高添富先生 您好:

感謝您選用行動基因所提供的檢測服務。行動基因經您的同意，於西元 2021 年 08 月 06 日取得您的檢體，進行 ACTOnco®+癌安克™癌症基因檢測與 ACTFusion™癌融克™癌症融合基因檢測。行動基因實驗室為通過美國病理學會 (The College of American Pathologists，CAP) (CAP#：9028096) 與臺灣衛生福利部食品藥物管理署「精準醫療分子檢測列冊登錄實驗室」(Laboratory Developed Tests and Services, LDTS) (列冊登錄編號：LDTS0001) 的認證機構。

ACTOnco®+癌安克™癌症基因檢測平台利用次世代定序分析技術，同時檢測 440 個與腫瘤相關的基因變異，並計算腫瘤突變負荷量。

ACTFusion™癌融克™癌症融合基因檢測能檢測 13 個融合基因轉錄片段。

行動基因的專業生物與醫藥資訊團隊根據您的基因檢測結果與參考文獻，評估您對藥物的反應，輔助醫師進行治療與預後分析，以體現癌症精準醫療。

本次檢測於腫瘤檢體偵測到的重要基因變異及其相對應的標靶用藥如下:

基因變異	具敏感性之標靶用藥	具抗藥性之標靶用藥
• PIK3CA E545K 基因突變 • NF1 Splice donor 基因突變 • NF1 基因減少	• Trametinib	-
• CDKN2A 基因減少	• Ribociclib • Abemaciclib • Palbociclib	-
• RAD51C 基因減少	• Niraparib	-
• BRCA1, BRCA2 基因減少	• Rucaparib	-
• RAD51C, BAP1, BRCA1, KMT2C, BRCA2 基因減少	• Olaparib	-
• PIK3CA E545K 基因突變	• Lapatinib* • Trastuzumab* • Alpelisib	-
• PIK3CA E545K 基因突變 • NF1 Splice donor 基因突變 • TSC1, NF2, NF1 基因減少	• Everolimus	-
• PIK3CA E545K 基因突變 • TSC1 基因減少	• Temsirolimus	-
• NF1 Splice donor 基因突變 • SMAD4, NF1 基因減少	-	• Cetuximab
• NF1 Splice donor 基因突變 • NF1 基因減少	-	• Lapatinib • Gefitinib • Afatinib • Erlotinib • Vemurafenib • Trastuzumab
• RB1 基因減少	-	• Ribociclib • Abemaciclib • Palbociclib

*研究結果顯示，帶有此基因變異者對此藥物的反應率較差。

腫瘤突變負荷量 (TMB): 5.2 mutations/megabase

微小衛星體不穩定性 (MSI): 穩定(stable)

融合基因: 未測得基因融合

詳細變異基因描述與用藥建議，請參閱以下完整基因檢測報告內容。

基因檢測報告所提供的資訊僅作為診療參考依據之一，您必須藉由醫師綜合評估過去的治療紀錄及專業判斷，選擇最適合您的治療策略。

若您對本檢測報告有任何疑問，請隨時與我們聯繫。

行動基因 敬上

ACTOnco®+ Report

REPORT SUMMARY

REPORT DETAILS

APPENDIX

PATIENT AND SAMPLE INFORMATION

PATIENT	SPECIMEN	ORDERING PHYSICIAN
Name: 高添富	Type: FFPE tissue	Name: 徐博奎醫師
Gender: Male	Date received: Aug 06, 2021	Facility: 臺北榮總
Date of Birth: Jul 27, 1949	Collection site: Esophagus	Tel: 886-228712121
Patient ID: 37977687	Specimen ID: S10944572	Address: 臺北市北投區石牌路二段 201 號
Diagnosis: Esophageal cancer	Lab ID: AA-21-03176	Cooperative ID: NA
	D/ID: NA	

VARIANT(S) WITH CLINICAL RELEVANCE

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

SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Gene	Amino Acid Change	Coverage	Allele Frequency	COSMIC ID
NF1	Splice donor	369	38.8%	-
PIK3CA	E545K	321	30.2%	COSM763
TGFBR2	Y281fs	432	44.0%	-
TP53	C135*	608	42.1%	COSM44319

COPY NUMBER VARIANTS (CNVS)

Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on **55%** tumor purity.

Amplification (Copy number ≥ 8)

Chr	Gene	Copy Number
chr7	*CDK6*	7¥

Homozygous deletion (Copy number=0)

Chr	Gene
chr9	*CDKN2A*

¥ Increased gene copy number was observed.

Heterozygous deletion (Copy number=1)

Chr	Gene
chr3	*BAP1, TGFBR2*
chr7	*KMT2C*
chr9	*TSC1*
chr13	*BRCA2, RB1*
chr17	*BRCA1, NF1, RAD51C*
chr18	*SMAD4*
chr22	*NF2*

TUMOR MUTATIONAL BURDEN (TMB)

5.2 muts/Mb

Muts/Mb, mutations per megabase

MICROSATELLITE INSTABILITY (MSI)

Microsatellite stable (MSS)

Note:
TMB was calculated by using the sequenced regions of ACTOnco®+ to estimate the number of somatic nonsynonymous mutations per megabase of all protein-coding genes (whole exome). The threshold for high mutation load is set at ≥ 7.5 mutations per megabase. TMB, microsatellite status and gene copy number deletion cannot be determined if calculated tumor purity is < 30%.

Variant Analysis:
醫檢師張筑芫 博士
Chu-Yuan Chang Ph.D.
檢字第 020115 號

Sign Off
醫檢師張筑芫 博士
Chu-Yuan Chang Ph.D.
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THERAPEUTIC IMPLICATIONS

TARGETED THERAPIES

Genomic Alterations	Therapies	Effect
Level 3A		
PIK3CA E545K	Alpelisib	**Sensitive**
Level 3B		
PIK3CA E545K	Everolimus, Temsirolimus	**Sensitive**
CDKN2A Homozygous deletion	Abemaciclib, Palbociclib, Ribociclib	**Sensitive**
RAD51C Heterozygous deletion	Niraparib	**Sensitive**
Level 4		
PIK3CA E545K	Trametinib	**Sensitive**
NF1 Splice donor	Everolimus, Trametinib	**Sensitive**
NF1 Heterozygous deletion	Everolimus, Trametinib	**Sensitive**
NF2 Heterozygous deletion	Everolimus	**Sensitive**
TSC1 Heterozygous deletion	Everolimus, Temsirolimus	**Sensitive**
BAP1 Heterozygous deletion	Olaparib	**Sensitive**
RAD51C Heterozygous deletion	Olaparib	**Sensitive**
KMT2C Heterozygous deletion	Olaparib	**Sensitive**
BRCA1 Heterozygous deletion	Olaparib, Rucaparib	**Sensitive**
BRCA2 Heterozygous deletion	Olaparib, Rucaparib	**Sensitive**
PIK3CA E545K	Lapatinib, Trastuzumab	**Less sensitive**
NF1 Splice donor	Afatinib, Erlotinib, Gefitinib, Lapatinib, Trastuzumab, Vemurafenib, Cetuximab	**Resistant**
NF1 Heterozygous deletion	Afatinib, Erlotinib, Gefitinib, Lapatinib, Trastuzumab, Vemurafenib, Cetuximab	**Resistant**
SMAD4 Heterozygous deletion	Cetuximab	**Resistant**
RB1 Heterozygous deletion	Abemaciclib, Palbociclib, Ribociclib	**Resistant**

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

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

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

IMMUNE CHECKPOINT INHIBITORS (ICI) THERAPIES

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

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

MMR, mismatch repair; ND, not detected

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

CHEMOTHERAPIES

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

HORMONAL THERAPIES

Therapies	Genomic Alterations	Effect	Gene / Variant Level Evidence	Cancer Type
Tamoxifen	***NF1*** Splice donor Heterozygous deletion	**Less sensitive**	Clinical	Breast cancer
	TGFBR2 Y281fs Heterozygous deletion	**Resistant**	Clinical	Breast cancer

OTHERS

Pharmacogenomic implication

Gene	Detection Site	Genotype	Drug Impact	Clinical Interpretation	Level of Evidence*
UGT1A1	rs4148323	AG	Irinotecan-based regimens	Patients with the AG genotype and cancer who are treated with irinotecan-based regimens may have an increased risk of diarrhea and neutropenia as compared to patients with the GG genotype, or a decreased risk of diarrhea and neutropenia compared to patients with the AA genotype. Other genetic and clinical factors may also influence a patient's risk of diarrhea and neutropenia.	**Level 1B**

* Level of evidence was defined by PharmGKB (https://www.pharmgkb.org/page/clinAnnLevels)

Level 1A: ***Clinical annotations describe variant-drug combinations that have variant-specific prescribing guidance available in a current clinical guideline annotation or an FDA-approved drug label annotation.***

Level 1B: ***Clinical annotations describe variant-drug combinations with a high level of evidence supporting the association but no variant-specific prescribing guidance in an annotated clinical guideline or FDA drug label.***

Level 2A: ***Variants in Level 2A clinical annotations are found in PharmGKB's Tier 1 Very Important Pharmacogenes (VIPs). These variants are in known pharmacogenes, implying causation of drug phenotype is more likely.***

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

VARIANT INTERPRETATION

NF1 Splice donor, Heterozygous deletion

Biological Impact

The neurofibromin 1 (NF1) gene encodes a GTPase activating protein (GAP) which is an important negative regulator of the Ras cellular proliferation pathways [1][2][3][4]. Besides, NF1 also physically interacts with the N-terminal domain of focal adhesion kinase (FAK) and involves in the regulation of cell adhesion, growth, and other pathways [5][6]. NF1 is considered a classical haploinsufficient tumor suppressor gene with loss of one allele through inherited or acquired mutation may lead to reduced protein expression and is insufficient to execute normal cellular functions contributing to tumor development [7][8][9][10][11]. NF1 syndrome is a germline condition resulting in a predisposition to several types of cancer such as neurofibromas, melanoma, lung cancer, ovarian cancer, breast cancer, colorectal cancer, hematological malignancies [12][13][14]. Meanwhile, sporadic NF1 mutations have been observed in multiple cancer types [15], including myelodysplastic syndromes, melanomas, colon cancer [16], glioblastomas [17], lung cancer [18], ovarian cancer, and breast cancer [12].

NF1 c.7869+1G>T is a variant located at the splice donor region, which may result in the exon skipping. Loss of the second wild-type allele resulted in the biallelic inactivation of the gene.

Therapeutic and prognostic relevance

Currently, there is no FDA-approved drug for NF1-based therapeutic regimen. A case study had reported that MEK inhibitor, trametinib, was effective in a treatment-refractory neurofibromatosis type I-associated glioblastoma [19]. Various preclinical data had also supported the activity of MEK [20][21][22][23] and mTOR [24][25] inhibitors in NF1-deficient tumors.

In an NGS-based study, patients harboring mutations in the mTOR pathway, including mTOR, TSC1, TSC2, NF1, PIK3CA, and PIK3CG responded to everolimus [26].

NF1 depletion has been associated with drug resistance to RAF and EGFR inhibitors, tamoxifen and retinoic acid [15][27]. For example, loss of NF1 was identified in patients with lung adenocarcinomas or colorectal cancer who presented resistance to anti-EGFR treatment, including erlotinib, gefitinib, afatinib, and cetuximab, respectively [28][29][30]. Notably, preclinical studies further revealed that the addition of a MEK inhibitor could restore the sensitivity to erlotinib [28].

In a liquid biopsy-based ctDNA profiling study of HER2-positive metastatic gastric cancer, NF1 loss (either induced by mutation or deletion) was suggested as a novel mechanism contributes to trastuzumab resistance. The cell-based study also showed that the trastuzumab resistance could be overcome with a combination of HER2 and MEK/ERK inhibitors [31].

Loss of NF1 in patients with BRAF-mutated melanomas was suggested conferring resistance to BRAF inhibitors [32][33][34][35].

PIK3CA E545K

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 [36][37][38][39]. 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 [37][40][41][42].

The PIK3CA E545K/E542K is the second most prevalent activating mutations in breast cancer and are also highly recurrent in other cancer types.

Therapeutic and prognostic relevance

In a preclinical study, cells harbored different activating PIK3CA mutations (H1047R, E545K, G1049R, Q546K, N345K, H1047L, E542K) were significantly more sensitive to PIK3 pathway inhibitors (dactolisib, MK2206, alpelisib), and MEK1/2 inhibitor trametinib, compared to wild-type [43]. According to ExteNET trial, PIK3CA activating mutation was not an appropriate predictive biomarker of response to neratinib in HER2-positive early breast cancer [44].

In 2019 May, alpelisib, the PI3K inhibitor, in combination with fulvestrant received U.S. FDA approval for the treatment of postmenopausal women and men, with HR+, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced breast cancer following progression on or after an endocrine-based regimen. The approval is based on the results of the SOLAR-1 trial (NCT02437318), a randomized phase III trial of 572 patients with breast cancers showed that addition of alpelisib to fulvestrant significantly prolonged progression-free survival (median of 11 months vs. 5.7 months) in patients whose tumors had a PIK3CA mutation including C420R, E542K, E545A, E545D, E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y [45].

An early phase study demonstrated that alpelisib has potential treatment benefits in a subset of PIK3CA-mutated tumors. Of the 114 patients with PIK3CA-mutated tumors, one patient with endometrial cancer achieved a complete response and seven had partial responses (including cervical, breast, endometrial, colon and rectal cancers). Besides, stable disease was observed in 70 patients and disease control rate was 58.2% [46].

Given that compared to patients with wild-type PIK3CA, those carrying PIK3CA mutations have a favorable response to mTOR inhibitors-containing monotherapy like everolimus and temsirolimus in several clinical studies of advanced malignancies [47][48][49][50][51] or temsirolimus in combination with doxorubicin and bevacizumab [52][53], combining PI3K-targeted agent with endocrine therapy was suggested.

Meanwhile, clinical benefit and response were observed when everolimus, an mTOR kinase inhibitor, was added to trastuzumab for the treatment of patients with HER2-overexpressing metastatic breast cancer, who progressed on trastuzumab-based therapy [54]. BOLERO-1 and BOLERO-3 (Breast Cancer Trials of Oral Everolimus) also suggested that patients with HER2-positive advanced breast cancer carrying PIK3CA mutations, PTEN loss, or hyperactive PI3K pathway could derive progression-free survival (PFS) benefit from everolimus [55]. In addition, BOLERO-3 study also showed that the addition of everolimus to trastuzumab plus vinorelbine significantly prolonged PFS in patients with trastuzumab-resistant and taxane-pretreated, HER2-positive, advanced breast cancer. However, the clinical benefit should be considered in the context of the adverse event profile in this population [56].

PIK3CA mutations have been determined as an inclusion criterion for the trials evaluating everolimus efficacy in patients with malignant glioma and different types of tumors (NCT03834740, NCT01827384) and trials examining temsirolimus in malignant solid tumors, multiple myeloma, B-cell non-Hodgkin lymphoma and malignant uterine neoplasm (NCT03297606, NCT02693535). On the other hand, there are several investigational PI3K inhibitors, including taselisib and buparlisib, which are currently in clinical development [57][58].

A retrospective study indicated that CRC patients with PIK3CA mutation and wild-type KRAS/BRAF showed fair responses to anti-EGFR therapies [59].

Hyperactivation of the PI3K signaling pathway is common and has been implicated in resistance to endocrine therapy [60][57] and HER2-targeting neoadjuvant therapies such as trastuzumab and lapatinib [61][62][63][64] [65][66] in patients with advanced breast cancer.

Results from a clinical study showed that PIK3CA mutations occur in approximately 5% (2/37) of EGFR-mutated lung cancers who developed acquired resistance to EGFR TKI therapy [67]. Preclinical data also showed that PI3K/AKT/mTOR signaling was implicated in gefitinib resistance in EGFR-mutated lung cancer cell lines [68]. Although preclinical data suggested that addition of everolimus restored gefitinib sensitivity in NSCLC cancer cell lines [69][70], the efficacy was not evidenced in the clinical settings [71][72].

Two meta-analyses involving five studies demonstrated a significant correlation of PIK3CA mutations with better recurrence-free survival (RFS) in patients with unsorted breast cancer [73][74][64].In patients with advanced EGFR- or KRAS-mutant lung adenocarcinoma, a concurrent PIK3CA mutation has been reported as a poor prognostic factor [75].

TGFBR2 Y281fs, Heterozygous deletion

Biological Impact

TGFBR2 (transforming growth factor-beta receptor 2) gene encodes a serine/threonine protein kinase that belongs to the transforming growth factor β (TGFβ) family. It heterodimerizes with other TGFβ receptor family members to initiate downstream signaling and plays an essential role in cellular proliferation, differentiation and tissue homeostasis [76][77][78]. Germline mutations in TGFBR2 have been reported to associate with connective-tissue disorders such as Loeys-Dietz aortic aneurysm syndrome (LDS) and Marfan syndrome (MFS) [79][80][81]. Loss-of-function mutations have been observed in pancreatic cancers and biliary adenocarcinomas [82][77]. Specifically, TGFBR2 mutations are frequently occurred in colon cancer with microsatellite instability (MSI) [83][84][85].

Y281fs mutation results in a change in the amino acid sequence beginning at 281, likely to cause premature truncation of the functional TGFBR2 protein (UniProtKB). This mutation is predicted to lead to a loss of TGFBR2 protein function, despite not being characterized in the literature. Loss of the second wild-type allele resulted in the biallelic inactivation of the gene.

Therapeutic and prognostic relevance

A study of invasive breast carcinoma patients (n = 564) demonstrated that acquisition of low TGFBR2 expression may confer resistance to tamoxifen therapy [86].

Several retrospective studies have shown that low expression of TGFBR2 associates with poor prognosis of patients with colon adenocarcinoma [87], breast cancer [88], and oral carcinoma [89].

TP53 C135*

Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis [90]. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation [91].

C135* mutation results in a premature truncation of the p53 protein at amino acid 135 (UniProtKB). This mutation is predicted to lead to a loss of p53 function, despite not having characterized in the literature.

Therapeutic and prognostic relevance

Despite having a high mutation rate in cancers, there are currently no approved targeted therapies for TP53 mutations. A phase II trial demonstrated that Wee1 inhibitor (AZD1775) in combination with carboplatin was well tolerated and showed promising anti-tumor activity in TP53-mutated ovarian cancer refractory or resistant (< 3 months) to standard first-line therapy (NCT01164995) [92].

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 [93]. 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 [94].

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 [95][96][97]. 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) [98]. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy [99][100]. 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 [101].

CDK6 Amplification

Biological Impact

CDK6 encodes the cyclin-dependent kinase 6, a serine/threonine kinase that controls the checkpoint at G1-S phase. Binding of CDK4/6 to cyclin D is negatively regulated by p16INK4a, a cyclin-dependent kinase inhibitor encoded by CDKN2A [102][103]. As CDK4 and CDK6 play overlapping and redundant physiological roles in the regulation of cell cycle, increased CDK6 activity could also promote tumorigenesis in a way similar to CDK4 [104]. Amplification of CDK6 has been observed in esophageal carcinoma [105][106][107], leukemia and lymphoma [108][109][110].

Therapeutic and prognostic relevance

CDK6 amplification has been determined as an inclusion criterion for the trial evaluating CDK4/6 inhibitors efficacy in several types of solid tumors (NCT02693535).

Results from two cohort studies (n=45 and n=46) showed that CDK6 overexpression was correlated with shorter median time to progression in ER+ breast cancer patients who had received fulvestrant (2.5 vs. 8.2 months and 3.4 vs. 8.9 months for CDK6 overexpression vs. normal expression) but was not correlated with other lines of treatment (N=68, tamoxifen or endocrine therapy). In vitro study further confirmed that cells exhibiting upregulation of CDK6 were resistant to fulvestrant [111].

BAP1 Heterozygous deletion

Biological Impact

Breast cancer type 1 susceptibility protein (BRCA1)-associated protein (BAP1) encodes an enzyme with ubiquitin carboxyl hydrolase activity involved in the regulation of cell cycle, transcription, and double-strand DNA repair [112][113][114]. BAP1 acts as a tumor suppressor by forming a complex with BRCA1 [115]. BAP1 is a haploinsufficient tumor suppressor with one copy loss may lead to weak protein expression and is related to renal cell carcinoma (RCC) [116]. Inactivating mutations of BAP1 were frequently observed in uveal melanoma with high metastatic risk, malignant mesothelioma and other carcinoma types, including a subtype of renal cell carcinoma and intrahepatic cholangiocarcinoma [113][117][118][119][120][121][122].

Therapeutic and prognostic relevance

The loss of BAP1 was shown to be associated with increased sensitivity to PARP inhibitor, olaparib, in renal cell carcinoma (RCC) [119] and mesothelioma cell lines [123]. However, no difference in sensitivity to the PARP inhibitor MK4827 was observed between BAP1-mutant and wild-type mesothelioma cell [113]. BAP1 deficiency was also linked to a high tumor grade and was correlated with metastasis development in uveal melanoma [117].

An open-label, non-randomized, Phase II study (NCT03207347) has been initiated, aimed at investigating the use of niraparib in mesothelioma, uveal melanoma, renal cell carcinoma, and cholangiocarcinoma patients with tumors known to have mutations in BAP1 and other selected DNA double-strand break repair pathway genes. BAP1 loss of function mutation has been selected as an inclusion criteria for the trial examining olaparib in urothelial cancer (NCT03375307) and malignant mesothelioma (NCT04515836).

BRCA1 Heterozygous deletion

Biological Impact

The breast cancer 1, early onset (BRCA1) gene encodes for a multifunctional ubiquitin E3 ligase, a tumor suppressor that has diverse cellular functions, including transcription, protein ubiquitination, cell cycle regulation and DNA damage response, with a particularly important role in homologous recombination, a DNA double-strand break repair pathway. BRCA1 germline mutations confer an increased lifetime risk of developing breast, ovarian and prostate cancer [124][125]. BRCA1 is also a Fanconi anemia susceptibility gene in FANCS, a rare Fanconi anemia subtype [126]. Prevalence of BRCA1 somatic mutation is in non-small cell lung cancer (NSCLC), pancreatic cancer, and colon cancer [127]. Deletion of BRCA1 gene has been correlated to significantly lower expression levels of the BRCA1 mRNA and reduced BRCA1 protein dosage, leading to a reduction in the efficiency of homologous recombination repair of DNA double-strand breaks [128][129][130]. Deleterious BRCA1 mutations have been detected in 8.5% of patients with triple-negative breast cancer (TNBC) (n=1824) unselected for family history and TNBC patients with mutations in BRCA1/2 and genes involved in homologous recombination (including PALB2, BARD1, RAD51D, RAD51C and BRIP1) were diagnosed at an earlier age and had higher-grade tumors than those

without mutations [131].

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 [132]; (2) in combination with bevacizumab as first-line maintenance treatment for patients with homologous recombination deficiency (HRD)-positive status [133]; (3) maintenance treatment for patients with germline BRCA-mutated recurrent ovarian cancer who are in complete or partial response to platinum-based chemotherapy [134][135]; (4) treatment for patients with germline BRCA-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy [136]. 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 [137] and germline BRCA-mutated metastatic pancreatic cancer [138]. 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 [139].

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 [140] and patients with BRCA-mutated epithelial ovarian, fallopian tube, or primary peritoneal cancer, who have been treated with two or more chemotherapies [141]. 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).

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 [142][143] and patients who have been treated with three or more prior lines of chemotherapy and associated with HRD positive status [144]. In addition, talazoparib for patients with deleterious or suspected deleterious germline BRCA-mutated, HER2 negative locally advanced or metastatic breast cancer [145].

BRCA2 Heterozygous deletion

Biological Impact

The BRCA2 gene encodes a tumor suppressor involved in the homologous recombination pathway for double-strand DNA repair [146]. 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 [147]. 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 [125]. Somatic mutations in BRCA2 are highest in colorectal, non-small cell lung cancer (NSCLC), and ovarian cancers [127].

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 [132]; (2) in combination with bevacizumab as first-line maintenance treatment for patients with homologous recombination deficiency (HRD)-positive status [133]; (3) maintenance treatment for patients with germline BRCA-mutated recurrent ovarian cancer who are in complete or partial response to platinum-based chemotherapy [134][135]; (4) treatment for patients with germline BRCA-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy [136]. 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 [137] and germline BRCA-mutated metastatic pancreatic cancer [138]. 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 [139].

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status [144]. In addition, talazoparib for patients with deleterious or suspected deleterious germline BRCA-mutated, HER2 negative locally advanced or metastatic breast cancer [145].

CDKN2A Homozygous deletion

Biological Impact

The Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A) gene encodes the p16 (p16INK4a) and p14 (ARF) proteins. p16INK4a binds to CDK4 and CDK6, inhibiting these CDKs from binding D-type cyclins and phosphorylating the retinoblastoma (RB) protein [148][149] whereas p14 (ARF) blocks the oncogenic activity of MDM2 by inhibiting MDM2-induced degradation of p53 [150]. 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 [151]. Loss of CDKN2A has been frequently found in human tumors that result in uncontrolled cell proliferation [152][153].

Therapeutic and prognostic relevance

Intact p16-Cdk4-Rb axis is known to be associated with sensitivity to cyclin-dependent kinase inhibitors [154][155]. Several case reports also revealed that patients with CDKN2A-deleted tumors respond to the CDK4/6-specific inhibitor treatments [156][157][158]. 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 [159][160][161]. 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 [155][162][163].

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 [157]. 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 [164].

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 [165].

KMT2C Heterozygous 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) [166] and regulates the gene expression during development and hematopoiesis [167][168][169]. KMT2C is ubiquitously expressed, and its function is essential for normal embryonal development and cell proliferation [170]. Genetic deletion of the region containing KMT2C is the most common chromosomal abnormality in acute myeloid leukemia [171][172], and KMT2C mutation has been reported in breast cancer, cutaneous squamous cell carcinoma, and leukemia [173][174][175][176][177]. 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 [178]. 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 [179].

A meta-analysis indicated that low levels of KMT2C expression was associated with better overall survival in pancreatic ductal adenocarcinoma (PDAC) patients [180]. However, another study of ER-positive breast cancer patients (n = 401) demonstrated that low KMT2C expression was associated with worse overall survival [181].

NF2 Heterozygous deletion

Biological Impact

The neurofibromin (NF2) gene encodes the protein Merlin, a tumor suppressor that functions as a negative regulator of the PI3K/AKT/mTOR pathway [182][183][184]. NF2 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 [185]. Inactivation germline mutations in the NF2 are associated with the hereditary neurofibromatosis type 2, a disorder characterized by the growth of noncancerous tumors in the nervous system [182][186]. Somatic mutations or deletion of NF2 are frequently observed in human cancers, including 20-50% of pleural mesotheliomas [113], 6% papillary renal cell carcinoma, 5% pancreas cancer, and 4% melanoma (cbioPortal; June 2015), and less frequently in other cancers [187].

Therapeutic and prognostic relevance

Genomic alterations with activating effects on the mTOR signaling pathway have been identified to confer sensitivity to everolimus across multiple cancer types [55][188][189][26]. There are at least two case studies indicating the clinical efficacy of everolimus in bladder cancer [190] and urothelial carcinoma [191], both harboring NF2 truncating mutations. Preclinical evidence has shown the efficacy of MEK1/2 inhibitor selumetinib in KRAS-mutant thyroid cancer model with NF2 loss [192].

Analysis of afatinib-plus-cetuximab-resistant biopsy specimens revealed a loss-of-function alteration in genes that modulate mTOR signaling pathway, including NF2 and TSC1 [193].

RAD51C Heterozygous deletion

Biological Impact

The RAD51C (RAD51 paralog C) encodes a member of the RAD51 protein family involved in the late phase of homologous recombination DNA repair. Germline mutations in RAD51C have been shown to confer increased susceptibility to ovarian cancer [194][195][196][197] and head and neck squamous cell carcinoma (HNSCC) [198]. Amplification of RAD51C has been implicated in tumor progression [199][200]. RAD51C is a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological function [201].

Therapeutic and prognostic relevance

In May 2020, the U.S. FDA approved olaparib for the treatment of adult patients with metastatic castration-resistant prostate cancer (mCRPC) who carry mutations in homologous recombination repair (HRR) genes, including BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L, and progressed following prior treatment with enzalutamide or abiraterone acetate [139].

A preclinical study using gastric cancer xenograft model showed that RAD51C deficiency caused sensitivity to PARP inhibitor olaparib [202].

RAD51C loss of function mutation has been determined as an inclusion criterion for the trials evaluating rucaparib efficacy in ovarian cancer [140] or prostate cancer [203]; talazoparib efficacy in HER2-negative breast cancer (NCT02401347) or prostate cancer (NCT03148795), and niraparib efficacies in pancreatic cancer (NCT03553004).

RB1 Heterozygous deletion

Biological Impact

The Retinoblastoma (RB1) gene encodes a tumor suppressor that negatively regulates the cell cycle, cell division, and DNA replication [204]. Loss-of-function RB1 could lead to unregulated cell division and growth, abrogation of multiple mechanisms that safeguard against cellular transformation, and tumorigenesis [205]. RB1 has also been implicated as a haploinsufficient tumor suppressor with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions [206][207][208]. Deletion or inactivating mutation of RB1 is found in a number of tumors, including lung, prostate, bladder, breast cancers and sarcomas. RB1 mutations are found in approximately half of all retinoblastoma cases [209].

Therapeutic and prognostic relevance

A deleterious mutation in one or more of the three DNA repair genes ATM, RB1, and FANCC predicted pathologic response and better overall survival to cisplatin-based chemotherapy for muscle-invasive bladder cancer patients [210]. High RB loss was found to be associated with improved pathologic clinical response in breast cancer patients treated with 5-fluorouracil/adriamycin/cytoxan (FAC), T/FAC, and Taxane/Adriamycin neoadjuvant therapy [211].

Clinical and experimental data suggested that a non-functional retinoblastoma pathway is associated with resistance to tamoxifen in breast cancer [212][213].

Acquired RB1 mutations were found in hormone receptor positive breast cancer patients who developed resistance to palbociclib or ribociclib treatment [214]. Preclinical data also showed that knockdown of RB1 would impair antitumor activity of CDK4/6 inhibitor, abemaciclib [215].

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-β 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-β-targeted genes [216]. 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 [217]. SMAD4 germline mutations are associated with juvenile polyposis syndrome (JPS) [218][219][220][78]. Somatic mutations of SMAD4 are commonly observed in pancreatic cancer [221], colorectal cancer (CRC) [220][222][223] and less frequently seen in other cancers such as lung adenocarcinoma [224], head and neck cancer [225][226] and cutaneous squamous cell carcinoma [227].

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 [29]. 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 [228].

SMAD4 is also suggested as a predictive marker for 5 fluorouracil-based chemotherapy in colorectal cancer (CRC) [229][230]. 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 [231].

TSC1 Heterozygous deletion

Biological Impact

The tuberous sclerosis complex 1 (TSC1) gene encodes a tumor suppressor, hamartin, a key negative regulator of the mammalian target of rapamycin (mTOR) pathway [232][233]. Mutations in TSC1/TSC2 tumor suppressor genes that result in inactivation of the complex are commonly found in patients with tuberous sclerosis [234][235][236], while LOH in TSC1/TSC2 has been identified in head and neck squamous cell carcinoma (HNSCC) [237] and endometrial cancer [238]. Loss of single TSC1 allele (haploinsufficiency) may provide a growth advantage to bladder epithelial cells, contributing to bladder cancer development [239]. Both TSC1 and TSC2 mutations cause the autosomal dominant genetic disorder tuberous sclerosis complex (TSC), in which individuals develop a variety of benign but often progressive neoplasms [240].

Therapeutic and prognostic relevance

Genomic alterations with activating effects of the mTOR signaling pathway (including deletion/inactivation of TSC1/TSC2) have been shown to confer sensitivity to everolimus across multiple neoplasms, such as bladder tumors [190], gastric, sarcoma, thyroid cancer, and HNSCC [26]. There were case reports demonstrated the efficacy of sirolimus in malignant uterine perivascular epithelioid cell tumors (PEComa) patients harboring mutations/deletions in TSC1 and TSC2 genes, and temsirolimus in PEComa patients with hyperactivated mTOR pathway. Genomic profiling analysis of GOG248, a Phase II study of temsirolimus or temsirolimus and alternating megestrol acetate and tamoxifen for advanced endometrial cancer showed that mutations in AKT1, TSC1 and TSC2 may predict clinical benefit from temsirolimus [241]. Recent studies indicate that there are mTORC1-independent signaling pathways downstream of hamartin-tuberin, which may represent new therapeutic targets [242].

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)

MONARCH 3[253] NCT00246621	**Breast cancer** (Approved on 2018/02/26) **HR-positive, HER2-negative** Abemaciclib + Anastrozole/Letrozole vs. Placebo + Anastrozole/Letrozole [PFS(m): 28.2 vs. 14.8]
MONARCH 1[252] NCT02102490	**Breast cancer** (Approved on 2017/09/28) **HR-positive, HER2-negative** Abemaciclib [ORR(%): 19.7 vs. 17.4]
MONARCH 2[163] NCT02107703	**Breast cancer** (Approved on 2017/09/28) **HR-positive, HER2-negative** Abemaciclib + Fulvestrant vs. Placebo + Fulvestrant [PFS(m): 16.4 vs. 9.3]

Alpelisib (PIQRAY)

Alpelisib is an inhibitor of phosphatidylinositol-3-kinase (PI3K) with inhibitory activity predominantly against PI3Kα. Gain-of-function mutations in the gene encoding the catalytic α-subunit of PI3K (PIK3CA) lead to activation of PI3Kα and Akt-signaling, cellular transformation and the generation of tumors in in vitro and in vivo models. Alpelisib is developed and marketed by Novartis under the trade name PIQRAY.

FDA Approval Summary of Alpelisib (PIQRAY)

SOLAR-1[45] NCT02437318	**Breast cancer** (Approved on 2019/05/24) **PIK3CA** Alpelisib plus fulvestrant vs. Placebo plus fulvestrant [PFS(m): 11 vs. 5.7]

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[247] NCT01524783	**Lung or gastrointestinal neuroendocrine tumor** (Approved on 2016/02/26)
	-
	Everolimus vs. Placebo [PFS(m): 11 vs. 3.9]
BOLERO-2[245] NCT00863655	**Breast cancer** (Approved on 2012/07/20)
	ER+/HER2-
	Everolimus + exemestane vs. Placebo + exemestane [PFS(m): 7.8 vs. 3.2]
RADIANT-3[246] NCT00510068	**Pancreatic neuroendocrine tumor** (Approved on 2011/05/05)
	-
	Everolimus vs. Placebo [PFS(m): 11 vs. 4.6]
EXIST-1[243] NCT00789828	**Subependymal giant cell astrocytoma** (Approved on 2010/10/29)
	-
	Everolimus vs. Placebo [ORR(%): 35.0 vs. 0]
RECORD-1[244] NCT00410124	**Renal cell carcinoma** (Approved on 2009/05/30)
	-
	Everolimus vs. Placebo [PFS(m): 4.9 vs. 1.9]

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)

QUADRA[144] NCT02354586	**Ovarian cancer** (Approved on 2019/10/23)
	HRD-positive (defined by either a deleterious or suspected deleterious BRCA mutation, and/or genomic instability)
	Niraparib [ORR(%): 24.0, DOR(m): 8.3]

NOVA[143] NCT01847274	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/03/27)
	Germline BRCA (wild-type)
	Niraparib vs. Placebo [PFS(m): 9.3 vs. 3.9]
NOVA[143] NCT01847274	**Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/03/27)
	Germline BRCA (mutation)
	Niraparib vs. Placebo [PFS(m): 21.0 vs. 5.5]
NOVA[143] NCT01847274	**Ovarian cancer** (Approved on 2017/03/27)
	Germline BRCA (mutation)
	Niraparib vs. Placebo [PFS(m): 21 vs. 5.5]

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)

PROfound[139] NCT02987543	**Prostate cancer** (Approved on 2020/05/19)
	ATMm, BRCA1m, BRCA2m, BARD1m, BRIP1m, CDK12m, CHEK1m, CHEK2m, FANCLm, PALB2m, RAD51Bm, RAD51Cm, RAD51Dm, RAD54Lm
	Olaparib vs. Enzalutamide or Abiraterone acetate [PFS(m): 5.8 vs. 3.5]
PAOLA-1[133] NCT02477644	**Ovarian cancer** (Approved on 2020/05/08)
	HRD-positive (defined by either a deleterious or suspected deleterious BRCA mutation, and/or genomic instability)
	Olaparib + Bevacizumab vs. Placebo + Bevacizumab [PFS(m): 37.2 vs. 17.7]
POLO[138] NCT02184195	**Pancreatic adenocarcinoma** (Approved on 2019/12/27)
	Germline BRCA mutation (deleterious/suspected deleterious)
	Olaparib vs. Placebo [ORR(%): 23.0 vs. 12.0, PFS(m): 7.4 vs. 3.8]

SOLO-1[132] NCT01844986	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2018/12/19)
	Germline or somatic BRCA-mutated (gBRCAm or sBRCAm)
	Olaparib vs. Placebo [PFS(m): NR vs. 13.8]
OlympiAD[137] NCT02000622	**Breast cancer** (Approved on 2018/02/06)
	Germline BRCA mutation (deleterious/suspected deleterious) HER2-negative
	Olaparib vs. Chemotherapy [PFS(m): 7 vs. 4.2]
Study19[257] NCT00753545	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/08/17)
	-
	Olaparib vs. Placebo [PFS(m): 8.4 vs. 4.8]
SOLO-2/ENGOT-Ov21[256] NCT01874353	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/08/17)
	Germline BRCA mutation (deleterious/suspected deleterious)
	Olaparib vs. Placebo [PFS(m): 19.1 vs. 5.5]
Study 42[255] 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[258] NCT01740427	**Breast cancer** (Approved on 2017/03/31)
	ER+, HER2-
	Palbociclib + letrozole vs. Placebo + letrozole [PFS(m): 24.8 vs. 14.5]

PALOMA-3[259] NCT01942135	**Breast cancer** (Approved on 2016/02/19)	
	ER+, HER2-	
	Palbociclib + fulvestrant vs. Placebo + fulvestrant [PFS(m): 9.5 vs. 4.6]	

Ribociclib (KISQALI)

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

FDA Approval Summary of Ribociclib (KISQALI)

MONALEESA-2[162] NCT01958021	**Breast cancer** (Approved on 2017/03/13)
	HR+, HER2-
	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	**Prostate cancer** (Approved on 2020/05/15)
	gBRCA+, sBRCA
	Rucaparib [ORR(%): 44.0, DOR(m): NE]
ARIEL3[140] NCT01968213	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2018/04/06)
	All patients|HRD group|tBRCA group
	Rucaparib vs. Placebo [PFS(m): 10.8|13.6|16.6 vs. 5.4|5.4|5.4]
ARIEL2[254] NCT01482715, NCT01891344	**Ovarian cancer** (Approved on 2016/12/19)
	Germline and/or somatic BRCA mutation
	Rucaparib [ORR(%): 54.0]

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)

[260] NCT00065468	**Renal cell carcinoma** (Approved on 2007/05/30)
	-
	Temsirolimus vs. IFN-α [OS(m): 10.9 vs. 7.3]

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[251] NCT02034110	**Anaplastic thyroid cancer** (Approved on 2018/05/04)
	BRAF V600E
	Dabrafenib + trametinib [ORR(%): 61.0]
BRF113928[250] NCT01336634	**Non-small cell lung cancer** (Approved on 2017/06/22)
	BRAF V600E
	Trametinib + dabrafenib vs. Dabrafenib [ORR(%): 63.0 vs. 27.0, DOR(m): 12.6 vs. 9.9]
COMBI-d[249] NCT01584648	**Melanoma** (Approved on 2014/01/10)
	BRAF V600E/K
	Trametinib + dabrafenib vs. Dabrafenib + placebo [PFS(m): 9.3 vs. 8.8]
METRIC[248] NCT01245062	**Melanoma** (Approved on 2013/05/29)
	BRAF V600E/K
	Trametinib vs. Dacarbazine or paclitaxel [PFS(m): 4.8 vs. 1.5]

d=day; w=week; m=month

ONGOING CLINICAL TRIALS

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

No trial has been found.

高添富
Project ID: C21-M001-00397
Report No.: AA-21-03176_ONC
Date Reported: Aug 19, 2021

DETAILED TEST RESULTS

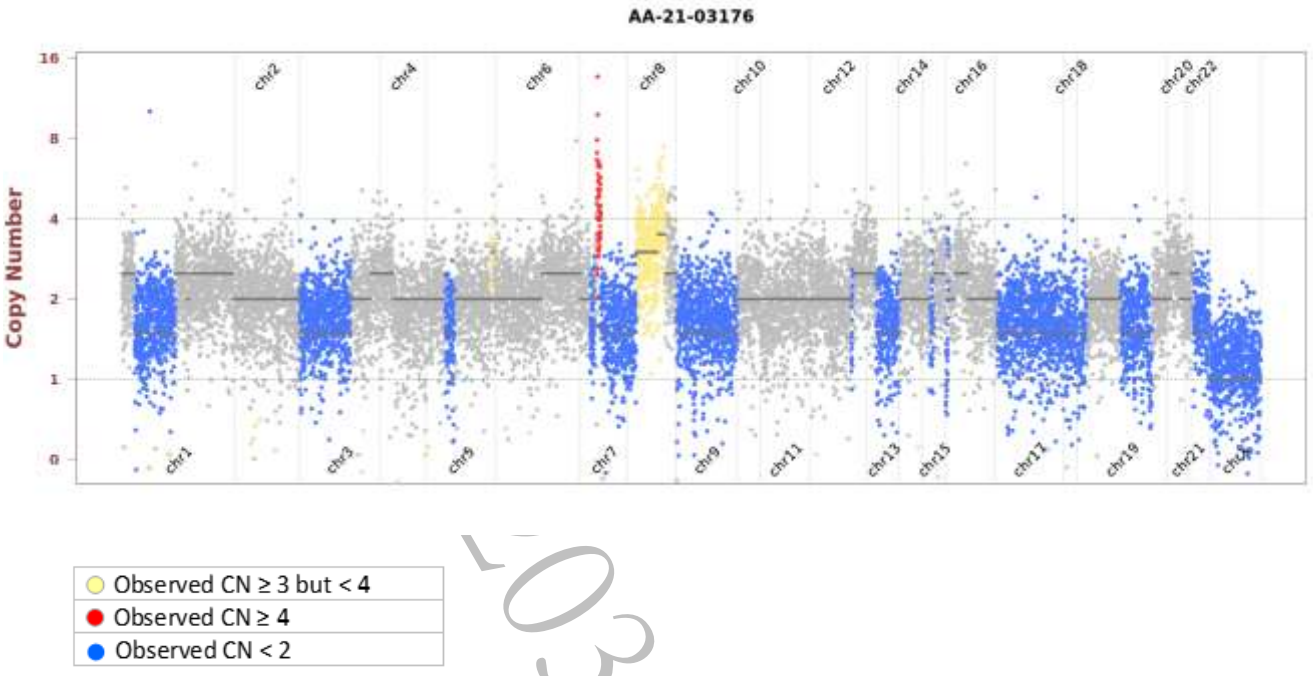
SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Gene	Chr	Exon	Accession Number	cDNA Change	Amino Acid Change	Coverage	Allele Frequency	COSMIC ID
ABL1	9	11	NM_005157	c.2951C>T	T984M	382	71.2%	COSM1460559
CYLD	16	19	NM_015247	c.2643C>G	D881E	1151	13.6%	-
CYLD	16	19	NM_015247	c.2610C>A	S870R	1134	13.1%	-
ERBB2	17	3	NM_004448	c.428G>A	R143Q	565	70.8%	COSM1382867
FGFR3	4	11	NM_000142	c.1513G>A	V505I	999	54.3%	COSM1428715
FGFR4	5	9	NM_213647	c.1205C>T	P402L	538	54.8%	-
FLT1	13	4	NM_002019	c.430G>A	E144K	382	41.1%	COSM1366253
GATA1	X	-	NM_002049	c.870+6G>A	Splice region	246	100.0%	-
KMT2D	12	34	NM_003482	c.8714G>A	R2905H	437	54.7%	-
MUC16	19	3	NM_024690	c.18379T>A	S6127T	902	48.2%	-
MUC6	11	31	NM_005961	c.6387_6389del	S2130del	831	20.1%	COSM150850
NF1	**17**	**-**	**NM_001042492**	**c.7869+1G>T**	**Splice donor**	**369**	**38.8%**	**-**
PIK3CA	**3**	**10**	**NM_006218**	**c.1633G>A**	**E545K**	**321**	**30.2%**	**COSM763**
ROS1	6	19	NM_002944	c.2872A>G	I958V	63	60.3%	-
TGFBR2	**3**	**4**	**NM_003242**	**c.840del**	**Y281fs**	**432**	**44.0%**	**-**
TNFAIP3	6	7	NM_006290	c.1812_1821del	S605fs	1339	13.1%	-
TP53	**17**	**5**	**NM_000546**	**c.405C>A**	**C135***	**608**	**42.1%**	**COSM44319**
TSC1	9	15	NM_000368	c.1460C>G	S487C	828	77.4%	COSM9177413

Mutations with clinical relevance are highlighted in red.

COPY NUMBER VARIANTS (CNVS)

Observed copy number (CN) for each evaluated position is shown on the y-axis. Regions referred to as amplification or deletion are shown in color. Regions without significant changes are represented in gray.



高添富
Project ID: C21-M001-00397
Report No.: AA-21-03176_ONC
Date Reported: Aug 19, 2021

HOTSPOT GENOTYPES

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

Gene	Variant	Genotype Detected
BRAF	V600X	Not detected
EGFR	A763_Y764insFQEA, E709K, E709_T710delinsD, Exon 19 deletion, Exon 19 insertion, Exon 20 insertion, G719A/C/D/S, L747P, L833V, L858R, L861Q/R, S768I, T790M	Not detected
IDH2	R140Q, R172G/K/M/S	Not detected
KIT	A502_Y503dup, D419del, D579del, D816F/V/Y, D820A/E/G/Y, E554_I571del, E554_K558del, E554_V559del, Exon 11 mutation, F522C, H697Y, I563_L576del, I653T, K550_W557del, K558N, K558_E562del, K558_V559del, K558delinsNP, K642E, M552_W557del, N505I, N564_Y578del, N822H/I/K/Y, P551_M552del, P573_D579del, P577_D579del, P577_W582delinsPYD, P838L, Q556_K558del, T417_D419delinsI, T417_D419delinsRG, T574_Q575insTQLPYD, V530I, V555_L576del, V555_V559del, V559A/C/D/G, V559_V560del, V559del, V560D/G, V560del, V569_L576del, V654A, W557G/R, W557_K558del, Y553N, Y553_K558del, Y570H, Y578C	Not detected
KRAS	A146T/V/P, G12X, G13X, Q61X	Not detected
MET	D1028H/N/Y	Not detected
NRAS	G12X, G13X, Q61X	Not detected
PDGFRA	A633T, C450_K451insMIEWMI, C456_N468del, C456_R481del, D568N, D842I/V, D842_H845del, D842_M844del, D846Y, E311_K312del, G853D, H650Q, H845Y, H845_N848delinsP, I843del, N659K/R/S, N848K, P577S, Q579R, R560_V561insER, R748G, R841K, S566_E571delinsR, S584L, V469A, V536E, V544_L545insAVLVLLVIVIISLI, V561A/D, V561_I562insER, V658A, W559_R560del, Y375_K455del, Y555C, Y849C/S	Not detected
PIK3CA	C420R, E542K/V, E545A/D/G/K, H1047X, Q546E/R	**E545K**

V600X= any mutation in the valine (V) at amino acid 600 being replaced by a different amino acid.
G12X = any mutation in the glycine (G) at amino acid 12 being replaced by a different amino acid.
G13X= any mutation in the glycine (G) at amino acid 13 being replaced by a different amino acid.
Q61X = any mutation in the glutamine (Q) at amino acid 61 being replaced by a different amino acid.
H1047X = any mutation in the histidine (H) at amino acid 1047 being replaced by a different amino acid.

Gene	Copy Number Detected
CDK4	2
EGFR	2
ERBB2	2
MET	1

Copy number ≥ 8 is considered amplification

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

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

Gene	Copy Number Detected
FGFR1	2
MDM2	2
MDM4	2

Copy number ≥ 8 is considered amplification

TEST DETAILS

ABOUT ACTOnco®+

The test is a next-generation sequencing (NGS)-based assay developed for efficient and comprehensive genomic profiling of cancers. This test interrogates coding regions of 440 genes associated with cancer treatment, prognosis and diagnosis. Genetic mutations detected by this test include small-scale mutations like single nucleotide variants (SNVs), small insertions and deletions (INDELs) (≤ 15 nucleotides) and large-scale genomic alterations like copy number variations (CNVs).

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

DATABASE USED

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

NEXT-GENERATION SEQUENCING (NGS) METHODS

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

Raw reads generated by the sequencer were mapped to the hg19 reference genome using the Ion Torrent Suite (version 5.10). Coverage depth was calculated using Torrent Coverage Analysis plug-in. Single nucleotide variants (SNVs) and short insertions/deletions (INDELs) were identified using the Torrent Variant Caller plug-in (version 5.10). The coverage was down-sampled to 4000. VEP (Variant Effect Predictor) (version 100) was used to annotate every variant using databases from Clinvar (version 20210208), COSMIC v.92 and Genome Aggregation database r2.1.1. Variants with coverage ≥ 25, allele frequency ≥ 5% and actionable variants with allele frequency ≥ 2% were retained.

This test provides uniform coverage of the targeted regions, enabling target base coverage at 100x ≥ 85% with a mean coverage ≥ 500x.

Variants reported in Genome Aggregation database r2.1.1 with > 1% minor allele frequency (MAF) were

considered as polymorphisms. ACT Genomics in-house database was used to determine technical errors. Clinically actionable and biologically significant variants were determined based on the published medical literature.

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

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

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

STANDARD OPERATING PROCEDURES (SOPS)

Standard operating procedures (SOPs) are shown below:

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

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

LIMITATIONS

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

NOTES

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

PATHOLOGY EVALUATION

- H&E-stained section No.: S10944572
- Collection site: Esophagus
- Examined by: Dr. Yeh-Han Wang
- Estimated neoplastic nuclei (whole sample): The percentage of viable tumor cells in total cells in the whole slide (%): 10%
  The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 30%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
  Additional comment: NA
- Manual macrodissection: Performed on the highlighted region

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

SPECIMEN PHOTO(S)

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

RUN QC

- Panel: ACTOnco®+
- Mean Depth: 688x
- Target Base Coverage at 100x: 91%

高添富
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ACTOnco®+ GENE LIST

ABCB1*	AURKB	CBL	CDKN2B	E2F3	FAT1	GRIN2A	JAK2	MED12	NOTCH4	PMS1	RAD51D	SLCO1B3*	TNFRSF14
ABCC2*	AXIN1	CCNA1	CDKN2C	EGFR	FBXW7	GSK3B	JAK3	MEF2B	NPM1	PMS2	RAD52	SMAD2	TNFSF11
ABCG2*	AXIN2	CCNA2	CEBPA*	EP300	FCGR2B	GSTP1*	JUN*	MEN1	NQO1*	POLB	RAD54L	SMAD3	TOP1
ABL1	AXL	CCNB1	CHEK1	EPCAM	FGF1*	GSTT1*	KAT6A	MET	NRAS	POLD1	RAF1	SMAD4	TP53
ABL2	B2M	CCNB2	CHEK2	EPHA2	FGF10	HGF	KDM5A	MITF	NSD1	POLE	RARA	SMARCA4	TPMT*
ADAMTS1	BAP1	CCNB3	CIC	EPHA3	FGF14	HIF1A	KDM5C	MLH1	NTRK1	PPARG	RB1	SMARCB1	TSC1
ADAMTS13	BARD1	CCND1	CREBBP	EPHA5	FGF19*	HIST1H1C*	KDM6A	MPL	NTRK2	PPP2R1A	RBM10	SMO	TSC2
ADAMTS15	BCL10	CCND2	CRKL	EPHA7	FGF23	HIST1H1E*	KDR	MRE11	NTRK3	PRDM1	RECQL4	SOCS1*	TSHR
ADAMTS16	BCL2*	CCND3	CRLF2	EPHB1	FGF3	HNF1A	KEAP1	MSH2	PAK3	PRKAR1A	REL	SOX2*	TYMS
ADAMTS18	BCL2L1	CCNE1	CSF1R	ERBB2	FGF4*	HR	KIT	MSH6	PALB2	PRKCA	RET	SOX9	U2AF1
ADAMTS6	BCL2L2*	CCNE2	CTCF	ERBB3	FGF6	HRAS*	KMT2A	MTHFR*	PARP1	PRKCB	RHOA	SPEN	UBE2A*
ADAMTS9	BCL6	CCNH	CTLA4	ERBB4	FGFR1	HSP90AA1	KMT2C	MTOR	PAX5	PRKCG	RICTOR	SPOP	UBE2K
ADAMTSL1	BCL9	CD19	CTNNA1	ERCC1	FGFR2	HSP90AB1	KMT2D	MUC16	PAX8	PRKCI	RNF43	SRC	UBR5
ADGRA2	BCOR	CD274	CTNNB1	ERCC2	FGFR3	HSPA4	KRAS	MUC4	PBRM1	PRKCQ	ROS1	STAG2	UGT1A1*
ADH1C*	BIRC2	CD58	CUL3	ERCC3	FGFR4	HSPA5	LCK	MUC6	PDCD1	PRKDC	RPPH1	STAT3	USH2A
AKT1	BIRC3	CD70*	CYLD	ERCC4	FH	IDH1	LIG1	MUTYH	PDCD1LG2	PRKN	RPTOR	STK11	VDR*
AKT2	BLM	CD79A	CYP1A1*	ERCC5	FLCN	IDH2	LIG3	MYC	PDGFRA	PSMB8	RUNX1	SUFU	VEGFA
AKT3	BMPR1A	CD79B	CYP2B6*	ERG	FLT1	IFNL3*	LMO1	MYCL	PDGFRB	PSMB9	RUNX1T1	SYK	VEGFB
ALDH1A1*	BRAF	CDC73	CYP2C19*	ESR1	FLT3	IGF1	LRP1B	MYCN	PDIA3	PSME1	RXRA	SYNE1	VHL
ALK	BRCA1	CDH1	CYP2C8*	ESR2	FLT4	IGF1R	LYN	MYD88	PGF	PSME2	SDHA	TAF1	WT1
AMER1	BRCA2	CDK1	CYP2D6	ETV1	FOXL2*	IGF2	MALT1	NAT2*	PHOX2B*	PSME3	SDHB	TAP1	XIAP
APC	BRD4	CDK12	CYP2E1*	ETV4	FOXP1	IKBKB	MAP2K1	NBN	PIK3C2B	PTCH1	SDHC	TAP2	XPO1
AR	BRIP1	CDK2	CYP3A4*	EZH2	FRG1	IKBKE	MAP2K2	NEFH	PIK3C2G	PTEN	SDHD	TAPBP	XRCC2
ARAF	BTG1*	CDK4	CYP3A5*	FAM46C	FUBP1	IKZF1	MAP2K4	NF1	PIK3C3	PTGS2	SERPINB3	TBX3	ZNF217
ARID1A	BTG2*	CDK5	DAXX	FANCA	GATA1	IL6	MAP3K1	NF2	PIK3CA	PTPN11	SERPINB4	TEK	
ARID1B	BTK	CDK6	DCUN1D1	FANCC	GATA2	IL7R	MAP3K7	NFE2L2	PIK3CB	PTPRD	SETD2	TERT	
ARID2	BUB1B	CDK7	DDR2	FANCD2	GATA3	INPP4B	MAPK1	NFKB1	PIK3CD	PTPRT	SF3B1	TET1	
ASXL1	CALR	CDK8	DICER1	FANCE	GNA11	INSR	MAPK3	NFKBIA	PIK3CG	RAC1	SGK1	TET2	
ATM	CANX	CDK9	DNMT3A	FANCF	GNA13	IRF4	MAX	NKX2-1*	PIK3R1	RAD50	SH2D1A*	TGFBR2	
ATR	CARD11	CDKN1A	DOT1L	FANCG	GNAQ	IRS1	MCL1	NOTCH1	PIK3R2	RAD51	SLC19A1*	TMSB4X*	
ATRX	CASP8	CDKN1B	DPYD	FANCL	GNAS	IRS2*	MDM2	NOTCH2	PIK3R3	RAD51B	SLC22A2*	TNF	
AURKA	CBFB	CDKN2A	DTX1	FAS	GREM1	JAK1	MDM4	NOTCH3	PIM1	RAD51C	SLCO1B1*	TNFAIP3	

*Analysis of copy number alteration not available.

DISCLAIMER

Legal Statement

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

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

Treatment Decisions are the Responsibility of the Physician

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

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

Genetic Alterations and Drugs Not Presented in Ranked Order

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

Level of Evidence Provided

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

No Guarantee of Clinical Benefit

This report makes no promises or guarantees about the effectiveness of a particular drug or any treatment procedure in any disease or in any patient. This report also makes no promises or guarantees that a drug without an association of reportable genomic alteration will, in fact, provide no clinical benefit.

Liability

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免責聲明

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。

本檢驗報告非經本公司許可，不得私自變造、塗改，或以任何方式作為廣告及其他宣傳之用途。
本公司於提供檢驗報告後，即已完成本次契約義務，後續之報告解釋、判讀及用藥、治療，應自行尋求相關專業醫師協助，若需將報告移件其他醫師，本人應取得該醫師同意並填寫移件申請書，主動告知行動基因，行動基因僅能配合該醫師意願與時間提供醫師解說。

醫療決策需由醫師決定

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

基因突變與用藥資訊並非依照有效性排序

本報告中列出之生物標記變異與藥物資訊並非依照潛在治療有效性排序。

證據等級

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

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Project ID: C21-M001-00397
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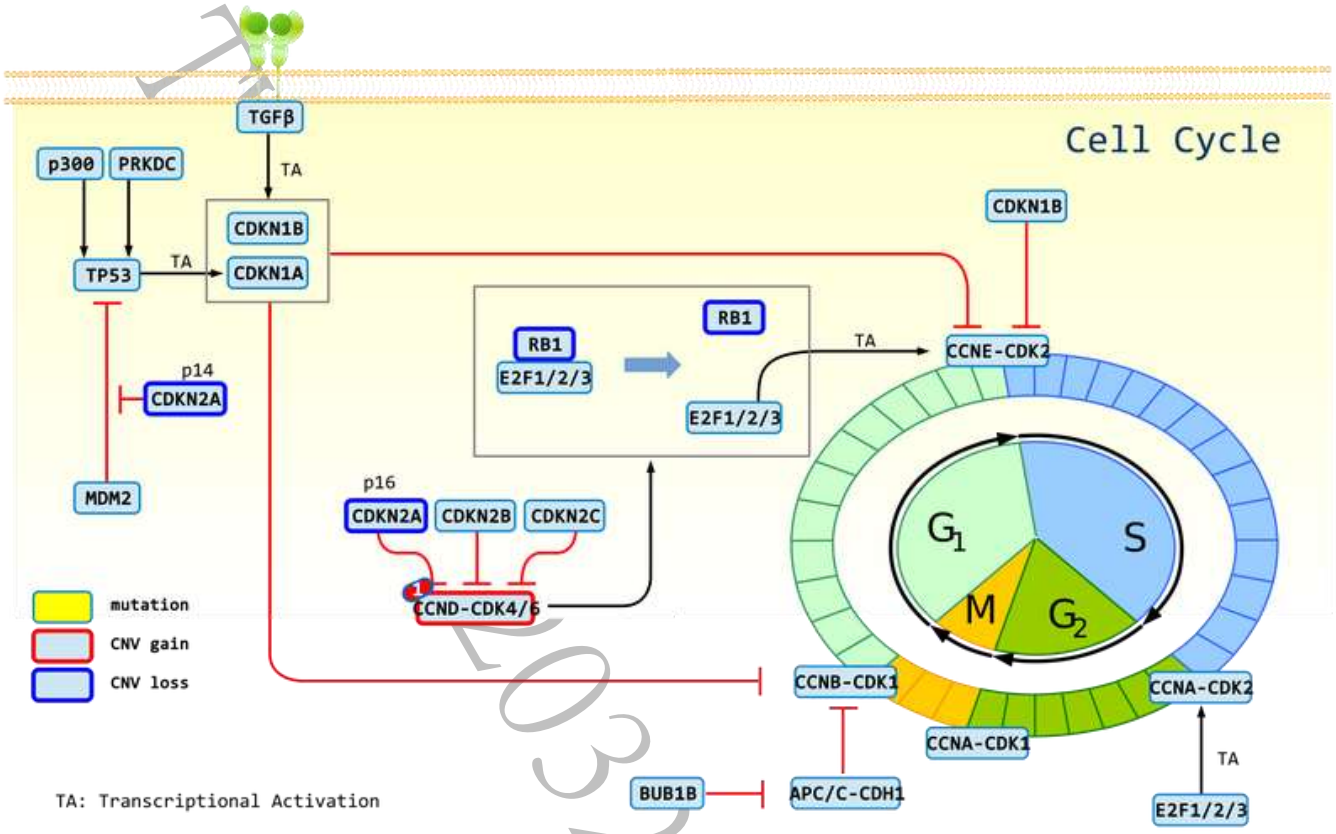
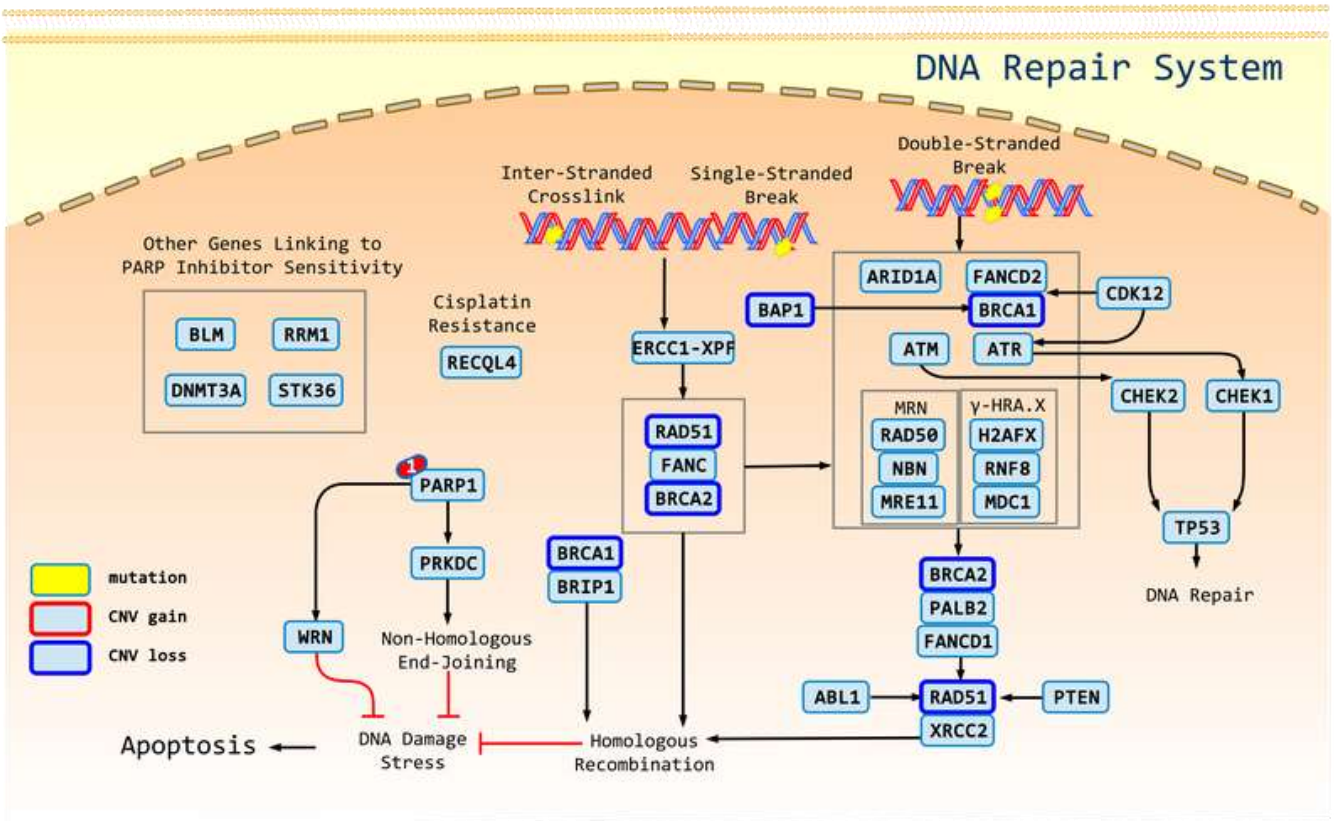
Date Reported: Aug 19, 2021

SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



1: Abemaciclib, Palbociclib, Ribociclib



1: Olaparib, Rucaparib, Niraparib

高添富

Project ID: C21-M001-00397
Report No.: AA-21-03176_ONC
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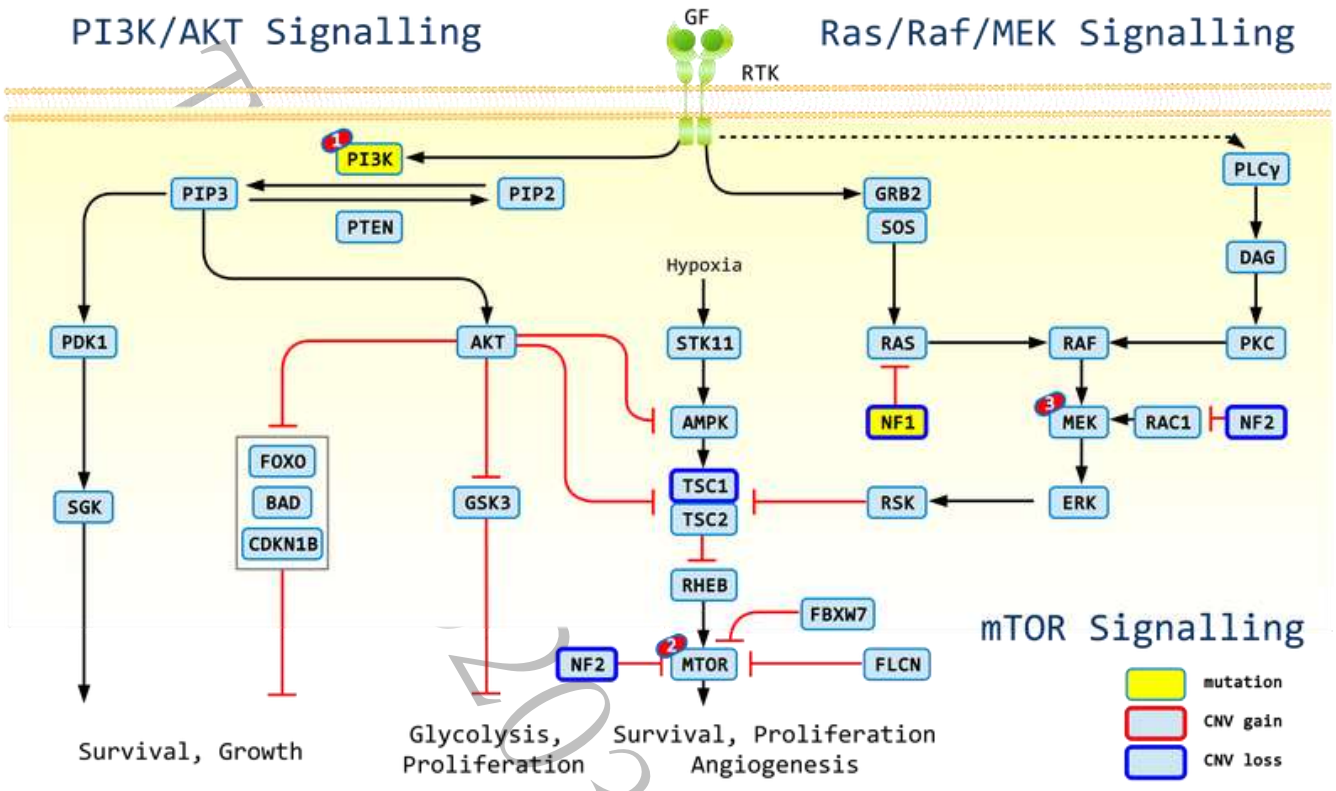

1: Alpelisib; 2: Everolimus, Temsirolimus; 3: Trametinib

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

ACTFusion™ Report

高添富
Project ID: C21-M001-00397
Report No.: AA-21-03176_FUS
Date Reported: Aug 19, 2021

PATIENT	SPECIMEN	ORDERING PHYSICIAN
Name: 高添富	Type: FFPE tissue	Name: 徐博奎醫師
Gender: Male	Date received: Aug 06, 2021	Facility: 臺北榮總
Date of Birth: Jul 27, 1949	Collection site: Esophagus	Tel: 886-228712121
Patient ID: 37977687	Specimen ID: S10944572	Address: 臺北市北投區石牌路二段 201 號
Diagnosis: Esophageal cancer	Lab ID: AA-21-03176	Cooperative ID: NA
	D/ID: NA	

ABOUT ACTFusion™

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

VARIANT(S) WITH CLINICAL RELEVANCE

FUSION RESULTS

No fusion gene detected in this sample.

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

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

高添富
Project ID: C21-M001-00397
Report No.: AA-21-03176_FUS
Date Reported: Aug 19, 2021

THERAPEUTIC IMPLICATIONS

TARGETED THERAPIES

Not Applicable.

VARIANT INTERPRETATION

Not Applicable.

US FDA-APPROVED DRUG(S)

Not Applicable.

高添富
Project ID: C21-M001-00397
Report No.: AA-21-03176_FUS
Date Reported: Aug 19, 2021

ONGOING CLINICAL TRIAL(S)

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

No trial has been found.

ACTFusion™ GENE LIST

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

TEST DETAILS

SPECIMEN RECEIVED

- H&E-stained section No.: S10944572
- Collection date: Dec 2020
- Collection site: Esophagus
- Facility retrieved: 臺北榮總
- Examined by: Dr. Yeh-Han Wang
- Estimated neoplastic nuclei (whole sample): The percentage of viable tumor cells in total cells in the whole slide (%): 10%
  The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 30%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
  Additional comment: NA
- Manual macrodissection: Performed on the highlighted region

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

NEXT-GENERATION SEQUENCING (NGS) METHODS

The extracted RNA was reverse-transcribed and subjected to library construction. The quality and quantity of the amplified library was determined using the fragment analyzer (AATI) and Qubit (Invitrogen). Sequencing was performed on the Ion 540™ Chip/ Ion 550™ Chip / Ion P1™ Chip and Ion GeneStudio™ S5 Prime System / Ion Proton™ System (Life Technologies). All assays were performed in accordance with ACT Genomics testing SOPs.

Data processing and statistical analysis for the identification of relevant fusions was performed using in-house fusion calling pipeline with default parameter setting. The four internal controls for the purpose of monitoring the overall sequencing quality of the sample were built into the assay, including CHMP2A, RABA7A, GPI, and VCP. Amplification of these genes using gene specific primers was performed, and the sequencing results were applied to the analysis pipeline to assess RNA quality. The inability of the software to detect these genes was considered a run failure. To ensure optimal sequencing quality for variant analysis, all samples had to meet the following sample quality control (QC) criteria: 1) Average unique RNA Start Sites (SS) per control Gene Specific Primer 2 (GSP 2) ≥ 10 (default), and 2) Total reads after sequencing ≥ 500,000 (recommended).

Samples passed the sample QC would be subjected to the fusion analysis pipeline for fusion transcript calling. Briefly, the analysis pipeline aligned sequenced reads to a reference genome, identified regions that map to noncontiguous regions of the genome, and applied filters to exclude probable false-positive events and annotate previously characterized fusion events. A minimum of 5 reads with 3 unique sequencing start sites that cross the breakpoints was set as the cutoff value to indicate strong evidence of fusions. RNA fusions would need to be in frame in order to generate productive transcripts. In addition, databases with details for documented fusions were used to authenticate the fusion sequence identified. Known fusions were queried using Quiver Gene Fusion Database, a curated database owned and maintained by ArcherDX. In summary, samples with detectable fusions had to meet the following criteria: 1) Number of unique start sites (SS) for the GSP2 ≥ 3. 2) Number of supporting reads spanning the fusion junction ≥ 5. 3) Percentage of supporting reads spanning the fusion junction ≥ 10%. 4) Fusions annotated in Quiver Gene Fusion Database.

DATABASE USED

Quiver Gene Fusion Database version 5.1.18

LIMITATIONS

This test has been designed to detect fusions in 13 genes sequenced. Therefore, fusion in genes not covered by this test would not be reported. For novel fusions detected in this test, Sanger sequencing confirmation is recommended if residue specimen is available.

STANDARD OPERATING PROCEDURES (SOPs)

Standard operating procedures (SOPs) are shown below:

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

RUN QC

- Panel: ACTFusion™
- Total reads: 1093441
- Average unique RNA Start Sites per control GSP2: 42

DISCLAIMER

Legal Statement

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

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

Treatment Decisions are the Responsibility of the Physician

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

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

Genetic Alterations and Drugs Not Presented in Ranked Order

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

Level of Evidence Provided

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

No Guarantee of Clinical Benefit

This report makes no promises or guarantees about the effectiveness of a particular drug or any treatment procedure in any disease or in any patient. This report also makes no promises or guarantees that a drug without an association of reportable genomic alteration will, in fact, provide no clinical benefit.

Liability

ACT Genomics is not affiliated with any medical facility or medical practitioner. We provide information for informational purposes only, therefore, ACT Genomics and their employees cannot be held responsible for any direct, indirect, special, incidental or consequential damages that may arise from the use of information provided in the report.

免責聲明

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。

本檢驗報告非經本公司許可，不得私自變造、塗改，或以任何方式作為廣告及其他宣傳之用途。
本公司於提供檢驗報告後，即已完成本次契約義務，後續之報告解釋、判讀及用藥、治療，應自行尋求相關專業醫師協助，若需將報告移件其他醫師，本人應取得該醫師同意並填寫移件申請書，主動告知行動基因，行動基因僅能配合該醫師意願與時間提供醫師解說。

醫療決策需由醫師決定

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

基因突變與用藥資訊並非依照有效性排序

本報告中列出之生物標記變異與藥物資訊並非依照潛在治療有效性排序。

證據等級

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

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REFERENCES

Not Applicable.