

# ACT Onco<sup>®</sup> + Report

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Date of Birth: Apr 26, 1957		Gender: Male
Diagnosis: Lung squamous cell carcinoma		
ORDERING PHYSICIAN		
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SPECIMEN		
Specimen ID: S11170347		Type: FFPE tissue
Collection site: Liver		
Date received: Mar 14, 2022	Lab ID: AA-22-01225	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
CDKN2A E69fs	Abemaciclib, Palbociclib, Ribociclib	-
KMT2C Homozygous deletion	Olaparib	-

## 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	E69fs	50.6%
TP53	G105V	46.1%

#### - Copy Number Alterations

Chromosome	Gene	Variation	Copy Number
Chr7	KMT2C	Homozygous deletion	0
Chr10	PTEN	Heterozygous deletion	1
Chr13	BRCA2	Heterozygous deletion	1
Chr4	FBXW7	Heterozygous deletion	1
Chr5	RAD50	Heterozygous deletion	1
Chr12	CDKN1B, KDM5A, KRAS	Amplification	6*
Chr3	PIK3CA	Amplification	6*
Chr3	PRKCI	Amplification	8

\* Increased gene copy number was observed.

#### - 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)	4.5 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 41% 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®+ 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 3B</b>		
<b>CDKN2A</b> E69fs	Abemaciclib, Palbociclib, Ribociclib	<b>sensitive</b>
<b>Level 4</b>		
<b>KMT2C</b> Homozygous deletion	Olaparib	<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

### CDKN2A E69fs

#### 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>[1][2][3]</sup>. CDKN2A has been reported as a haploinsufficient tumor suppressor with one copy loss that may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[4]</sup>. Loss of CDKN2A has been frequently found in human tumors that result in uncontrolled cell proliferation<sup>[5][6]</sup>.

E69fs mutation results in a change in the amino acid sequence beginning at 69, likely to cause premature truncation of the functional CDKN2A protein (UniProtKB). This mutation is predicted to lead to a loss of CDKN2A protein function, despite not being characterized in the literature.

#### Therapeutic and prognostic relevance

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

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

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

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

### TP53 G105V

#### Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis<sup>[19]</sup>. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation<sup>[20]</sup>.

TP53 G105V lies within the DNA-binding domain (DBD) 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. A phase II trial demonstrated that Wee1 inhibitor (AZD1775) in combination with carboplatin was well tolerated and

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showed promising anti-tumor activity in TP53-mutated ovarian cancer refractory or resistant (< 3 months) to standard first-line therapy (NCT01164995)<sup>[21]</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>[22]</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>[23]</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>[24][25][26]</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>[27]</sup>. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy<sup>[28][29]</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>[30]</sup>.

## **BRCA2 Heterozygous deletion**

### **Biological Impact**

The BRCA2 gene encodes a tumor suppressor involved in the homologous recombination pathway for double-strand DNA repair<sup>[31]</sup>. BRCA2 has been implicated as a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[32]</sup>. BRCA2 germline mutations confer an increased lifetime risk of developing breast, ovarian, prostate and pancreatic cancer, limited reports of related gastric cancer, and Fanconi anemia subtype D1-associated risk of brain cancer, medulloblastoma, pharyngeal cancer, chronic lymphocytic leukemia and acute myeloid leukemia<sup>[33]</sup>. Somatic mutations in BRCA2 are highest in colorectal, non-small cell lung cancer (NSCLC), and ovarian cancers<sup>[34]</sup>.

### **Therapeutic and prognostic relevance**

The U.S. FDA has approved olaparib in advanced ovarian cancer under several settings including (1) first-line maintenance treatment for patients with deleterious or suspected deleterious germline or somatic BRCA mutation who are in complete or partial response to first-line platinum-based chemotherapy<sup>[35]</sup>; (2) in combination with bevacizumab as first-line maintenance treatment for patients with homologous recombination deficiency (HRD)-positive status<sup>[36]</sup>; (3) maintenance treatment for patients with germline BRCA-mutated recurrent ovarian cancer who are in complete or partial response to platinum-based chemotherapy<sup>[37][38]</sup>; (4) treatment for patients with germline BRCA-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy<sup>[39]</sup>. In addition, olaparib has also been approved in patients with deleterious or suspected deleterious germline BRCA-mutated, HER2-negative metastatic breast cancer who have been treated with chemotherapy in either neoadjuvant, adjuvant, or metastatic setting<sup>[40]</sup> and germline BRCA-mutated metastatic pancreatic cancer<sup>[41]</sup>. Of note, 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>[42]</sup>.

Rucaparib has been approved for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy and patients with BRCA-mutated epithelial ovarian, fallopian tube, or primary peritoneal cancer, who have been treated with two or more chemotherapies<sup>[43][44]</sup>. In May 2020, the U.S. FDA also approved rucaparib to treat adult patients with a deleterious BRCA mutation-associated metastatic castration-resistant prostate cancer (mCRPC) who have been treated with androgen receptor-directed therapy and a taxane-based chemotherapy (TRITON2, NCT02952534).



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The U.S. FDA also approved niraparib for the maintenance treatment of patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in response to platinum-based chemotherapy and patients who have been treated with three or more prior lines of chemotherapy and associated with HRD positive status<sup>[45][46][47]</sup>. In addition, talazoparib for patients with deleterious or suspected deleterious germline BRCA-mutated, HER2 negative locally advanced or metastatic breast cancer<sup>[48]</sup>.

## CDKN1B Amplification

### Biological Impact

The CDKN1B gene encodes cyclin-dependent kinase (CDK) inhibitor 1B, also called p27, which is a member of the Cip/Kip protein family. The p27 protein is ubiquitously expressed and located both in the nucleus and in the cytoplasm. Nuclear p27 functions as a tumor suppressor by controlling cell cycle progression from G1 to S phase, specifically by inhibiting the binding of cyclin A and E to CDK2<sup>[49]</sup>. It has been demonstrated that haploinsufficiency of CDKN1B contributed to leukemogenesis in T-cell prolymphocytic leukemia<sup>[50]</sup>.

### Therapeutic and prognostic relevance

Low CDKN1B levels due to increased protein degradation are prevalent in several different types of epithelial tumors and are commonly correlated with aggressive tumor growth and poor clinical outcome<sup>[51][52][53]</sup>. Loss of p27 expression is associated with poor prognosis in a variety of tumors, including pancreatic cancer<sup>[54]</sup>, colorectal cancer<sup>[55]</sup>, gastroenteropancreatic neuroendocrine tumors<sup>[56]</sup>, and breast cancer<sup>[57]</sup>.

In vitro data demonstrated that Src inhibitors could increase p27 stability and restore tamoxifen sensitivity in tamoxifen-resistant breast cancer cells<sup>[58]</sup>.

CDKN1B amplification has been found to correlate with poor prognosis in gastric cancer<sup>[59]</sup>.

## FBXW7 Heterozygous deletion

### Biological Impact

The F-box/WD repeat-containing protein 7 (FBXW7) gene encodes a protein that belongs to the SCF (SKP1-CUL1-F-box protein) E3 ligase complex. FBXW7 is recognized as a tumor suppressor which is involved in the negative regulation of oncogenes such as c-Myc<sup>[60][61]</sup>, c-Jun<sup>[62]</sup>, cyclin E<sup>[63]</sup>, Notch family members<sup>[64][65]</sup>, Aurora-A<sup>[66]</sup>, mTOR<sup>[67]</sup>, KLF5<sup>[68]</sup>, and MCL-1<sup>[69]</sup>. Inactivating FBXW7 mutation or copy number loss may result in the accumulation of oncoproteins and therefore lead to malignant transformation<sup>[70]</sup>. FBXW7 is a haploinsufficient tumor suppressor gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[68][69][71]</sup>.

### Therapeutic and prognostic relevance

Clinical efficacy of mTOR inhibitors was seen in patients harboring aberrations in the FBXW7 gene (one patient with refractory fibrolamellar hepatocellular carcinoma, and one patient with lung adenocarcinoma)<sup>[72][73]</sup>. Moreover, in vitro assay also suggested that loss or inactivation of FBXW7 may confer sensitivity to mTOR inhibitor<sup>[67]</sup>.

Preclinical studies suggested that mutations or loss of FBXW7 were associated with regorafenib and oxaliplatin resistance in CRC cell lines and gefitinib resistance in lung cancer cells<sup>[74][75][76][77]</sup>.

Retrospective studies have indicated that a relatively low expression level of FBXW7 is an independent prognostic marker of poor survival for patients with hepatocellular carcinoma, lung adenocarcinoma and squamous cell carcinoma<sup>[78][79]</sup>.

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## KDM5A Amplification

### Biological Impact

KDM5A (lysine demethylase 5A) gene encodes a histone demethylase for histone 3 lysine 4 (H3K4)<sup>[79]</sup> which regulates cell cycle progression and cellular differentiation by chromatin remodeling and transcriptional silencing<sup>[80][81][82][83]</sup>. KDM5A gene amplification has been reported in breast cancer, glioblastoma, and head and neck cancer<sup>[84][85][86]</sup> which is associated with angiogenesis, tumor progression, and treatment resistance<sup>[87][88][89]</sup>. Rearrangements of KDM5A with NUP98 has been reported in acute leukemia patients<sup>[90]</sup>.

### Therapeutic and prognostic relevance

Several in vitro studies have reported that amplification of KDM5A was correlated with drug resistance such as temozolomide in glioblastoma<sup>[85]</sup>, gefitinib in NSCLC<sup>[89]</sup>, and erlotinib in breast cancer<sup>[84]</sup>.

## KMT2C Homozygous deletion

### Biological Impact

Lysine methyltransferase 2C (KMT2C) gene encodes the histone methyltransferase MLL3, which methylates lysine residue four on the tail of histone H3 (H3K4)<sup>[91]</sup> and regulates the gene expression during development and hematopoiesis<sup>[92][93][94]</sup>. KMT2C is ubiquitously expressed, and its function is essential for normal embryonal development and cell proliferation<sup>[95]</sup>. Genetic deletion of the region containing KMT2C is the most common chromosomal abnormality in acute myeloid leukemia<sup>[96][97]</sup>, and KMT2C mutation has been reported in breast cancer, cutaneous squamous cell carcinoma, and leukemia<sup>[98][99][100][101][102]</sup>. KMT2C was implicated as a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[103]</sup>. Animal studies revealed that MLL3 haploinsufficiency enhances hematopoietic stem cells (HSCs) self-renewal capacity and induces extensive division of HSCs (AACR; Cancer Res 2018;78(13 Suppl): Abstract nr 4996).

### Therapeutic and prognostic relevance

Preclinical studies of cell lines and xenograft models demonstrated that cells with reduced KMT2C expression and activity are deficient in homologous recombination-mediated double-strand break DNA repair and therefore, are more sensitive to olaparib, a PARP1/2 inhibitor<sup>[104]</sup>.

A meta-analysis indicated that low levels of KMT2C expression was associated with better overall survival in pancreatic ductal adenocarcinoma (PDAC) patients<sup>[105]</sup>. However, another study of ER-positive breast cancer patients (n = 401) demonstrated that low KMT2C expression was associated with worse overall survival<sup>[106]</sup>.

## KRAS Amplification

### 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>[107]</sup>. KRAS mutations occur primarily in three hotspots G12, G13 and Q61, and less frequently in codon A146<sup>[107][108]</sup>. These are activating mutations that lead to constitutive activation and persistent stimulation of the downstream signaling pathways<sup>[109][110]</sup>. Mutations in KRAS have been reported in a diverse spectrum of human malignancies, including pancreatic carcinomas (>80%)<sup>[107][111]</sup>, colon carcinomas (40-50%)<sup>[112][113]</sup>, and lung carcinomas (30-50%)<sup>[114][115]</sup>, but are also present in biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, myeloid leukemia and breast cancer<sup>[108]</sup>.



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## 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>[116]</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>[117][118][119][120]</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>[121]</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>[122]</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>[123]</sup>.

Both clinical and preclinical studies demonstrated a limited response to monotherapy using MEK inhibitors<sup>[124]</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>[125]</sup>, and in patient-derived xenografts of RAS-mutant CRC, inhibition of MEK and mTOR suppressed tumor growth, but not tumor regression<sup>[126]</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>[127]</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>[128]</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>[129][130]</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>[131][132][133][134][135][136][137]</sup>.

Sorafenib, a multi-kinase inhibitor, has been shown to be beneficial in KRAS-mutant CRC<sup>[138]</sup>, KRAS-mutant NSCLC<sup>[139]</sup>, and KRAS-amplified melanoma<sup>[140]</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>[141][142]</sup>.

In NCCN guidelines for NSCLC (version 5. 2021), 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>[143][144][145]</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>[146]</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>[147]</sup>. In ovarian serous borderline tumor with recurrent low-grade serous carcinoma, patient harboring KRAS G12V mutation appeared to have shorter survival time<sup>[148]</sup>.

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Metastatic colorectal cancer patients harboring KRAS amplification were resistant to anti-EGFR therapy such as cetuximab and panitumumab<sup>[149][150]</sup>.

Some in vitro studies showed that activation of the RAS, due to either KRAS/NRAS mutations or to KRAS amplification, rendered lung cancer cells resistant to ROS1 inhibition by crizotinib<sup>[151][152][153]</sup>.

## PIK3CA Amplification

### Biological Impact

The PIK3CA gene encodes the catalytic subunit (p110α) of phosphatidylinositol 3-kinase (PI3K) that plays a key role in the PI3K/AKT signaling pathway and is involved in the regulation of cellular functions such as proliferation, metabolism and protein synthesis, angiogenesis and apoptosis. PIK3CA has long been described as an oncogene and the PIK3CA gene amplification, deletion, and mutations have been reported in a wide range of cancers, including colorectal, breast, brain, liver, ovarian, stomach and lung cancers<sup>[154][155][156][157]</sup>. Mutations located in the exon 9 that encodes the PI3K helical (like E542K, E545K) and the exon 20 that encodes the catalytic/kinase domain (like H1047R, H1047L, H1047Y) have been shown to result in the constitutively activated mutant, which could enhance downstream signaling and oncogenic transformation in vitro and in vivo<sup>[155][158][159][160]</sup>.

### Therapeutic and prognostic relevance

Results of a phase I study (n=60) showed that one platinum-refractory epithelial ovarian cancer patient with PTEN loss and PIK3CA amplification had a partial response to PI3K inhibitor pictilisib (GDC-0941)<sup>[161]</sup>.

In a preclinical study, buparlisib (BKM120) exerted antitumor activity in lung squamous cell carcinoma cells overexpressing wild-type PIK3CA in culture<sup>[162]</sup>. Other preclinical studies of triple-negative breast cancer (TNBC), head and neck squamous cell carcinoma, ovarian cancer, lung small cell carcinoma, and PIK3CA-amplified cell lines were sensitive to PI3K inhibitors pictilisib<sup>[163]</sup>, BEZ235<sup>[164]</sup>, pilaralisib<sup>[165]</sup>, and PIK3CA/PIK3CD inhibitor PF-4989216<sup>[166]</sup>, respectively.

PIK3CA amplification is a biomarker predicting ovarian cancer lack of response to taxane- and platinum-based chemotherapy in ovarian cancer patients<sup>[167]</sup>.

PIK3CA amplification is associated with poor prognosis among patients with gastric cancer<sup>[168]</sup>, esophageal squamous cell carcinoma<sup>[169]</sup>, and non-lymph node metastatic head and neck squamous cell carcinoma<sup>[170]</sup>.

## PRKCI Amplification

### Biological Impact

The PRKCI gene encodes a member of the atypical protein kinase C (PKC). PKCs are a family of lipid-dependent serine/threonine kinases that represent a branch of the AGC kinase group which are central components of many signaling pathways that regulate diverse cellular functions including proliferation, cell cycle, differentiation, survival, cell migration, and polarity<sup>[171][172][173]</sup>. PRKCI resides on chromosome 3q26, one of the most frequently amplified genomic regions in human cancers, including cervical, head and neck, lung squamous and serous ovarian cancers<sup>[174][175][176][177]</sup>. PKCi is frequently overexpressed in the majority of tumor types<sup>[178]</sup>. As reviewed in<sup>[179][178][180]</sup>, PKCi is required for multiple aspects of the transformed phenotype and appears to participate in the initiation, progression and metastatic stages of cancer.

### Therapeutic and prognostic relevance

In NSCLC, the PB1-PB1 interaction between PKCi and Par6 is required for the transformed phenotype and Rac1 activation<sup>[181]</sup>. A high throughput screen for small molecular weight compounds identified gold-containing compounds such as aurothioglucose (ATG), aurothiomalate (ATM) (FDA-approved for treatment of rheumatoid arthritis patients),

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and auranofin (ANF) as selective and potent inhibitors of PKCi and Par6 binding<sup>[182]</sup>. Phase I studies suggested that ATM is well tolerated in patients with NSCLC and ovarian cancer (NCT00575393). However, currently, there is no FDA-approved PKCi inhibitor for patients with cancer.

Increased PRKCi gene copy number and/or increased PKCi protein expression level are associated with decreased progression-free survival and overall survival in various cancer types, including lung<sup>[175]</sup>, pancreatic cancer<sup>[183]</sup>, cholangiocarcinoma<sup>[184]</sup>, ovarian cancer<sup>[176][185]</sup>. Moreover, a correlation between PKCi expression and prostate cancer recurrence was reported<sup>[186]</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>[187][188]</sup>. PTEN acts as an essential tumor suppressor by antagonizing the PI3K/AKT/mTOR signaling pathway<sup>[189]</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>[20][190][191]</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>[192][193][194]</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>[195][196][197][198][199]</sup>.

### 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>[200][201]</sup>. Preclinical studies demonstrated that PTEN deficiency was associated with increased sensitivity to PI3K pathway inhibitors in selected cancer subtypes<sup>[202][203][204][205][206][207]</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>[208]</sup>, advanced pancreatic neuroendocrine tumors<sup>[209]</sup>, and metastatic castration-resistant prostate cancer treated with mTORC1 inhibitor, everolimus<sup>[210]</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>[211][212][213][214][215]</sup>.

Loss of PTEN expression in advanced colorectal cancer (CRC) has been linked with resistance to anti-EGFR mAbs like cetuximab and panitumumab<sup>[216][217][218][219][220][134]</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>[221][222]</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>[223]</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>[224][225][226]</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),

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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>[227]</sup>.

## **RAD50 Heterozygous deletion**

### **Biological Impact**

The RAD50 gene encodes a highly-conserved DNA double-strand break (DSB) repair factor. It forms MRN complex with NBS1 and MRE11 protein and is involved in sensing and early processing of DSB, cell cycle checkpoints, DNA recombination and maintenance of telomeres<sup>[228][229]</sup>. Mutations in the components of the MRN complex could increase susceptibility to familial breast cancer<sup>[230][231]</sup>, gastric cancer<sup>[232]</sup>, colorectal cancer<sup>[233]</sup>, and urothelial cancer<sup>[234]</sup>. RAD50 has been implicated as a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions<sup>[235]</sup>. Besides, RAD50 deletion was also suggested as a marker of BRCAness, a phenotype shared between non-BRCA1/2-mutated ovarian cancers and BRCA1/2-mutated ovarian cancers<sup>[236]</sup>.

### **Therapeutic and prognostic relevance**

Preclinical data showed that knockdown of the RAD50 gene in ovarian cancer cell lines was significantly associated with better responses to two PARP inhibitors, olaparib and rucaparib<sup>[236]</sup>. RAD50 has been selected as an inclusion criterion for the trials examining talazoparib efficacy in HER2-negative breast cancer, olaparib efficacy in breast cancer, rucaparib efficacy in metastatic prostate cancer and niraparib efficacy in any malignancy (except prostate) (NCT02401347, NCT03207347, NCT03344965, NCT03413995).

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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>[237]</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>[16]</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>[238]</sup> NCT02102490	<b>Breast cancer</b> (Approved on 2017/09/28)
	<b>HR-positive, HER2-negative</b> Abemaciclib [ORR(%): 19.7 vs. 17.4]

### Everolimus (AFINITOR)

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

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

<b>RADIANT-4</b> <sup>[239]</sup> NCT01524783	<b>Lung or gastrointestinal neuroendocrine tumor</b> (Approved on 2016/02/26)
	- Everolimus vs. Placebo [PFS(M): 11 vs. 3.9]
<b>BOLERO-2</b> <sup>[240]</sup> NCT00863655	<b>Breast cancer</b> (Approved on 2012/07/20)
	<b>ER+/HER2-</b> Everolimus + exemestane vs. Placebo + exemestane [PFS(M): 7.8 vs. 3.2]
<b>EXIST-2</b> NCT00790400	<b>Tuberous sclerosis complex (tsc)-associated renal angiomyolipoma</b> (Approved on 2012/04/26)
	- Everolimus vs. Placebo [ORR(%): 41.8 vs. 0]
<b>RADIANT-3</b> <sup>[209]</sup> NCT00510068	<b>Pancreatic neuroendocrine tumor</b> (Approved on 2011/05/05)
	- Everolimus vs. Placebo [PFS(M): 11 vs. 4.6]
<b>EXIST-1</b> <sup>[241]</sup> NCT00789828	<b>Subependymal giant cell astrocytoma</b> (Approved on 2010/10/29)
	- Everolimus vs. Placebo [ORR(%): 35.0]
<b>RECORD-1</b> <sup>[242]</sup> NCT00410124	<b>Renal cell carcinoma</b> (Approved on 2009/05/30)
	- Everolimus vs. Placebo [PFS(M): 4.9 vs. 1.9]



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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>[47]</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>[46]</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>[42]</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>[36]</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>[41]</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>[35]</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>[40]</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>[243]</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>[244]</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>[245]</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]

## 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>[246]</sup> NCT01740427	<b>Breast cancer</b> (Approved on 2017/03/31)
	<b>ER+, HER2-</b>
	Palbociclib + letrozole vs. Placebo + letrozole [PFS(M): 24.8 vs. 14.5]
PALOMA-3 <sup>[247]</sup> NCT01942135	<b>Breast cancer</b> (Approved on 2016/02/19)
	<b>ER+, HER2-</b>
	Palbociclib + fulvestrant vs. Placebo + fulvestrant [PFS(M): 9.5 vs. 4.6]

## Ribociclib (KISQALI)

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

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

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

## 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>[43]</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]
ARIEL2 <sup>[248]</sup> NCT01482715, NCT01891344	<b>Ovarian cancer</b> (Approved on 2016/12/19)
	<b>Germline and/or somatic BRCA mutation</b>
	Rucaparib [ORR(%): 54.0]

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## 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>[48]</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)

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

D=day; W=week; M=month

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## ONGOING CLINICAL TRIALS

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

No trial has been found.

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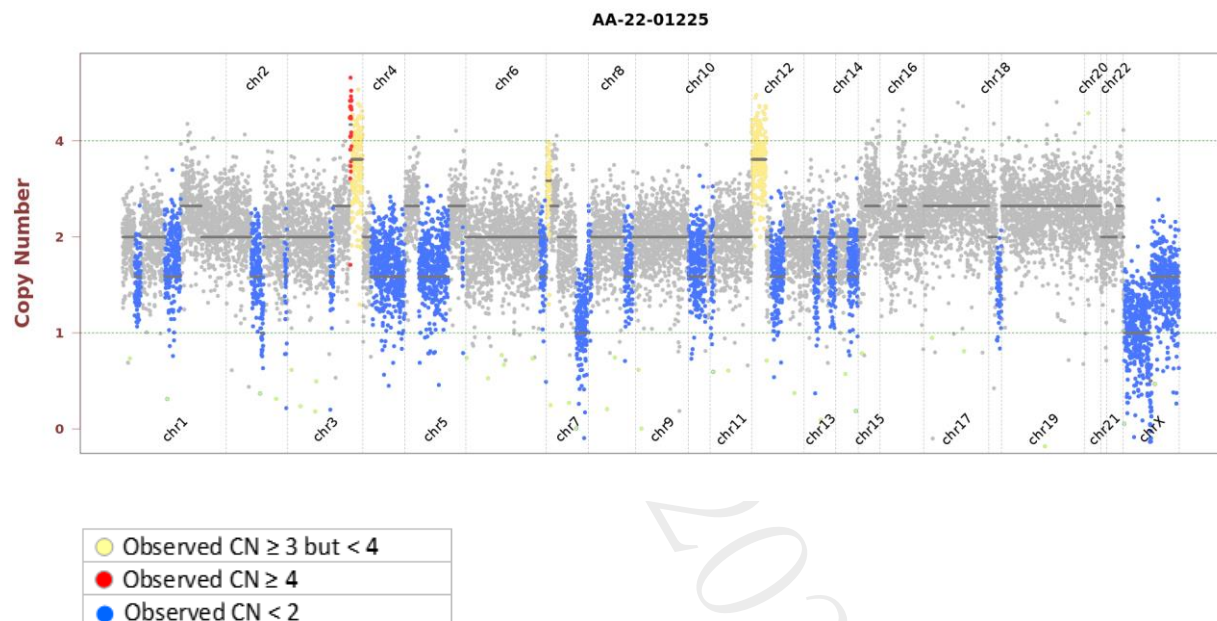
## SUPPLEMENTARY INFORMATION OF TESTING RESULTS DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

### - Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
CDKN2A	E69fs	2	c.205del	NM_000077	COSM13677	50.6%	449
TP53	G105V	4	c.314G>T	NM_000546	COSM46161	46.1%	1012

### - 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
ADAMTS9	M1100T	22	c.3299T>C	NM_182920	-	70.2%	1624
BRAF	M279L	6	c.835A>T	NM_004333	-	45.8%	742
GNAS	H69N	1	c.205C>A	NM_016592	-	45.9%	464
KDR	S231C	6	c.692C>G	NM_002253	-	31.0%	1072
MUC16	I2455T	1	c.7364T>C	NM_024690	-	51.7%	325
MUC16	S10457L	4	c.31370C>T	NM_024690	COSM3404848	59.5%	967
NEFH	A380T	3	c.1138G>A	NM_021076	COSM2936486	31.3%	772
NFE2L2	D29N	2	c.85G>A	NM_006164	COSM124735	33.9%	2287
NSD1	T1098A	5	c.3292A>G	NM_022455	-	43.7%	1202
NTRK1	R220Q	6	c.659G>A	NM_002529	COSM4140875	51.8%	1039
POLE	Splice region	-	c.3061-4C>G	NM_006231	-	33.2%	1520
RET	L481V	7	c.1441C>G	NM_020975	-	30.1%	831
RUNX1T1	R458S	11	c.1372C>A	NM_175634	-	27.8%	1154
TP53	Splice region	4	c.375G>T	NM_000546	COSM45940	9.8%	1035
USH2A	G1947A	29	c.5840G>C	NM_206933	-	36.3%	1244
USH2A	L4030P	62	c.12089T>C	NM_206933	-	17.3%	612

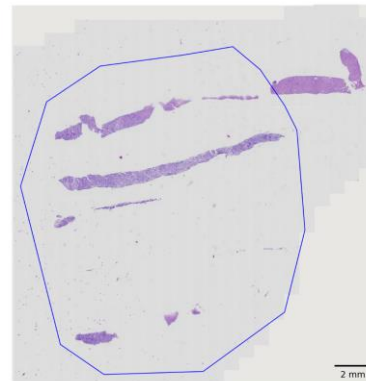
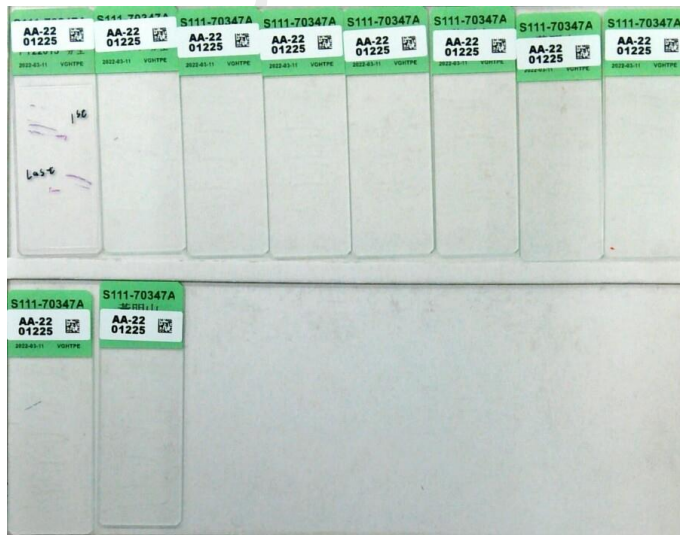
### Note:

- 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: Mar 2022
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11170347
- Collection site: Liver
- Examined by: Dr. Yeh-Han Wang
  1. The percentage of viable tumor cells in total cells in the whole slide (%): 30%
  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: 884x
- Target Base Coverage at 100x: 93%

### RNA test

- Average unique RNA Start Sites per control GSP2: 144



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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:

醫檢師陳韻仔 博士  
Yun-Yu Chen Ph.D.  
檢字第 015647 號

Yun Yu Chen

## Sign Off

醫檢師陳韻仔 博士  
Yun-Yu Chen Ph.D.  
檢字第 015647 號

Yun Yu Chen

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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	BMPR1A	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	CYP11A1*	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*
SLC18A3*	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	TGFB2	TMSB4X*	TNF	TNFAIP3	TNFRSF14	TNFSF11	TOP1	TP53
TPMT*	TSC1	TSC2	TSHR	TYMS	U2AF1	UBE2A*	UBE2K	UBR5	UGT1A1*	USH2A	VDR*
VEGFA	VEGFB	VHL	WT1	XIAP	XPO1	XRCC2	ZNF217				

\*Analysis of copy number alterations NOT available.

## FUSION

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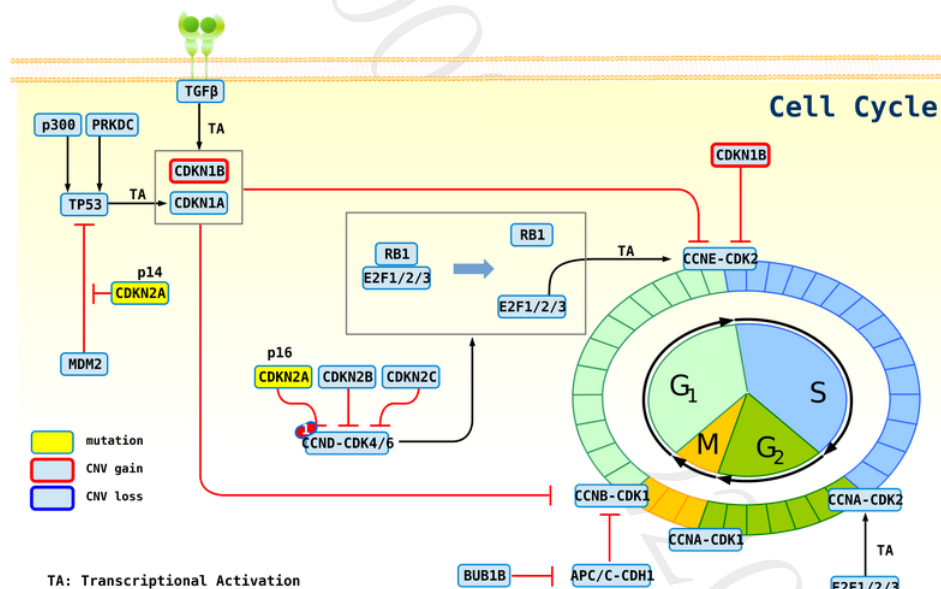
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## APPENDIX

### POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

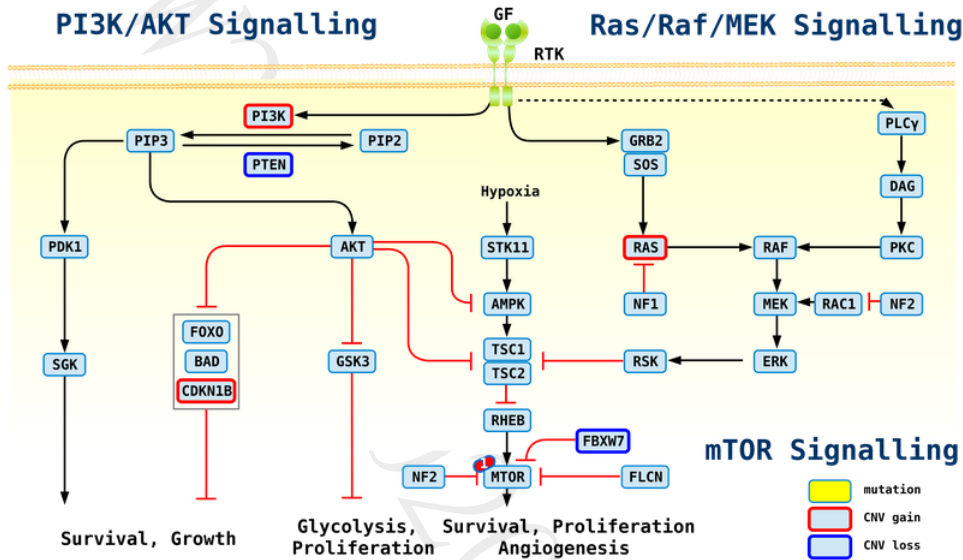
Gene	Therapies	Possible effect
<i>PTEN</i>	Everolimus, Niraparib, Olaparib, Rucaparib, Talazoparib, Temsirolimus	<b>sensitive</b>
<i>FBXW7</i>	Everolimus, Temsirolimus	<b>sensitive</b>
<i>BRCA2</i>	Niraparib, Olaparib, Rucaparib, Talazoparib	<b>sensitive</b>
<i>RAD50</i>	Niraparib, Olaparib, Rucaparib, Talazoparib	<b>sensitive</b>
<i>PTEN</i>	Cetuximab, Erlotinib, Gefitinib, Panitumumab, Trastuzumab	<b>resistant</b>
<i>FBXW7</i>	Gefitinib, Regorafenib	<b>resistant</b>

### SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS

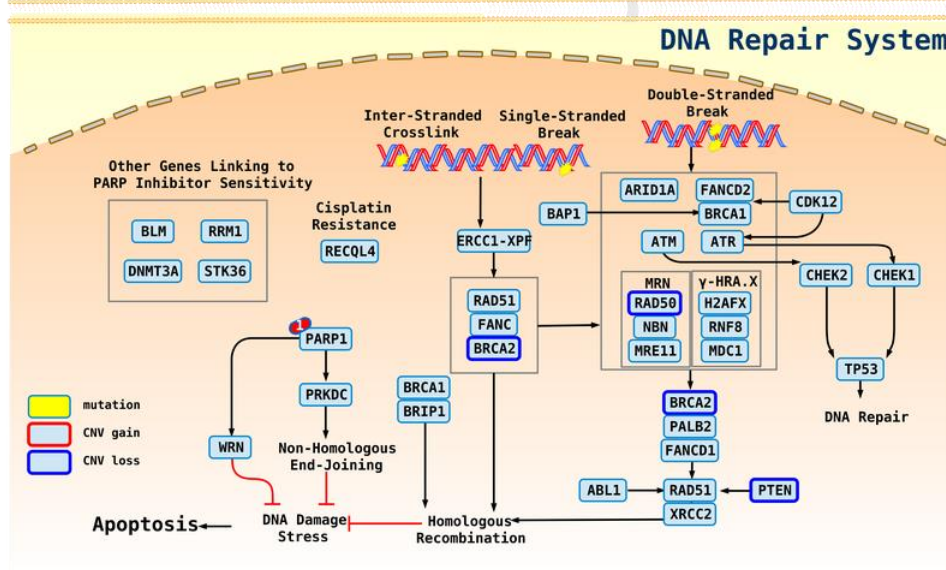


#### 1: Abemaciclib, Ribociclib, Palbociclib

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1: Everolimus, Temsirolimus



1: Olaparib, Niraparib, Rucaparib, Talazoparib

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### 法律聲明

本檢驗報告僅提供專業醫療參考，結果需經專業醫師解釋及判讀。基因突變資訊非必具備藥物或治療有效性指標，反之亦然。本檢驗報告提供之用藥指引不聲明或保證其臨床有效性，反之亦然。本基因檢測方法係由本公司研究開發，已經過有效性測試。

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

### 醫療決策需由醫師決定

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

### 基因突變與用藥資訊並非依照有效性排序

本報告中列出之生物標記變異與藥物資訊並非依照潛在治療有效性排序。

### 證據等級

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

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