008, Pathogenetics;1(1):2  
Smad4 haploinsufficiency: a matter of dosage.
59. PMID: 9545410; 1998, Am J Hum Genet;62(5):1129-36  
A gene for familial juvenile polyposis maps to chromosome 18q21.1.
60. PMID: 8553070; 1996, Science;271(5247):350-3  
DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1.
61. PMID: 8673134; 1996, Nat Genet;13(3):343-6  
Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers.
62. PMID: 18662538; 2008, Cell;134(2):215-30  
TGFbeta in Cancer.
63. PMID: 9135016; 1997, Cancer Res;57(9):1731-4  
Tumor-suppressive pathways in pancreatic carcinoma.
64. PMID: 23139211; 2013, Cancer Res;73(2):725-35  
SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer.
65. PMID: 22810696; 2012, Nature;487(7407):330-7  
Comprehensive molecular characterization of human colon and rectal cancer.
66. PMID: 25890228; 2015, World J Surg Oncol;13():128  
Clinical outcome and expression of mutant P53, P16, and Smad4 in lung adenocarcinoma: a prospective study.
67. PMID: 19841540; 2009, J Clin Invest;119(11):3208-11  
Smad4: gatekeeper gene in head and neck squamous cell carcinoma.
68. PMID: 15867212; 2005, Clin Cancer Res;11(9):3191-7  
Differences in Smad4 expression in human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck squamous cell carcinoma.
69. PMID: 25589618; 2015, Clin Cancer Res;21(6):1447-56  
Genomic analysis of metastatic cutaneous squamous cell carcinoma.
70. PMID: 29703253; 2018, BMC Cancer;18(1):479  
SMAD4 and NF1 mutations as potential biomarkers for poor prognosis to cetuximab-based therapy in Chinese metastatic colorectal cancer patients.
71. PMID: 28522603; 2017, Clin Cancer Res;23(17):5162-5175  
SMAD4 Loss Is Associated with Cetuximab Resistance and Induction of MAPK/JNK Activation in Head and Neck Cancer Cells.
72. PMID: 16144935; 2005, Clin Cancer Res;11(17):6311-6  
SMAD4 levels and response to 5-fluorouracil in colorectal cancer.
73. PMID: 24384683; 2014, Br J Cancer;110(4):946-57  
Loss of Smad4 in colorectal cancer induces resistance to 5-fluorouracil through activating Akt pathway.
74. PMID: 12237773; 2002, Br J Cancer;87(6):630-4  
SMAD4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer.

# ACT Onco<sup>®</sup> + Report

75. PMID: 25749173; 2015, Transl Oncol;8(1):18-24  
A Meta-Analysis of SMAD4 Immunohistochemistry as a Prognostic Marker in Colorectal Cancer.
76. PMID: 19478385; 2009, Cell Oncol;31(3):169-78  
Presence of a high amount of stroma and downregulation of SMAD4 predict for worse survival for stage I-II colon cancer patients.
77. PMID: 25681512; 2015, J Clin Pathol;68(5):341-5  
Smad4 inactivation predicts for worse prognosis and response to fluorouracil-based treatment in colorectal cancer.
78. PMID: 26861460; 2016, Clin Cancer Res;22(12):3037-47  
Reduced Expression of SMAD4 Is Associated with Poor Survival in Colon Cancer.
79. PMID: 26947875; 2016, Transl Oncol;9(1):1-7  
Prognostic Value of SMAD4 in Pancreatic Cancer: A Meta-Analysis.
80. PMID: 25760429; 2015, Pancreas;44(4):660-4  
SMAD4 expression predicts local spread and treatment failure in resected pancreatic cancer.
81. PMID: 22504380; 2012, Pancreas;41(4):541-6  
SMAD4 genetic alterations predict a worse prognosis in patients with pancreatic ductal adenocarcinoma.
82. PMID: 19584151; 2009, Clin Cancer Res;15(14):4674-9  
SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer.
83. PMID: 18425078; 2008, Mod Pathol;21(7):866-75  
Expression of Smad2 and Smad4 in cervical cancer: absent nuclear Smad4 expression correlates with poor survival.
84. PMID: 24739573; 2014, Nat Rev Cancer;14(5):359-70  
Unravelling mechanisms of p53-mediated tumour suppression.
85. PMID: 21125671; 2011, J Pathol;223(2):137-46  
Haplo-insufficiency: a driving force in cancer.
86. PMID: 27998224; 2016, J Clin Oncol;34(36):4354-4361  
Phase II Study of WEE1 Inhibitor AZD1775 Plus Carboplatin in Patients With TP53-Mutated Ovarian Cancer Refractory or Resistant to First-Line Therapy Within 3 Months.
87. PMID: 26646755; 2016, Ann Oncol;27(3):539-43  
TP53 mutational status is predictive of pazopanib response in advanced sarcomas.
88. PMID: 25669829; 2015, Ann Oncol;26(5):1012-8  
Phase I study of pazopanib and vorinostat: a therapeutic approach for inhibiting mutant p53-mediated angiogenesis and facilitating mutant p53 degradation.
89. PMID: 27466356; 2016, Mol Cancer Ther;15(10):2475-2485  
TP53 Alterations Correlate with Response to VEGF/VEGFR Inhibitors: Implications for Targeted Therapeutics.
90. PMID: 23670029; 2013, Oncotarget;4(5):705-14  
P53 mutations in advanced cancers: clinical characteristics, outcomes, and correlation between progression-free survival and bevacizumab-containing therapy.
91. PMID: 17145525; 2006, Semin Oncol;33(5 Suppl 10):S8-14  
Bevacizumab in combination with chemotherapy: first-line treatment of patients with metastatic colorectal cancer.
92. PMID: 21399868; 2011, Int J Oncol;38(5):1445-52  
p53, HER2 and tumor cell apoptosis correlate with clinical outcome after neoadjuvant bevacizumab plus chemotherapy in breast cancer.
93. PMID: 20549698; 2011, Int J Cancer;128(8):1813-21  
p53 status influences response to tamoxifen but not to fulvestrant in breast cancer cell lines.

# ACT Onco<sup>®</sup> + Report

94. PMID: 10786679; 2000, Cancer Res;60(8):2155-62  
Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer.
95. PMID: 25672981; 2015, Cancer Res;75(7):1187-90  
VEGF-A Expression Correlates with TP53 Mutations in Non-Small Cell Lung Cancer: Implications for Antiangiogenesis Therapy.
96. PMID: 21088254; 2011, Clin Cancer Res;17(3):401-5  
Tumor suppressor CHK2: regulator of DNA damage response and mediator of chromosomal stability.
97. PMID: 15261141; 2004, Cancer Cell;6(1):45-59  
Chk1 is haploinsufficient for multiple functions critical to tumor suppression.
98. PMID: 15539958; 2005, Cell Cycle;4(1):131-9  
Chk1 is essential for tumor cell viability following activation of the replication checkpoint.
99. PMID: 23296741; 2013, Fam Cancer;12(3):473-8  
The risk of gastric cancer in carriers of CHEK2 mutations.
100. PMID: 24713400; 2014, Hered Cancer Clin Pract;12(1):10  
A risk of breast cancer in women - carriers of constitutional CHEK2 gene mutations, originating from the North - Central Poland.
101. PMID: 25583358; 2015, Int J Cancer;137(3):548-52  
CHEK2 mutations and the risk of papillary thyroid cancer.
102. PMID: 12052256; 2002, Breast Cancer Res;4(3):R4  
Mutation analysis of the CHK2 gene in breast carcinoma and other cancers.
103. PMID: 15125777; 2004, Mol Cancer;3():14  
CHK2 kinase expression is down-regulated due to promoter methylation in non-small cell lung cancer.
104. PMID: 32343890; 2020, N Engl J Med;382(22):2091-2102  
Olaparib for Metastatic Castration-Resistant Prostate Cancer.
105. PMID: 28916367; 2017, Lancet;390(10106):1949-1961  
Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial.
106. PMID: 32086346; 2020, Clin Cancer Res;26(11):2487-2496  
Non-BRCA DNA Damage Repair Gene Alterations and Response to the PARP Inhibitor Rucaparib in Metastatic Castration-Resistant Prostate Cancer: Analysis From the Phase II TRITON2 Study.
107. PMID: 26510020; 2015, N Engl J Med;373(18):1697-708  
DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer.
108. PMID: 30948273; 2019, Lancet Oncol;20(5):636-648  
Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial.
109. PMID: 27717299; 2016, N Engl J Med;375(22):2154-2164  
Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer.
110. PMID: 31851799; 2019, N Engl J Med;381(25):2416-2428  
Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer.
111. PMID: 31157963; 2019, N Engl J Med;381(4):317-327  
Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer.
112. PMID: 30345884; 2018, N Engl J Med;379(26):2495-2505  
Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer.
113. PMID: 28578601; 2017, N Engl J Med;377(6):523-533

# ACT Onco<sup>®</sup> + Report

Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation.

114. PMID: 28754483; 2017, Lancet Oncol;18(9):1274-1284  
Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial.
115. PMID: 27617661; 2016, Lancet Oncol;17(11):1579-1589  
Overall survival in patients with platinum-sensitive recurrent serous ovarian cancer receiving olaparib maintenance monotherapy: an updated analysis from a randomised, placebo-controlled, double-blind, phase 2 trial.
116. PMID: 25366685; 2015, J Clin Oncol;33(3):244-50  
Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation.
117. PMID: 27908594; 2017, Lancet Oncol;18(1):75-87  
Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial.
118. PMID: 30110579; 2018, N Engl J Med;379(8):753-763  
Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation.