

# ACT Onco<sup>®</sup> + Report

PATIENT		
Name: 何家敏		Patient ID: 8122723
Date of Birth: Nov 21, 1941		Gender: Female
Diagnosis: Cholangiocarcinoma		
ORDERING PHYSICIAN		
Name: 周德盈醫師		Tel: 886-228712121
Facility: 臺北榮總		
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SPECIMEN		
Specimen ID: S11029477	Collection site: Lung	Type: FFPE tissue
Date received: Mar 09, 2022	Lab ID: AA-22-01127	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	
FGFR2(17)-XPNPEP1(11) fusion	Infigratinib, Pemigatinib	-	-

### VARIANTS/BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Genomic Alterations/Biomarkers	Possibly Sensitive	Possibly Resistant
CDKN2A P114L	Abemaciclib, Palbociclib, Ribociclib	-
FGFR2(17)-XPNPEP1(11) fusion	Erdafitinib	-

#### 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
CDKN2A	P114L	41.0%
SERPINB4	Q375R	73.2%
TP53	G266R	27.8%
TP53	M237V	26.6%

#### - Copy Number Alterations

Chromosome	Gene	Variation	Copy Number
Chr10	PTEN	Heterozygous deletion	1
Chr18	SMAD4	Heterozygous deletion	1
Chr19	STK11	Heterozygous deletion	1
Chr3	MLH1	Heterozygous deletion	1

#### - Fusions

Fusion Gene & Exon	Transcript ID
FGFR2(17)-XPNPEP1(11) fusion*	FGFR2(NM_000141.4), XPNPEP1(NM_020383.3)

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

Biomarker	Results
Tumor Mutational Burden (TMB)	3.2 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 55% tumor purity.
- For more therapeutic agents which are possibly respond to heterozygous deletion of genes listed above, please refer to APPENDIX for more information.
- The fusion gene reported above is confirmed to be in-frame and includes the kinase/functional domain. Such alteration may indicate potential benefits from kinase inhibitors. However, for a novel fusion, its functional significance and response to kinase inhibitors are undetermined.
- \*The result is a novel fusion. Confirmation by Sanger sequencing is necessary if residual specimen is available.
- TMB was calculated by using the sequenced regions of ACTOnco<sup>®</sup> to estimate the number of somatic nonsynonymous mutations per megabase of all protein-coding genes (whole exome). The threshold for high mutation load is set at  $\geq 7.5$  mutations per megabase. TMB, microsatellite status and gene copy number deletion cannot be determined if calculated tumor purity is  $< 30\%$ .

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## THERAPEUTIC IMPLICATIONS

### TARGETED THERAPIES

Genomic Alterations	Therapies	Effect
<b>Level 1</b>		
<i>FGFR2(17)-XPNPEP1(11)</i> fusion	Infigratinib, Pemigatinib	<b>sensitive</b>
<b>Level 3B</b>		
<i>CDKN2A</i> P114L	Abemaciclib, Palbociclib, Ribociclib	<b>sensitive</b>
<i>FGFR2(17)-XPNPEP1(11)</i> fusion	Erdafitinib	<b>sensitive</b>

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

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

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## IMMUNE CHECKPOINT INHIBITORS (ICIs)

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

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

Genomic Alterations	Potential Clinical Effects
Not detected	

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

## CHEMOTHERAPIES

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

## HORMONAL THERAPIES

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

## OTHERS

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

### Note:

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

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## VARIANT INTERPRETATION

### FGFR2(17)-XPNPEP1(11) fusion

#### Biological Impact

The fibroblast growth factor receptor 2 (FGFR2) gene encodes a transmembrane receptor tyrosine kinase. Upon binding of the FGF ligand, the receptor activates downstream signaling pathways like RAS-MAPK and PI3K-AKT<sup>[1][2][3]</sup>. Aberrant activation of FGFR2 through genomic alterations like gain-of-function mutations, fusions, and amplification enhances tumorigenesis, angiogenesis, cell proliferation, and migration<sup>[1][2]</sup>. FGFR2 alterations are recurrently observed in prostate<sup>[4]</sup>, breast, lung, uterine, and ovarian cancers<sup>[5]</sup>. While FGFR2 amplification and FGFR2 fusions are commonly detected in gastric cancer<sup>[6]</sup> and intrahepatic cholangiocarcinoma<sup>[3]</sup>, respectively.

FGFR1/2/3 fusions with genes encoding other signaling proteins can result in FGFR kinases dimerized through protein-protein interactions through N-terminal regions derived from fusion partners in hematological malignancies (type I) and C-terminal regions derived from fusion partners in solid tumors (type II), leading to increased ligand-independent receptor activation<sup>[7]</sup>.

#### Therapeutic and prognostic relevance

Erdafitinib (JNJ-42756493), a small molecule inhibitor of FGFR, was granted by the U.S. FDA approval in patients with locally advanced or metastatic urothelial carcinoma in April 2019. This approval is based on results from a phase II clinical trial BLC2001 (NCT02365597), a multicenter, open-label, single-arm study of patients (N = 87) with progressive urothelial carcinoma harboring at least one of the genetic alterations including FGFR3 mutations (R248C, S249C, G370C, Y373C) or FGFR gene fusions (FGFR3-TACC3, FGFR3-BAIAP2L1, FGFR2-BICC1, FGFR2-CASP7)<sup>[8]</sup>.

Pemigatinib, a kinase inhibitor, was granted approval by the U.S. FDA in April 2020 for the treatment of patients with previously treated, unresectable locally advanced or metastatic cholangiocarcinoma with a FGFR2 fusion or other rearrangement. This approval is based on results from a phase II clinical trial FIGHT-202 (NCT02924376), and the most commonly identified FGFR2 fusion was FGFR2-BICC1 (34%)<sup>[9]</sup>.

Infigratinib has been approval by the U.S. FDA in May 2021 for previously treated, unresectable locally advanced or metastatic cholangiocarcinoma patients harboring FGFR2 fusion or other rearrangement. This approval is based on the results from a phase II clinical study CBGJ398X2204 (n=108) (NCT02150967). The ORR of 108 patients was 23.1% including 1 complete response and 24 partial responses. The median DoR was 5 months; in the 23 responders, 8 patients showed response for at least 6 months<sup>[10]</sup> (DOI: 10.1200/JCO.2021.39.3\_suppl.265).

Several FGFR2-targeting tyrosine kinase inhibitors, including ponatinib (NCT02265341), infigratinib (NCT02150967), erdafitinib (NCT02699606, NCT03390504), and pemigatinib (NCT03914794) are currently under investigation in multiple cancer types<sup>[3][11]</sup>.

### CDKN2A P114L

#### 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>[12][13][14]</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>[15]</sup>. Loss of CDKN2A has been frequently found in human tumors that result in uncontrolled cell proliferation<sup>[16][17]</sup>.

CDKN2A P114L mutation is located within the ANK repeat 4 of the CDKN2A protein (UniProtKB). P114L confers a loss of function to the CDKN2A protein as demonstrated by loss of RB phosphorylation inhibition and loss of cell cycle

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control in vitro<sup>[18]</sup>.

## Therapeutic and prognostic relevance

Intact p16-Cdk4-Rb axis is known to be associated with sensitivity to cyclin-dependent kinase inhibitors<sup>[19][20]</sup>. Several case reports also revealed that patients with CDKN2A-deleted tumors respond to the CDK4/6-specific inhibitor treatments<sup>[21][22][23]</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>[24][25][26]</sup>. However, CDKN2A loss or mutation has been determined as an inclusion criterion for the trial evaluating CDK4/6 inhibitors efficacy in different types of solid tumors (NCT02693535, NCT02187783).

Notably, the addition of several CDK4/6 inhibitors to hormone therapies, including palbociclib in combination with letrozole, ribociclib plus letrozole, and abemaciclib combines with fulvestrant, have been approved by the U.S. FDA for the treatment of ER+ and HER2- breast cancer<sup>[20][27][28]</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>[22]</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>[29]</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>[30]</sup>.

## SERPINB4 Q375R

### Biological Impact

SERPINB4 encodes a protein of the serpin family of serine protease inhibitors. SERPINB4 is a close human homolog of SERPINB3 with which shares 92% protein sequence identity. SERPINB3 and SERPINB4 proteins have overlapping functions and are involved in both oncogenesis and immunity<sup>[31][32]</sup>.

### Therapeutic and prognostic relevance

Results from a clinical study showed that somatic mutations in SERPINB3 and SERPINB4 predicted improved survival from treatment with anti-CTLA4 therapy in two independent cohorts of patients with melanoma (n=174)<sup>[33]</sup>.

## TP53 G266R, M237V

### Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis<sup>[34]</sup>. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation<sup>[35]</sup>.

TP53 G266R mutation is located in the DNA-binding domain of the p53 protein (UniProtKB) and has been shown to have decreased transactivation activity in vitro<sup>[36][37]</sup>.

TP53 M237V is a missense mutation lies within the DNA-binding domain of the p53 protein (UniProtKB). This mutation has not been characterized in the scientific literature; therefore, its effect on the p53 protein function remains unknown.

### Therapeutic and prognostic relevance

Despite having a high mutation rate in cancers, there are currently no approved targeted therapies for TP53 mutations.



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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>[38]</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>[39]</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>[40]</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>[41][42][43]</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>[44]</sup>. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy<sup>[45][46]</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>[47]</sup>.

## MLH1 Heterozygous deletion

### Biological Impact

The MutL protein homolog 1 (MLH1) gene encodes a tumor suppressor that dimerizes with PMS2 protein to form a component of the DNA mismatch repair (MMR) system<sup>[48]</sup>. Deletion of one copy of the MLH1 gene resulted in haploinsufficiency in the correction of small insertions/deletions (indels), and could be a driving force in pancreatic and renal carcinogenesis<sup>[49]</sup>. Genetic alterations such as mutation, loss of heterozygosity or epigenetic silencing could lead to inactivation of MLH1 and are associated with a broad spectrum of cancers, including a subset of sporadic colon, gastric and endometrial cancers, as well as the hereditary non-polyposis colon cancer (HNPCC, also known as Lynch syndrome)<sup>[50][51][52]</sup>.

### Therapeutic and prognostic relevance

Currently, there are no FDA-approved medications specifically targeting MLH1. A screening test for microsatellite instability (MSI) is commonly used to identify an MMR-deficient tumor in the clinic<sup>[53][54]</sup>. Pembrolizumab (KEYTRUDA), an inhibitor targeting programmed cell death 1 (PD-1), has been approved by the U.S. FDA for adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair-deficient cancer. Down-regulation of genes involved in the MMR pathway such as MLH1, MSH2 and, MSH6 in high-grade serous epithelial ovarian cancer cell lines rendered cells sensitive to PARP inhibitors<sup>[55]</sup>.

## PTEN Heterozygous deletion

### Biological Impact

The phosphatase and tensin homolog deleted on chromosome ten (PTEN) gene encodes a lipid/protein phosphatase that is important for the regulation of cell proliferation, survival, homologous recombination and maintenance of genomic integrity<sup>[56][57]</sup>. PTEN acts as an essential tumor suppressor by antagonizing the PI3K/AKT/mTOR signaling pathway<sup>[58]</sup>. PTEN is a haploinsufficient tumor suppressor gene, in which having only one copy of the wild-type allele does not produce enough protein product to execute wild-type functions<sup>[35][59][60]</sup>. Germline loss-of-function PTEN mutations are found in approximately 80% of patients with Cowden syndrome, a disorder that is associated with high-penetrance breast and thyroid cancer<sup>[61][62][63]</sup>. Somatic mutations or monoallelic loss of PTEN is regularly observed in a significant fraction of human cancers, including sporadic breast cancer, colon cancer, endometrial cancer, prostate cancer, and glioblastoma<sup>[64][65][66][67][68]</sup>.

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## Therapeutic and prognostic relevance

Somatic loss of PTEN results in aberrant activation of PI3K/AKT/mTOR signaling pathway and provides a mechanistic rationale for PI3K pathway inhibitors treatment<sup>[69][70]</sup>. Preclinical studies demonstrated that PTEN deficiency was associated with increased sensitivity to PI3K pathway inhibitors in selected cancer subtypes<sup>[71][72][73][74][75][76]</sup>. Moreover, early clinical data also indicated that PTEN loss was associated with improved response and longer PFS in patients with advanced breast cancer<sup>[77]</sup>, advanced pancreatic neuroendocrine tumors<sup>[78]</sup>, and metastatic castration-resistant prostate cancer treated with mTORC1 inhibitor, everolimus<sup>[79]</sup>.

Several groups found that PTEN loss was generally associated with poor response to trastuzumab therapy, whether this agent was administered in the neoadjuvant, adjuvant, or metastatic settings<sup>[80][81][82][83][84]</sup>.

Loss of PTEN expression in advanced colorectal cancer (CRC) has been linked with resistance to anti-EGFR mAbs like cetuximab and panitumumab<sup>[85][86][87][88][89][90]</sup>. However, encouraging anti-tumor activity of the combination of an EGFR antibody and a mTORC1 inhibitor (everolimus or temsirolimus) have been reported in early-phase clinical studies (J Clin Oncol. 2011;29 (suppl): abstr 3587; J Clin Oncol. 2013;31 (suppl): abstr 608). Ongoing phase I/II studies testing combinations of EGFR antibodies and PI3K/AKT/mTOR pathway inhibitors (e.g., NCT01816984, NCT01252628, NCT01719380) will provide larger numbers of patients to assess the role of PTEN status in therapeutic response.

Preclinical studies showed that loss of PTEN expression in EGFR mutant cells was associated with decreased sensitivity to EGFR TKIs, erlotinib and gefitinib<sup>[91][92]</sup>. Inhibition of the PI3K/AKT/mTOR signal pathway has been shown to be an effective strategy to radiosensitize NSCLC cells harboring the EGFR activating mutation that acquires resistance to both TKIs due to PTEN loss or inactivation mutations<sup>[93]</sup>.

Loss or biallelic inactivation of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapies, including pembrolizumab and nivolumab in melanoma and leiomyosarcoma patients<sup>[94][95][96]</sup>.

PTEN loss of function mutation has been determined as an inclusion criterion for the trial evaluating olaparib efficacy in metastatic biliary tract cancer (NCT04042831); talazoparib efficacy in HER2-negative breast cancer (NCT02401347), and niraparib efficacy in breast cancer (NCT04508803) or any malignancy (except prostate) cancer (NCT03207347). Clinical data also suggested that PTEN deficient cancers may be sensitive to olaparib<sup>[97]</sup>.

## SMAD4 Heterozygous deletion

### 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>[98]</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>[99]</sup>. SMAD4 germline mutations are associated with juvenile polyposis syndrome (JPS)<sup>[100][101][102][103]</sup>. Somatic mutations of SMAD4 are commonly observed in pancreatic cancer<sup>[104]</sup>, colorectal cancer (CRC)<sup>[102][105][106]</sup>, and less frequently seen in other cancers such as lung adenocarcinoma<sup>[107]</sup>, head and neck cancer<sup>[108][109]</sup>, and cutaneous squamous cell carcinoma<sup>[110]</sup>.

## 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>[111]</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>[112]</sup>.

SMAD4 is also suggested as a predictive marker for 5-fluorouracil-based chemotherapy in colorectal cancer



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(CRC)<sup>[113][114]</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>[115]</sup>.

Results from clinical and meta-analyses showed that loss of SMAD4 in CRC, pancreatic cancer was correlated with poor prognosis<sup>[116][117][118][119][120][121][122][123]</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>[124]</sup>.

## STK11 Heterozygous deletion

### Biological Impact

The serine/threonine kinase 11 (STK11, also known as LKB1) gene encodes the multifunctional serine/threonine kinase, a tumor suppressor that functions as an inhibitor for the mTOR signaling pathway<sup>[125][126]</sup>. STK11 is a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[127][128]</sup>. In the mouse model, loss of STK11 promotes aggressive endometrial and squamous cell carcinomas<sup>[129][130]</sup>. Mutations in STK11 have been found in lung, breast, cervical, testicular, and liver cancers, as well as malignant melanoma, pancreatic and biliary carcinoma<sup>[131]</sup>. Germline mutations in STK11 are found in 30-70% of Peutz-Jeghers syndrome<sup>[132]</sup>.

### Therapeutic and prognostic relevance

A clinical study in a pancreatic cancer patient with Peutz-Jeghers syndrome whose tumor harboring an STK11 D194E mutation coupled with the loss of heterozygosity of the other STK11 allele displayed partial response to the everolimus treatment<sup>[133]</sup>. In another clinical case study, an adrenocorticotrophic pituitary carcinoma patient whose tumor bearing an STK11 inactivating mutation responded to a combination of everolimus and radiotherapy<sup>[134]</sup>.

Preclinical data suggested that lung cancer cell lines with STK11 inactivating mutations may confer increased sensitivity to the MEK-1 and MEK-2 inhibitor, trametinib<sup>[135]</sup>.

Inactivating mutations of STK11 was shown to be associated with resistance to immune checkpoint blockade in KRAS-mutant lung adenocarcinoma (LUAC) and NSCLC (DOI: 10.1200/JCO.2017.35.15\_suppl.9016)<sup>[136][137][138]</sup>. It was proposed that loss of STK11 negatively impacts the number and function of tumor-infiltrating T cells (TILs) and PD-L1 expression on tumor cells and therefore results in an ineffective response to PD-1-targeting antibodies<sup>[139]</sup>.

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## US FDA-APPROVED DRUG(S)

### Abemaciclib (VERZENIO)

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

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

<b>monarchE</b> NCT03155997	<b>Breast cancer</b> (Approved on 2021/10/12)
	<b>HR-positive, HER2-negative</b> Abemaciclib + tamoxifen/aromatase inhibitor vs. Tamoxifen/aromatase inhibitor [IDFS at 36 months(%): 86.1 vs. 79.0]
<b>MONARCH 3</b> <sup>[140]</sup> NCT00246621	<b>Breast cancer</b> (Approved on 2018/02/26)
	<b>HR-positive, HER2-negative</b> Abemaciclib + anastrozole/letrozole vs. Placebo + anastrozole/letrozole [PFS(M): 28.2 vs. 14.8]
<b>MONARCH 2</b> <sup>[28]</sup> NCT02107703	<b>Breast cancer</b> (Approved on 2017/09/28)
	<b>HR-positive, HER2-negative</b> Abemaciclib + fulvestrant vs. Placebo + fulvestrant [PFS(M): 16.4 vs. 9.3]
<b>MONARCH 1</b> <sup>[141]</sup> NCT02102490	<b>Breast cancer</b> (Approved on 2017/09/28)
	<b>HR-positive, HER2-negative</b> Abemaciclib [ORR(%): 19.7 vs. 17.4]

### Binimetinib (MEKTOVI)

Binimetinib is an oral kinase inhibitor that targets MEK. Binimetinib is developed and marketed by Array BioPharma under the trade name MEKTOVI.

#### - FDA Approval Summary of Binimetinib (MEKTOVI)

<b>MEKTOVI</b> <sup>[142]</sup> NCT01909453	<b>Melanoma</b> (Approved on 2018/06/27)
	<b>BRAF V600E/K</b> Encorafenib + binimetinib vs. Vemurafenib [PFS(M): 14.9 vs. 7.3]

### Cobimetinib (COTELLIC)

Cobimetinib is a reversible inhibitor which targets MEK1 and MEK2. Cobimetinib is developed by Exelixis and Genentech, and marketed by Genentech under the trade name COTELLIC.

#### - FDA Approval Summary of Cobimetinib (COTELLIC)

<b>coBRIM</b> <sup>[143]</sup> NCT01689519	<b>Melanoma</b> (Approved on 2015/11/10)
	<b>BRAF V600E/K</b> Cobimetinib + vemurafenib vs. Placebo + vemurafenib [PFS(M): 12.3 vs. 7.2]

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## Erdafitinib (BALVERSA)

Erdafitinib is a kinase inhibitor that binds to and inhibits enzymatic activity of FGFR1, FGFR2, FGFR3 and FGFR4 based on in vitro data. Erdafitinib also binds to RET, CSF1R, PDGFRA, PDGFRB, FLT4, KIT, and VEGFR2. Erdafitinib is developed and marketed by Janssen under the trade name BALVERSA.

### - FDA Approval Summary of Erdafitinib (BALVERSA)

Study BLC2001 NCT02365597	<b>Bladder urothelial carcinoma</b> (Approved on 2019/04/12)
	-
	Erdafitinib [ORR(%): 32.2]

## 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)

RADIANT-4 <sup>[144]</sup> NCT01524783	<b>Lung or gastrointestinal neuroendocrine tumor</b> (Approved on 2016/02/26)
	-
	Everolimus vs. Placebo [PFS(M): 11 vs. 3.9]
BOLERO-2 <sup>[145]</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]
EXIST-2 NCT00790400	<b>Tuberous sclerosis complex (tsc)-associated renal angiomyolipoma</b> (Approved on 2012/04/26)
	-
	Everolimus vs. Placebo [ORR(%): 41.8 vs. 0]
RADIANT-3 <sup>[78]</sup> NCT00510068	<b>Pancreatic neuroendocrine tumor</b> (Approved on 2011/05/05)
	-
	Everolimus vs. Placebo [PFS(M): 11 vs. 4.6]
EXIST-1 <sup>[146]</sup> NCT00789828	<b>Subependymal giant cell astrocytoma</b> (Approved on 2010/10/29)
	-
	Everolimus vs. Placebo [ORR(%): 35.0]
RECORD-1 <sup>[147]</sup> NCT00410124	<b>Renal cell carcinoma</b> (Approved on 2009/05/30)
	-
	Everolimus vs. Placebo [PFS(M): 4.9 vs. 1.9]

## Infigratinib (TRUSELTIQ)

Infigratinib a kinase inhibitor. Infigratinib is developed and marketed by QED Therapeutics, Inc. under the trade name TRUSELTIQ.

### - FDA Approval Summary of Infigratinib (TRUSELTIQ)

CBGJ398X2204 NCT02150967	<b>Cholangiocarcinoma</b> (Approved on 2021/05/28)
	<b>FGFR2 fusion</b>
	Infigratinib [ORR(%): 23.0, DOR(M): 5]

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## 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</b> <sup>[148]</sup> 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</b> <sup>[149]</sup> 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>PROfound</b> <sup>[150]</sup> 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</b> <sup>[151]</sup> 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</b> <sup>[152]</sup> 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</b> <sup>[153]</sup> 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</b> <sup>[154]</sup> 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</b> <sup>[155]</sup> 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]
<b>Study19</b> <sup>[156]</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]

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Study 42 <sup>[157]</sup> NCT01078662	Ovarian cancer (Approved on 2014/12/19)
	Germline BRCA mutation (deleterious/suspected deleterious)
	Olaparib [ORR(%): 34.0, DOR(M): 7.9]

## Palbociclib (IBRANCE)

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

### - FDA Approval Summary of Palbociclib (IBRANCE)

PALOMA-2 <sup>[158]</sup> NCT01740427	Breast cancer (Approved on 2017/03/31)
	ER+, HER2-
	Palbociclib + letrozole vs. Placebo + letrozole [PFS(M): 24.8 vs. 14.5]
PALOMA-3 <sup>[159]</sup> NCT01942135	Breast cancer (Approved on 2016/02/19)
	ER+, HER2-
	Palbociclib + fulvestrant vs. Placebo + fulvestrant [PFS(M): 9.5 vs. 4.6]

## Pemigatinib (PEMAZYRE)

Pemigatinib is a small molecule kinase inhibitor that targets FGFR1, 2, and 3 with IC50 values less than 2 nM. Pemigatinib is developed and marketed by Incyte under the tradename PEMAZYRE.

### - FDA Approval Summary of Pemigatinib (PEMAZYRE)

FIGHT-202 <sup>[9]</sup> NCT02924376	Cholangiocarcinoma (Approved on 2020/04/17)
	FGFR2 fusion
	Pemigatinib [ORR(%): 36.0]

## Ribociclib (KISQALI)

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

### - FDA Approval Summary of Ribociclib (KISQALI)

MONALEESA-2 <sup>[27]</sup> NCT01958021	Breast cancer (Approved on 2017/03/13)
	HR+, HER2-
	Ribociclib vs. Letrozole [PFS(M): NR vs. 14.7]



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## 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)

TRITON2 NCT02952534	<b>Prostate cancer</b> (Approved on 2020/05/15)
	<b>gBRCA+, sBRCA</b> Rucaparib [ORR(%): 44.0, DOR(M): NE]
ARIEL3 <sup>[160]</sup> NCT01968213	<b>Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma</b> (Approved on 2018/04/06)
	<b>AI/HRD/tBRCA</b> Rucaparib vs. Placebo [PFS (AI)(M): 10.8 vs. 5.4, PFS (HRD)(M): 13.6 vs. 5.4, PFS (tBRCA)(M): 16.6 vs. 5.4]
ARIEL2 <sup>[161]</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)

EMBRACA <sup>[162]</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]

## 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)

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

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## Trametinib (MEKINIST)

Trametinib is an anti-cancer inhibitor which targets MEK1 and MEK2. Trametinib is developed and marketed by GlaxoSmithKline (GSK) under the trade name MEKINIST.

### - FDA Approval Summary of Trametinib (MEKINIST)

BRF117019 <sup>[164]</sup> NCT02034110	<b>Anaplastic thyroid cancer</b> (Approved on 2018/05/04)
	<b>BRAF V600E</b> Dabrafenib + trametinib [ORR(%): 61.0]
BRF113928 <sup>[165]</sup> NCT01336634	<b>Non-small cell lung cancer</b> (Approved on 2017/06/22)
	<b>BRAF V600E</b> Trametinib + dabrafenib vs. Dabrafenib [ORR(%): 63.0 vs. 27.0, DOR(M): 12.6 vs. 9.9]
COMBI-d <sup>[166]</sup> NCT01584648	<b>Melanoma</b> (Approved on 2014/01/10)
	<b>BRAF V600E/K</b> Trametinib + dabrafenib vs. Dabrafenib + placebo [PFS(M): 9.3 vs. 8.8]
METRIC <sup>[167]</sup> NCT01245062	<b>Melanoma</b> (Approved on 2013/05/29)
	<b>BRAF V600E/K</b> Trametinib vs. Dacarbazine or paclitaxel [PFS(M): 4.8 vs. 1.5]

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.

### Erdafitinib

(NCT04083976, Phase 2)

The purpose of this study is to evaluate the efficacy of erdafitinib in terms of overall response rate (ORR) in adult and pediatric participants with advanced solid tumors with fibroblast growth factor receptor (FGFR) alterations (mutations or gene fusions). It will also evaluate ORR in pediatric participants with advanced solid tumors and FGFR alterations.

### - Contact

Name: Study Contact  
Phone: 844-434-4210  
Email: [JNJ.CT@sylogent.com](mailto:JNJ.CT@sylogent.com)

### - Location

Status: Recruiting Country: Taiwan City: Changhua Name: Changhua Christian Hospital	Status: Recruiting Country: Taiwan City: Kaohsiung City Name: E-Da Cancer Hospital
Status: Recruiting Country: Taiwan City: Kaohsiung Name: Kaohsiung Medical University Chung-Ho Memorial Hospital	Status: Recruiting Country: Taiwan City: Kaohsiung Name: Chang Gung Medical Foundation
Status: Recruiting Country: Taiwan City: Taichung Name: China Medical University Hospital	Status: Recruiting Country: Taiwan City: Taichung Name: Taichung Veterans General Hospital
Status: Recruiting Country: Taiwan City: Tainan Name: Chi Mei Medical Center - Yong Kang	Status: Recruiting Country: Taiwan City: Tainan Name: National Cheng Kung University Hospital
Status: Recruiting Country: Taiwan City: Tainan Name: Chi Mei Medical Center - Liu Ying	Status: Recruiting Country: Taiwan City: Taipei Name: National Taiwan University Hospital

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Status: Recruiting Country: Taiwan City: Taipei Name: Taipei Veterans General Hospital	Status: Recruiting Country: Taiwan City: Taoyuan City Name: Chang Gung Memorial Hospital Linkou Branch
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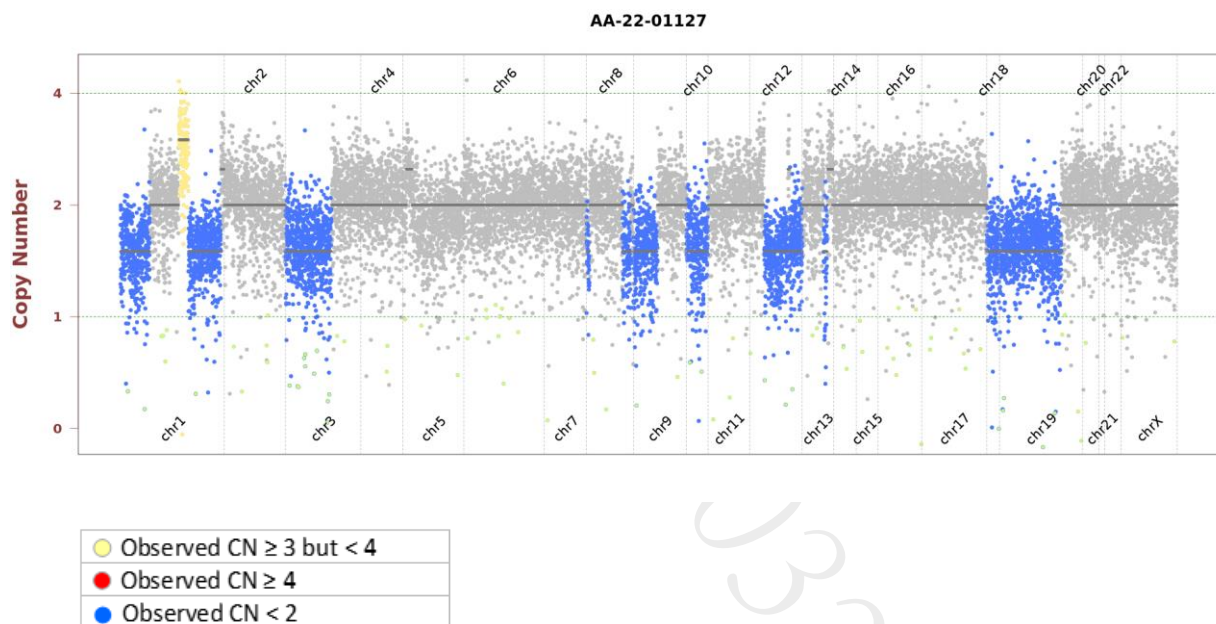
## SUPPLEMENTARY INFORMATION OF TESTING RESULTS DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

### - Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
CDKN2A	P114L	2	c.341C>T	NM_000077	COSM12476	41.0%	315
SERPINB4	Q375R	8	c.1124A>G	NM_002974	-	73.2%	613
TP53	G266R	8	c.796G>A	NM_000546	COSM10794	27.8%	468
TP53	M237V	7	c.709A>G	NM_000546	COSM44525	26.6%	1214

### - 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
ADAMTS15	A503T	4	c.1507G>A	NM_139055	-	51.6%	448
ADAMTS16	R957Q	19	c.2870G>A	NM_139056	-	57.4%	646
BAP1	K659del	15	c.1975_1977del	NM_004656	-	34.6%	572
CARD11	S694L	16	c.2081C>T	NM_032415	COSM5505215	54.7%	497
CD70	T118M	3	c.353C>T	NM_001252	-	30.7%	791
ERBB2	L137M	3	c.409C>A	NM_004448	COSM6950459	53.4%	822
ESR1	V103A	1	c.308T>C	NM_000125	-	55.8%	215
FANCA	Splice region	-	c.3935-6T>C	NM_000135	-	49.1%	782
FGFR1	M437V	10	c.1309A>G	NM_023110	-	54.6%	535
IGF1R	E685K	10	c.2053G>A	NM_000875	COSM5649239	28.5%	1035
KDR	T384M	9	c.1151C>T	NM_002253	-	6.2%	714
KDR	V136M	4	c.406G>A	NM_002253	-	27.2%	147
MUC16	P7134L	3	c.21401C>T	NM_024690	-	71.2%	886
POLD1	D644E	16	c.1932C>G	NM_001256849	-	53.0%	557
RARA	Splice region	-	c.1171+7C>T	NM_000964	-	27.9%	642
SYNE1	V5258I	82	c.15772G>A	NM_182961	-	50.6%	875
TSC2	S802G	22	c.2404A>G	NM_000548	-	50.4%	423

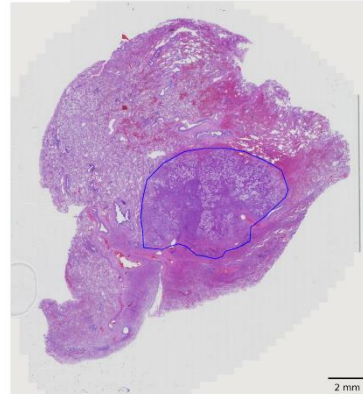
### Note:

- 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: Oct 2021
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11029477
- Collection site: Lung
- 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 (%): 50%
- 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: 741x
- Target Base Coverage at 100x: 92%

### RNA test

- Average unique RNA Start Sites per control GSP2: 111

## 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.

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## 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  $\geq 10$ .

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.

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## 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:

醫檢師黃靖婷 博士  
Ching-Ting Huang Ph.D.  
檢字第 016511 號

CT Huang

## Sign Off

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

Yeh

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## GENE LIST SNV & CNV

ABCB1*	ABCC2*	ABCG2*	ABL1	ABL2	ADAMTS1	ADAMTS13	ADAMTS15	ADAMTS16	ADAMTS18	ADAMTS6	ADAMTS9
ADAMTS11	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	BMP1A	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
-----	------	------	-------	-------	-------	-----	------	-------	-------	-------	-----	------



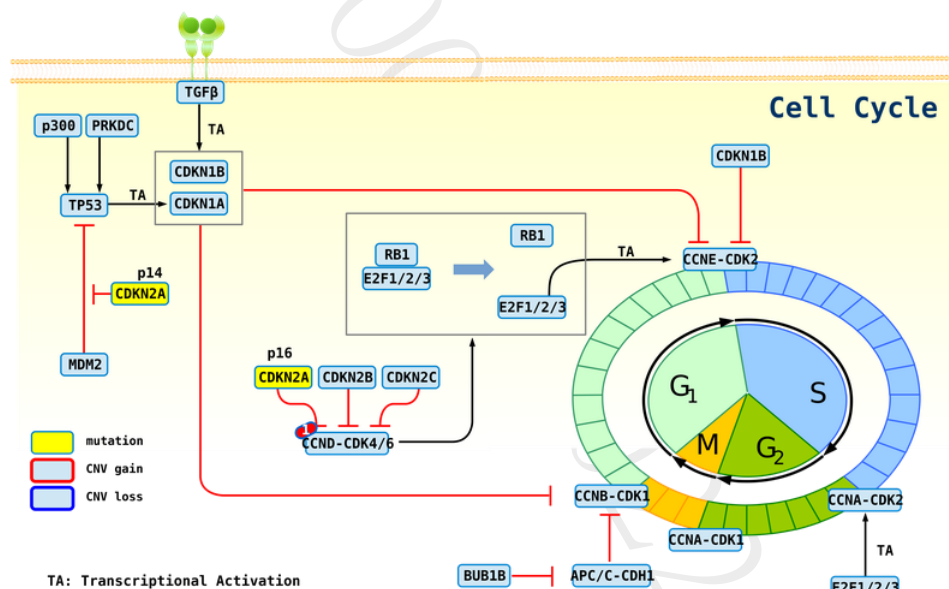
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## APPENDIX

### POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

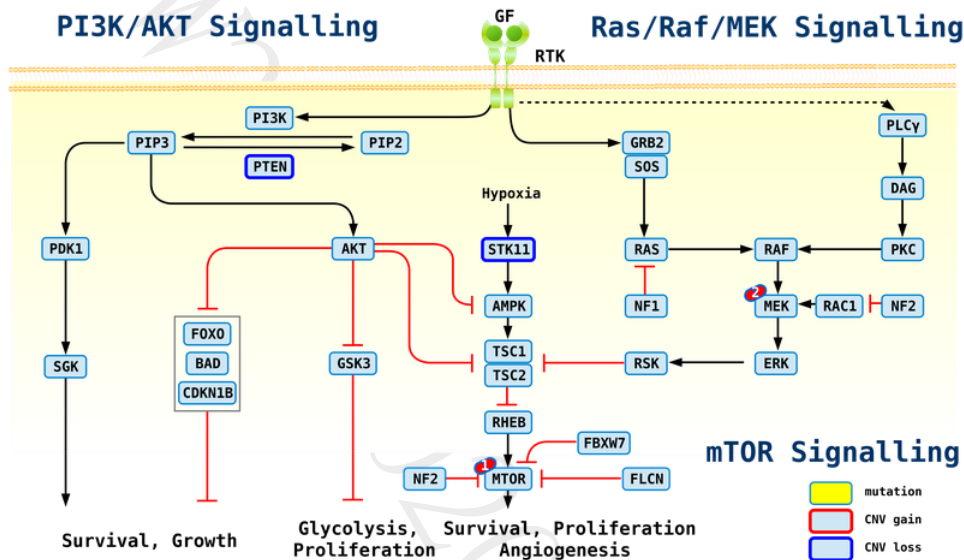
Gene	Therapies	Possible effect
<i>STK11</i>	Binimetinib, Cobimetinib, Everolimus, Temsirolimus, Trametinib	sensitive
<i>PTEN</i>	Everolimus, Niraparib, Olaparib, Rucaparib, Talazoparib, Temsirolimus	sensitive
<i>MLH1</i>	Niraparib, Olaparib, Rucaparib, Talazoparib	sensitive
<i>SMAD4</i>	Cetuximab	resistant
<i>PTEN</i>	Cetuximab, Erlotinib, Gefitinib, Panitumumab, Trastuzumab	resistant

### SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS

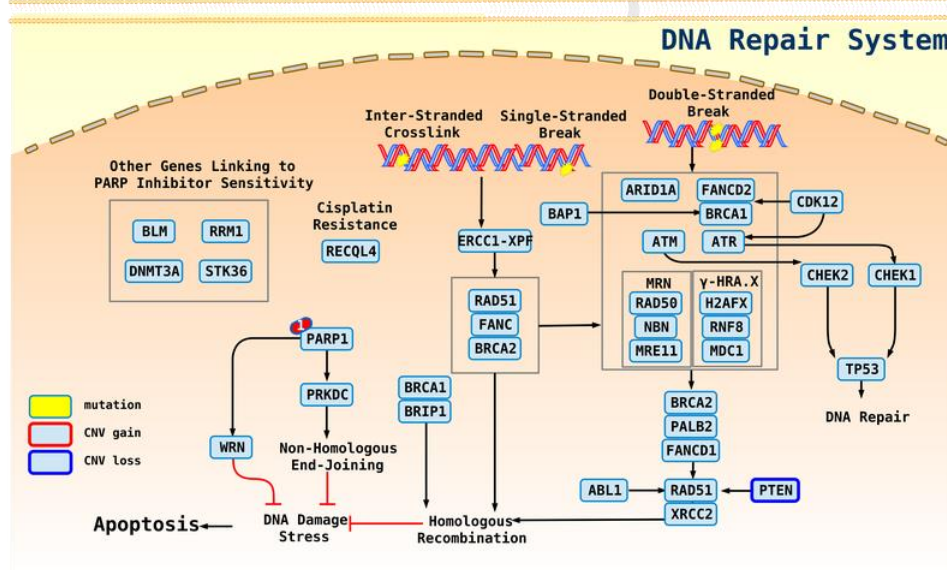


#### 1: Abemaciclib, Ribociclib, Palbociclib

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1: Everolimus, Temsirolimus; 2: Trametinib, Binimetinib, Cobimetinib



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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