張惠昆女士 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 10 月 08 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
• MET-MET fusion (Exon 14 skipping)基因突變 • MET D1028N (Exon 14 skipping)基因突變	• Cabozantinib • Crizotinib • Capmatinib • Tepotinib	-
• PIK3CA E542K 基因突變	• Alpelisib • Temsirolimus • Trametinib • Lapatinib* • Trastuzumab*	-
• FLCN 基因減少 • PIK3CA E542K 基因突變	• Everolimus	-
• CHEK2 基因減少	• Olaparib • Rucaparib • Niraparib	-
• PTCH1 基因減少	• Sonidegib • Vismodegib	-
• SMAD4 基因減少	-	• Cetuximab

*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): 1.9 mutations/megabase

微小衛星體不穩定性 (MSI): 穩定(stable)

融合基因: MET(13)-MET(15)基因融合(Exon 14 skipping)

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
Name: 張惠昆
Gender: Female
Date of Birth: Sep 28, 1974
Patient ID: 44621365
Diagnosis: Adenosquamous lung carcinoma

SPECIMEN
Type: FFPE tissue
Date received: Oct 08, 2021
Collection site: Lung
Specimen ID: S10720970
Lab ID: AA-21-04411
D/ID: NA

ORDERING PHYSICIAN
Name: 江起陸醫師
Facility: 臺北榮總
Tel: 886-228712121
Address: 臺北市北投區石牌路二段 201 號

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
MET	D1028N (Exon 14 skipping)	1100	49.1%	COSM6056852
PIK3CA	E542K	1504	3.8%	COSM760
TP53	R280*	352	19.0%	-

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 **32%** tumor purity.

Amplification (Copy number ≥ 8)

Chr	Gene	Copy Number
ND	ND	ND

Homozygous deletion (Copy number=0)

Chr	Gene
ND	ND

Heterozygous deletion (Copy number=1)

Chr	Gene
chr9	*PTCH1*
chr17	*FLCN*
chr18	*SMAD4*
chr22	*CHEK2*

ND, Not Detected

TUMOR MUTATIONAL BURDEN (TMB)

1.9 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:
醫檢師陳韻伃 博士
Yun-Yu Chen Ph.D.
檢字第 015647 號
Yun Yu Chen

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

THERAPEUTIC IMPLICATIONS

TARGETED THERAPIES

Genomic Alterations	Therapies	Effect
Level 1		
MET D1028N (Exon 14 skipping)	Capmatinib, Tepotinib	sensitive
Level 2		
MET D1028N (Exon 14 skipping)	Crizotinib	sensitive
Level 3A		
PIK3CA E542K	Alpelisib	sensitive
Level 3B		
MET D1028N (Exon 14 skipping)	Cabozantinib	sensitive
PIK3CA E542K	Everolimus, Temsirolimus	sensitive
CHEK2 Heterozygous deletion	Niraparib, Rucaparib	sensitive
PTCH1 Heterozygous deletion	Sonidegib, Vismodegib	sensitive
Level 4		
PIK3CA E542K	Trametinib	sensitive
CHEK2 Heterozygous deletion	Olaparib	sensitive
FLCN Heterozygous deletion	Everolimus	sensitive
PIK3CA E542K	Lapatinib, Trastuzumab	less sensitive
SMAD4 Heterozygous deletion	Cetuximab	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

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

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

MET D1028N (Exon 14 skipping)

Biological Impact

The Mesenchymal-Epithelial Transition (MET) is an oncogene that encodes the MET receptor tyrosine kinase (c-MET, also called HGFR, hepatocyte growth factor receptor). Binding of HGF leads to autophosphorylation and activation of MET and downstream effectors through the PI3K/AKT and RAS/RAF/MEK pathways, which regulates cell growth, proliferation, migration, and angiogenesis[1][2]. Gene amplification or overexpression of the MET occur in a wide range of cancers, including breast cancer[3], non-small cell lung cancer (NSCLC)[4], prostate cancer[5], renal papillary carcinoma[6][7], glioblastoma[8], hepatocellular carcinoma[9], and gastric cancer[10].

D1028 point mutations (including D1028N, D1028H and D1028Y) in the last nucleotide of MET exon 14 tend to cause abnormal splicing and result in exon 14 skipping[11][12][13].

MET exon 14 skipping alteration (METex14) is introduced by mutations in the MET exon 14 splice acceptor and donor sites which results in delayed receptor turnover upon HGF stimulation, enhanced MET receptor-mediated signaling, and oncogenic transformation[14][15][16][17].

MET exon 14 splice site mutations have been observed in multiple tumor types[15] including non-small cell lung cancer (NSCLC)[17][18], small cell lung cancer[19], glioblastoma multiforme[8] and squamous cell head and neck cancers[20].

Therapeutic and prognostic relevance

A partial response was observed within five weeks of crizotinib therapy in a lung cancer patient with tumor harboring MET exon 14 donor splice site mutation D1028N[13].

MET exon 14 skipping alteration (METex14) is one of the emerging biomarkers that the National Comprehensive Cancer Network (NCCN) guidelines recommend for all patients diagnosed with non-small cell lung cancer, for evaluating the availability of first or subsequent lines of targeted therapy with capmatinib, crizotinib and tepotinib (Version 5. 2021)[21].

In May 2020, the U.S. FDA approved TABRECTA (Capmatinib), a kinase inhibitor, to treat patients with metastatic NSCLC harboring MET exon 14 skipping based on a multicenter, open-label, multi-cohort clinical trial (GEOMETRY mono-1, NCT02414139). In the GEOMETRY mono-1 trial, the ORR of 28 treatment-naïve patients ORR was 68% (95% CI: 48, 84) with a response duration of 12.6 months; the he ORR of 69 previously treated patients, the ORR was 41% with a response duration of 9.7 months.

In February 2021, the U.S. FDA approved Tepmetko (Tepotinib), a kinase inhibitor, to treat patients with metastatic NSCLC harboring MET exon 14 skipping based on a multicenter, non-randomized, open-label, multi-cohort clinical trial (VISION, NCT02864992). In the VISION trial, the ORR or 69 treatment naïve patients was 43% with a median response duration of 10.8 months; the ORR of 83 previously treated patients was 43% with a median response duration of 11.1 months.

Clinical studies of lung adenocarcinoma and histiocytic sarcoma reported that cancer patients harboring oncogenic mutations in the MET exon 14 splice sites responded to MET inhibitors including crizotinib, cabozantinib, and capmatinib[16][22][15][23][24][25]. A retrospective analysis suggested that treatment with a MET inhibitor crizotinib can prolong survival in patients with advanced non–small cell lung cancer (NSCLC) with a MET exon 14 mutation (J Clin Oncol. 2017;35 (suppl): abstr 8511). A lung adenocarcinoma patient with concurrent EGFR L858R, METex14 alteration, and MET amplification showed ongoing clinical benefit and stable disease under osimertinib and crizotinib combination treatment. Furthermore, in vitro studies demonstrated that METex14 induces resistance to osimertinib in EGFR-mutant NSCLC cell lines[26].

PIK3CA **E542K**

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[27][28][29][30]. 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[28][31][32][33].

PIK3CA E545K/E542K are 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[34]. According to ExteNET trial, PIK3CA activating mutation was not an appropriate predictive biomarker of response to neratinib in HER2-positive early breast cancer[35].

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[36].

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%[37].

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[38][39][40][41][42] or temsirolimus in combination with doxorubicin and bevacizumab[43][44], 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[45]. 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[46]. 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[47].

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[48][49].

A retrospective study indicated that CRC patients with PIK3CA mutation and wild-type KRAS/BRAF showed fair responses to anti-EGFR therapies[50].

Hyperactivation of the PI3K signaling pathway is common and has been implicated in resistance to endocrine therapy[51][48] and HER2-targeting neoadjuvant therapies such as trastuzumab and lapatinib[52][53][54][55][56][57] 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[58]. Preclinical data also showed that PI3K/AKT/mTOR signaling was implicated in gefitinib resistance in EGFR-mutated lung cancer cell lines[59]. Although preclinical data suggested that addition of everolimus restored gefitinib sensitivity in NSCLC cancer cell lines[60][61], the efficacy was not evidenced in the clinical settings[62][63].

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[64][65][55].

In patients with advanced EGFR- or KRAS-mutant lung adenocarcinoma, a concurrent PIK3CA mutation has been reported as a poor prognostic factor[66].

TP53 R280*

Biological Impact

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

R280* mutation results in a premature truncation of the p53 protein at amino acid 280 (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)[69].

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[70]. 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[71].

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[72][73][74]. 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)[75]. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy[76][77]. 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[78].

CHEK2 Heterozygous deletion

Biological Impact

The checkpoint kinase 2 (CHEK2 or CHK2) gene encodes a serine/threonine protein kinase involved in transducing DNA damage signals that are required for both the intra-S phase and G2/M checkpoints[79]. CHEK2 heterozygosity has been shown to cause haploinsufficient phenotypes that can contribute to tumorigenesis through inappropriate S phase entry, accumulation of DNA damage during replication, and failure to restrain mitotic entry[80][81]. CHEK2 aberrations are associated with glioblastoma, breast, ovarian, prostate, colorectal, gastric, thyroid, and lung cancers[82][83][84][85][86].

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[87].

In addition, CHEK2 has been determined as an inclusion criterion for the trials evaluating rucaparib efficacy in ovarian cancer[88] or prostate cancer[89](NCT03533946), niraparib efficacy in melanoma (NCT03925350), pancreatic cancer (NCT03553004), prostate cancer (NCT02854436), and any malignancy, except prostate (NCT03207347), and talazoparib efficacy in HER2-negative breast cancer (NCT02401347), prostate cancer (NCT03148795), and lung cancer (NCT03377556), respectively.

In a phase 2 trial, two prostate cancer patients harboring CHEK2 homozygous deletion was enrolled. One of the two patients had a response to olaparib[90].

FLCN Heterozygous deletion

Biological Impact

The FLCN gene encodes the tumor suppressor, Folliculin, a GTPase activating protein (GAP) for RagC/D GTPase proteins involved in amino acid sensing and signaling to mTORC1[91]. FLCN 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[92][93]. Inactivation of the FLCN gene by mutation or deletion results in the activation of the mTOR pathway and AKT signaling[94][95]. Germline mutation of the FLCN gene causes the Birt-Hogg-Dubé syndrome, a rare disorder that is characterized by benign hamartomatous skin lesions and an increased risk of pneumothorax and renal tumors[96].

Therapeutic and prognostic relevance

In a prospective Phase 2 study, four anaplastic thyroid cancer (ATC)/ poorly differentiated thyroid cancer (PDTC) patients who had PI3K/mTOR/AKT alterations, including TSC2, FLCN or NF1, showed impressive progression-free survival (PFS) of 15.2 months after receiving everolimus[97]. mTOR inhibition via rapamycin also demonstrated potential in inhibiting the growth of renal cells deficient in FLCN in the preclinical setting[98].

PTCH1 Heterozygous deletion

Biological Impact

The PTCH1 (protein patched homolog 1) gene encodes a multi-pass transmembrane receptor for sonic hedgehog (shh), a tumor suppressor that acts to repress shh signaling in the absence of ligand[99]. Inactivation of PTCH1 results in hedgehog ligand-independent activation of SMO, causing a downstream activation of the pathway and lead to the neoplastic growth[100][101]. Recurrent PTCH1 mutations have been reported in sporadic basal cell carcinoma (BCCs) and medulloblastoma[102][103][104][105]. Germline PTCH1 mutations are associated with the nevoid basal cell carcinoma syndrome (NBCCS, Gorlin syndrome), predisposing patients to basal cell carcinoma and medulloblastoma[103]. PTCH1 is a haploinsufficient tumor suppressor gene with one copy loss may be sufficient to promote tumor development in mice[100][106].

Therapeutic and prognostic relevance

Vismodegib is a small molecule inhibitor of SMO approved by the FDA for the treatment of patients with basal cell carcinoma. A heavily-pretreated patient with metastatic medulloblastoma harboring loss of heterozygosity and somatic mutation of PTCH1, showed rapid regression of the tumor after treated with vismodegib[107]. Furthermore, a phase II study demonstrated that vismodegib treatment results in extended progression-free survival (PFS) in patients with loss-of-heterozygosity, SHH-driven medulloblastoma[108][109]. In a phase II trial (MyPathway), 3 advanced solid tumors patients harboring PTCH1 loss-of-function mutations had partial responses to vismodegib treatment[110].

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[111]. 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[112]. SMAD4 germline mutations are associated with juvenile polyposis syndrome (JPS)[113][114][115][116]. Somatic mutations of SMAD4 are commonly observed in pancreatic cancer[117], colorectal cancer (CRC)[115][118][119]and less frequently seen in other cancers such as lung adenocarcinoma[120], head and neck cancer[121][122]and cutaneous squamous cell carcinoma[123].

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[124]. 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[125].

SMAD4 is also suggested as a predictive marker for 5-fluorouracil-based chemotherapy in colorectal cancer (CRC)[126][127]. 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[128].

Results from clinical and meta-analyses showed that loss of SMAD4 in CRC, pancreatic cancer was correlated with poor prognosis[129][130][131][132][133][134][135][136]. In cervical cancer patients, weak cytoplasmic SMAD4 expression and absent nuclear SMAD4 expression were shown to be significantly associated with poor disease-free and overall 5-year survival[137]

US FDA-APPROVED DRUG(S)

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 tradename PIQRAY.

FDA Approval Summary of Alpelisib (PIQRAY)

SOLAR-1[36] NCT02437318	**Hr-positive, her2-negative breast cancer** (Approved on 2019/05/24)
	PIK3CA mutation
	Alpelisib plus fulvestrant vs. Placebo plus fulvestrant [PFS(M): 11 vs. 5.7]

Cabozantinib (COMETRIQ)

Cabozantinib is a small molecule inhibitors of multiple tyrosine kinases, including RET, MET, VEGFR-1, -2 and -3, KIT, TRKB, FLT-3, AXL, and TIE-2. Cabozantinib is developed and marketed by Exelixis under the trade names COMETRIQ (capsule) and CABOMETYX (tablet).

FDA Approval Summary of Cabozantinib (COMETRIQ)

EXAM[138] NCT00704730	**Thyroid cancer** (Approved on 2012/11/29)
	-
	Cabozantinib vs. Placebo [PFS(M): 11.2 vs. 4]

Cabozantinib (CABOMETYX)

Cabozantinib is a small molecule inhibitors of multiple tyrosine kinases, including RET, MET, VEGFR-1, -2 and -3, KIT, TRKB, FLT-3, AXL, and TIE-2. Cabozantinib is developed and marketed by Exelixis under the trade names COMETRIQ (capsule) and CABOMETYX (tablet).

FDA Approval Summary of Cabozantinib (CABOMETYX)

COSMIC-311 NCT03690388	**Differentiated thyroid cancer (dtc)** (Approved on 2021/09/17)
	-
	Cabozantinib vs. Placebo [PFS(M): 11 vs. 1.9, ORR(%): 18.0 vs. 0]

CHECKMATE-9ER NCT03141177	**Renal cell carcinoma** (Approved on 2021/01/22)
	-
	Nivolumab + cabozantinib vs. Sunitinib [ORR(%): 55.7 vs. 27.1, PFS(M): 16.6 vs. 8.3, OS(M): NR vs. NR]
CELESTIAL [139] NCT01908426	**Hepatocellular carcinoma** (Approved on 2019/01/14)
	-
	Cabozantinib vs. Placebo [OS(M): 10.2 vs. 8]
CABOSUN[140] NCT01835157	**Renal cell carcinoma** (Approved on 2017/12/09)
	-
	Cabozantinib vs. Sunitinib [PFS(M): 8.6 vs. 5.3]
METEOR[141] NCT01865747	**Renal cell carcinoma** (Approved on 2016/04/25)
	-
	Cabozantinib vs. Everolimus [PFS(M): 7.4 vs. 3.8]

Capmatinib (TABRECTA)

Capmatinib is an orally bioavailable inhibitor of the proto-oncogene c-Met (also known as hepatocyte growth factor receptor (HGFR)) with potential antineoplastic activity. Capmatinib is developed and marketed by Novartis under the tradename TABRECTA.

FDA Approval Summary of Capmatinib (TABRECTA)

GEOMETRY mono-1[142] NCT02414139	**Non-small cell lung carcinoma** (Approved on 2020/05/06)
	MET exon 14 skipping
	Capmatinib [ORR (Treatment naive) (%): 68, ORR (Previously treated)(%): 41]

Crizotinib (XALKORI)

Crizotinib is an inhibitor of the tyrosine kinases anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1), by competitively binding with the ATP-binding pocket. Crizotinib is developed and marketed by Pfizer under the trade name XALKORI.

FDA Approval Summary of Crizotinib (XALKORI)

ADVL0912 NCT00939770	**Alk fusion-positive anaplastic large cell lymphoma (alcl)** (Approved on 2021/01/14) **ALK fusion** Crizotinib [ORR(%): 88.0, DOR(M): 39 (maintained response for at least 6 months) vs. 22 (maintained response for at least 12 months)]
PROFILE 1001[143] NCT00585195	**Non-small cell lung carcinoma** (Approved on 2016/03/11) **ROS1-positive** Crizotinib [ORR(%): 66.0]
PROFILE 1014[144] NCT01154140	**Non-small cell lung carcinoma** (Approved on 2015/03/20) **ALK-positive** Crizotinib vs. Pemetrexed + cisplatin or pemetrexed + carboplatin [PFS(M): 10.9 vs. 7]
PROFILE 1007[145] NCT00932893	**Non-small cell lung carcinoma** (Approved on 2013/11/20) **ALK-positive** Crizotinib vs. Pemetrexed or docetaxel [PFS(M): 7.7 vs. 3]

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[146] NCT01524783	**Lung or gastrointestinal neuroendocrine tumor** (Approved on 2016/02/26) - Everolimus vs. Placebo [PFS(M): 11 vs. 3.9]

BOLERO-2[147] NCT00863655	**Breast cancer** (Approved on 2012/07/20) **ER+/HER2-** Everolimus + exemestane vs. Placebo + exemestane [PFS(M): 7.8 vs. 3.2]
RADIANT-3[148] NCT00510068	**Pancreatic neuroendocrine tumor** (Approved on 2011/05/05) - Everolimus vs. Placebo [PFS(M): 11 vs. 4.6]
EXIST-1[149] NCT00789828	**Subependymal giant cell astrocytoma** (Approved on 2010/10/29) - Everolimus vs. Placebo [ORR(%): 35.0]
RECORD-1[150] 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[151] 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[152] NCT01847274	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/03/27) **gBRCA+ CR/PR to platinum-based chemotherapy** Niraparib vs. Placebo [PFS(M): 21 vs. 5.5]
NOVA[152] NCT01847274	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/03/27) **gBRCA- CR/PR to platinum-based chemotherapy** Niraparib vs. Placebo [PFS(M): 9.3 vs. 3.9]

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[87] 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[153] 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[154] 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[155] 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[156] 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]
SOLO-2/ENGOT-Ov21[157] NCT01874353	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/08/17) **gBRCA+** Olaparib vs. Placebo [PFS(M): 19.1 vs. 5.5]

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Study19[158] NCT00753545	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2017/08/17)
	-
	Olaparib vs. Placebo [PFS(M): 8.4 vs. 4.8]
Study 42[159] NCT01078662	**Ovarian cancer** (Approved on 2014/12/19)
	Germline BRCA mutation (deleterious/suspected deleterious)
	Olaparib [ORR(%): 34.0, DOR(M): 7.9]

Rucaparib (RUBRACA)

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

FDA Approval Summary of Rucaparib (RUBRACA)

TRITON2 NCT02952534	**Prostate cancer** (Approved on 2020/05/15)
	gBRCA+, sBRCA
	Rucaparib [ORR(%): 44.0, DOR(M): NE]
ARIEL3[88] NCT01968213	**Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma** (Approved on 2018/04/06)
	All|HRD|tBRCA
	Rucaparib vs. Placebo [PFS(M): 10.8|13.6|16.6 vs. 5.4|5.4|5.4]
ARIEL2[160] NCT01482715, NCT01891344	**Ovarian cancer** (Approved on 2016/12/19)
	Germline and/or somatic BRCA mutation
	Rucaparib [ORR(%): 54.0]

Sonidegib (ODOMZO)

Sonidegib is a Hedgehog signaling pathway inhibitor by blocking its key component, smoothened (smo). Sonidegib is developed and marketed by Novartis under the trade name ODOMZO.

FDA Approval Summary of Sonidegib (ODOMZO)

BOLT[161] NCT01327053	**Basal cell carcinoma** (Approved on 2015/07/24)
	-
	Sonidegib [ORR(%): 58.0]

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)

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

Tepotinib (TEPMETKO)

Tepotinib is a potent and selective c-Met inhibitor.Tepotinib is developed and marketed by EMD Serono, Inc. under the trade name TEPMETKO.

FDA Approval Summary of Tepotinib (TEPMETKO)

VISION NCT02864992	**Non-small cell lung carcinoma** (Approved on 2021/02/03)
	MET exon 14 skipping
	Tepotinib [ORR (Treatment naive)(%): 43, ORR (Previously treated)(%): 43]

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[163] NCT02034110	**Anaplastic thyroid cancer** (Approved on 2018/05/04) **BRAF V600E** Dabrafenib + trametinib [ORR(%): 61.0]
BRF113928[164] 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[165] NCT01584648	**Melanoma** (Approved on 2014/01/10) **BRAF V600E/K** Trametinib + dabrafenib vs. Dabrafenib + placebo [PFS(M): 9.3 vs. 8.8]
METRIC[166] NCT01245062	**Melanoma** (Approved on 2013/05/29) **BRAF V600E/K** Trametinib vs. Dacarbazine or paclitaxel [PFS(M): 4.8 vs. 1.5]

Vismodegib (ERIVEDGE)

Vismodegib is a cyclopamine-competitive antagonist and acts as a first-in-class Hedgehog signaling pathway inhibitor by blocking its key component smoothened (smo). Vismodegib is developed by Genentech and marketed by Roche under the trade name ERIVEDGE.

FDA Approval Summary of Vismodegib (ERIVEDGE)

ERIVANCE BCC[167] NCT00833417	**Basal cell carcinoma** (Approved on 2012/01/30) - Vismodegib [ORR(%): 30.3 (mBCC) 42.9 (laBCC)]

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.

DETAILED TEST RESULTS

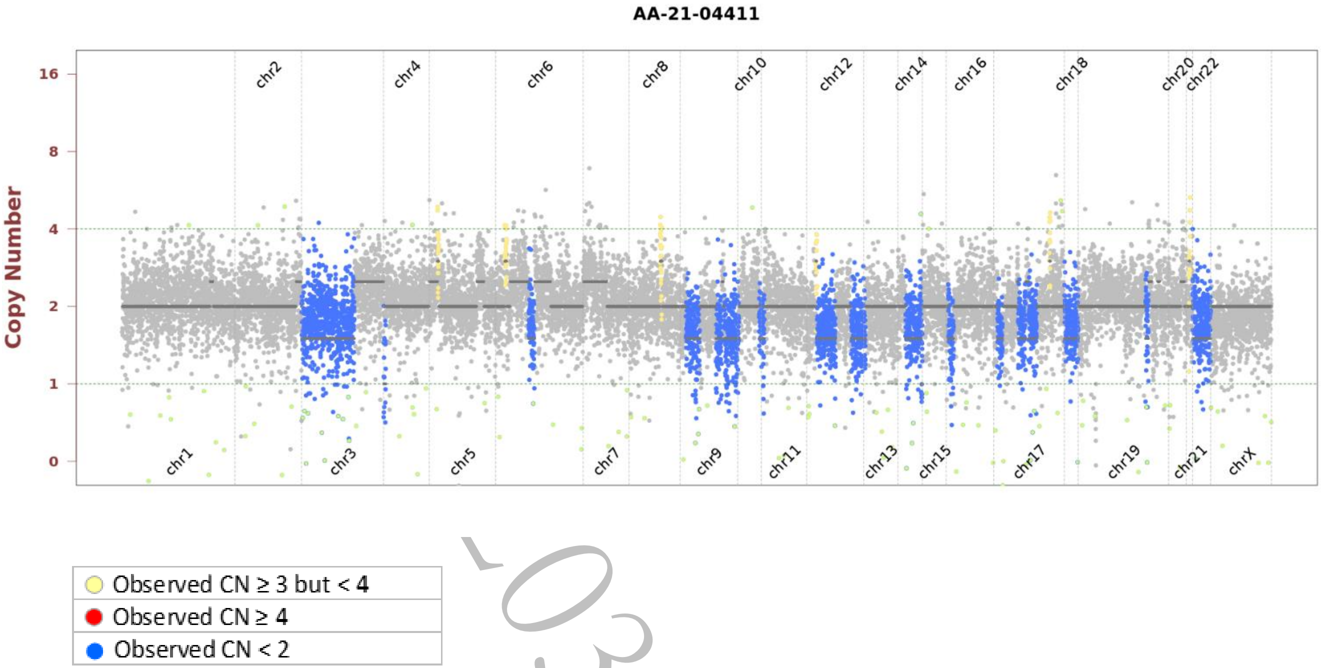
SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Gene	Chr	Exon	Accession Number	cDNA Change	Amino Acid Change	Coverage	Allele Frequency	COSMIC ID
ADAMTSL1	9	4	NM_001040272	c.305A>G	H102R	145	38.6%	-
BCL6	3	5	NM_001130845	c.511A>G	S171G	1254	27.8%	-
FANCA	16	-	NM_000135	c.1007-7C>T	Splice region	931	56.9%	-
FAT1	4	19	NM_005245	c.11080G>A	V3694I	1053	48.7%	-
GNAS	20	1	NM_080425	c.549C>A	D183E	146	43.2%	-
GNAS	20	1	NM_080425	c.913T>C	S305P	174	38.5%	-
INSR	19	22	NM_000208	c.3809G>A	R1270H	164	36.6%	COSM1003128
MET	**7**	**14**	**NM_001127500**	**c.3082G>A**	**D1028N (Exon 14 skipping)**	**1100**	**49.1%**	**COSM6056852**
PIK3C2B	1	13	NM_002646	c.1990G>A	E664K	537	49.9%	-
PIK3CA	**3**	**10**	**NM_006218**	**c.1624G>A**	**E542K**	**1504**	**3.8%**	**COSM760**
RBM10	X	12	NM_005676	c.1175A>G	N392S	363	55.9%	-
TP53	**17**	**8**	**NM_000546**	**c.837_838delinsTT**	**R280***	**352**	**19.0%**	**-**
XIAP	X	3	NM_001167	c.962C>G	A321G	1004	43.3%	-

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.



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	**D1028N**
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	**E542K**

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	1
MET	3

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

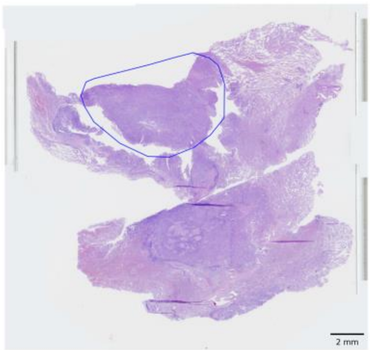
This test does not provide information of variant causality and does not detect variants in non-coding regions that could affect gene expression. This report does not report polymorphisms and we do not classify whether a mutation is germline or somatic. Variants identified by this assay were not subject 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.: S10720970
- Collection site: Lung
- Examined by: Dr. Yeh-Han Wang
- Estimated neoplastic nuclei (whole sample): The percentage of viable tumor cells in total cells in the whole slide (%): 30%
  The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%):50%
  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: Jun 2018
- Facility retrieved: 臺北榮總

RUN QC

- Panel: ACTOnco®+
- Mean Depth: 741x
- Target Base Coverage at 100x: 91%

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.

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

基因突變與用藥資訊並非依照有效性排序

本報告中列出之生物標記變異與藥物資訊並非依照潛在治療有效性排序。

證據等級

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

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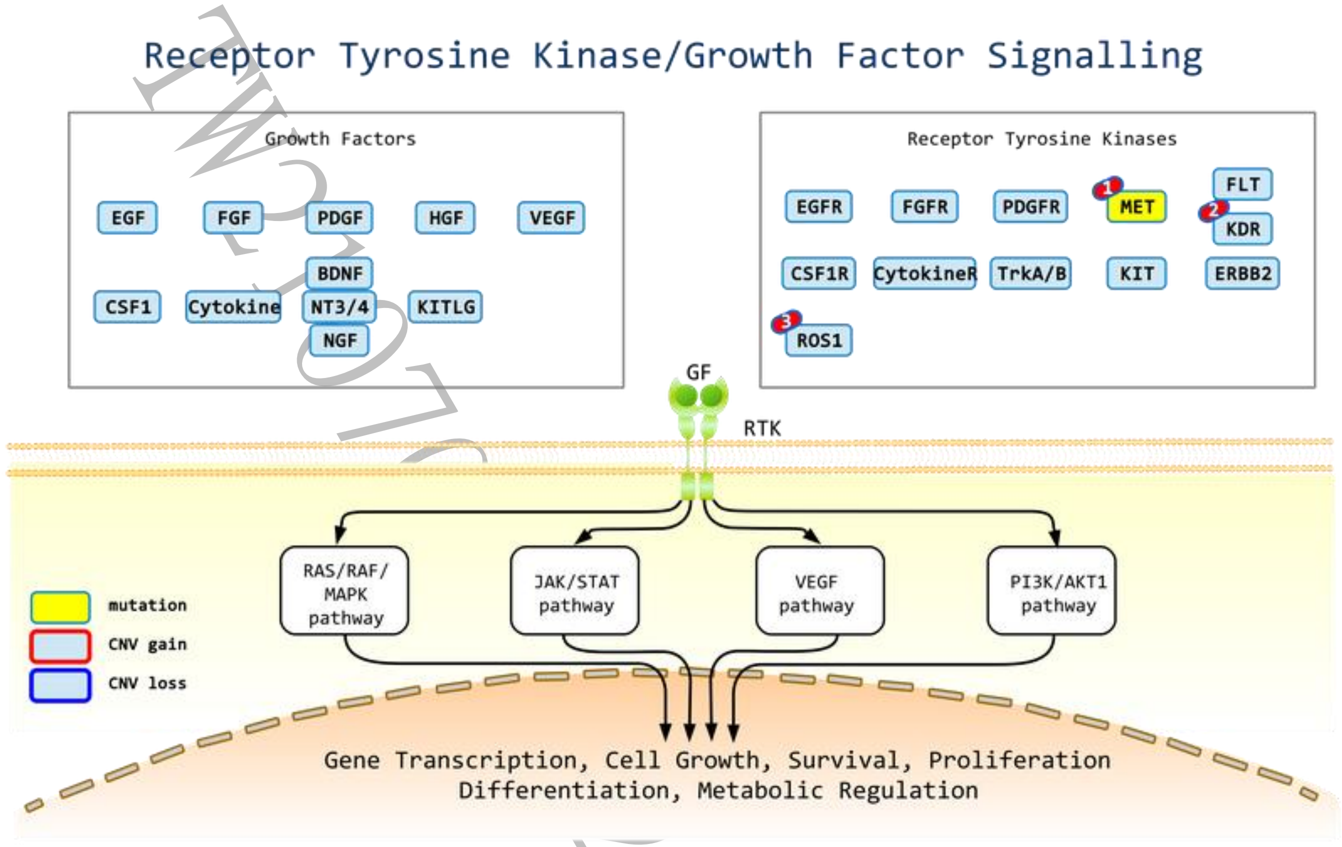
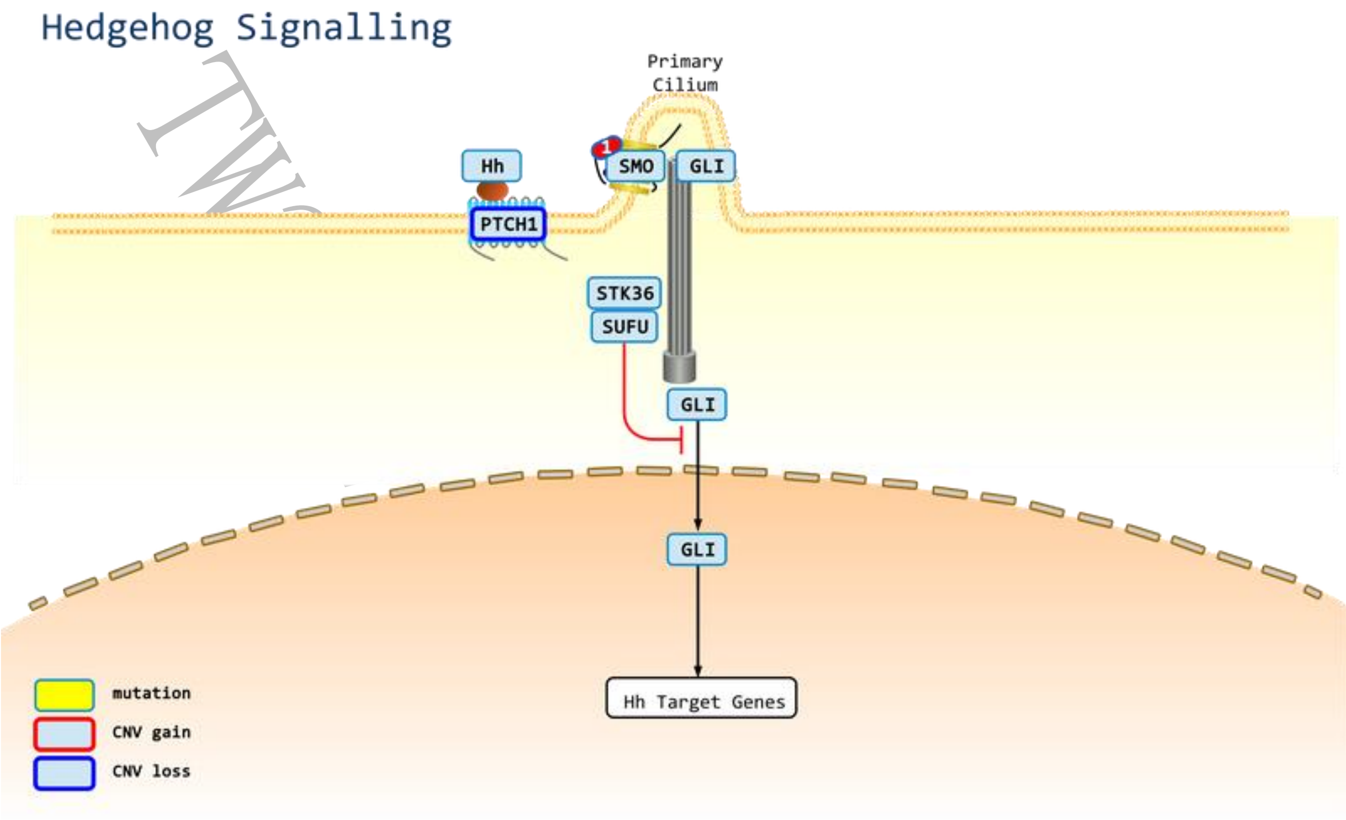
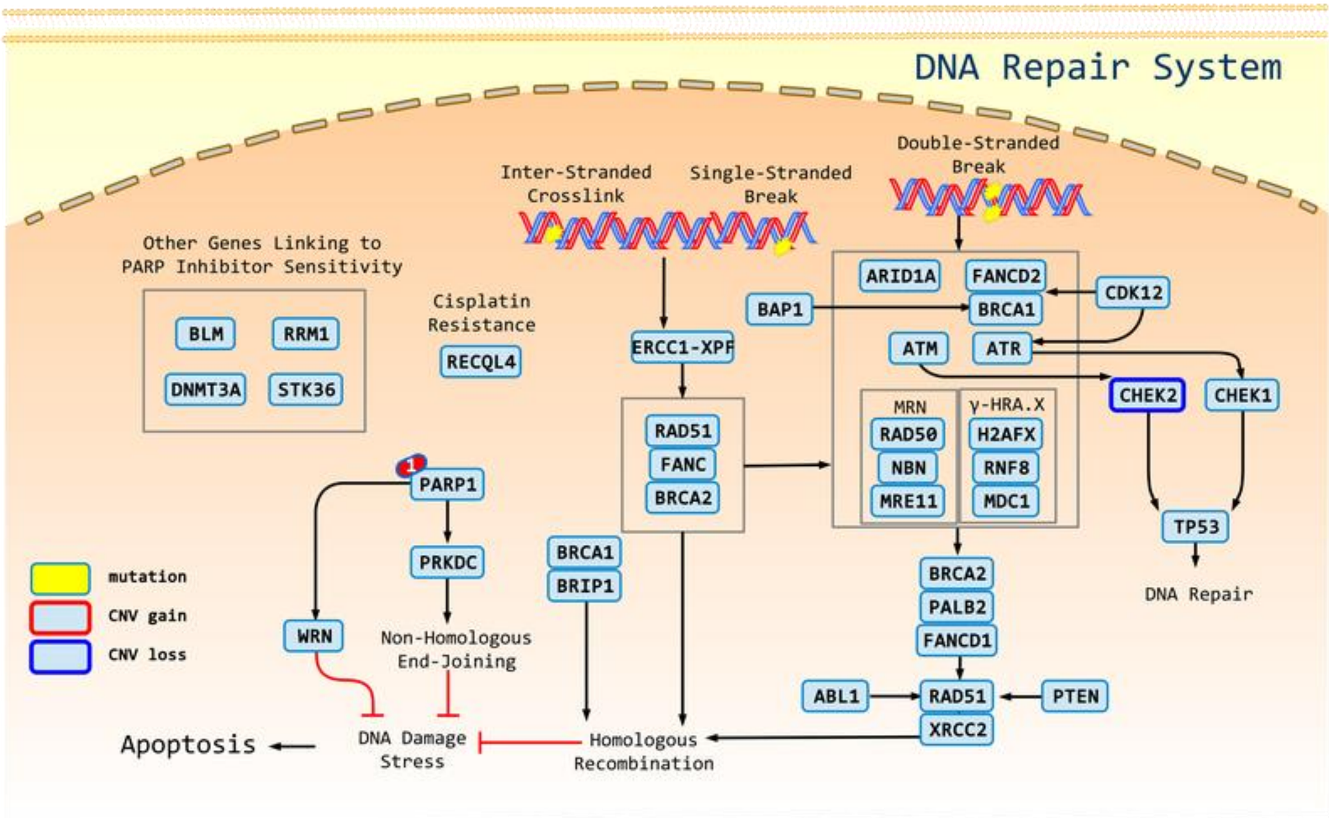
SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



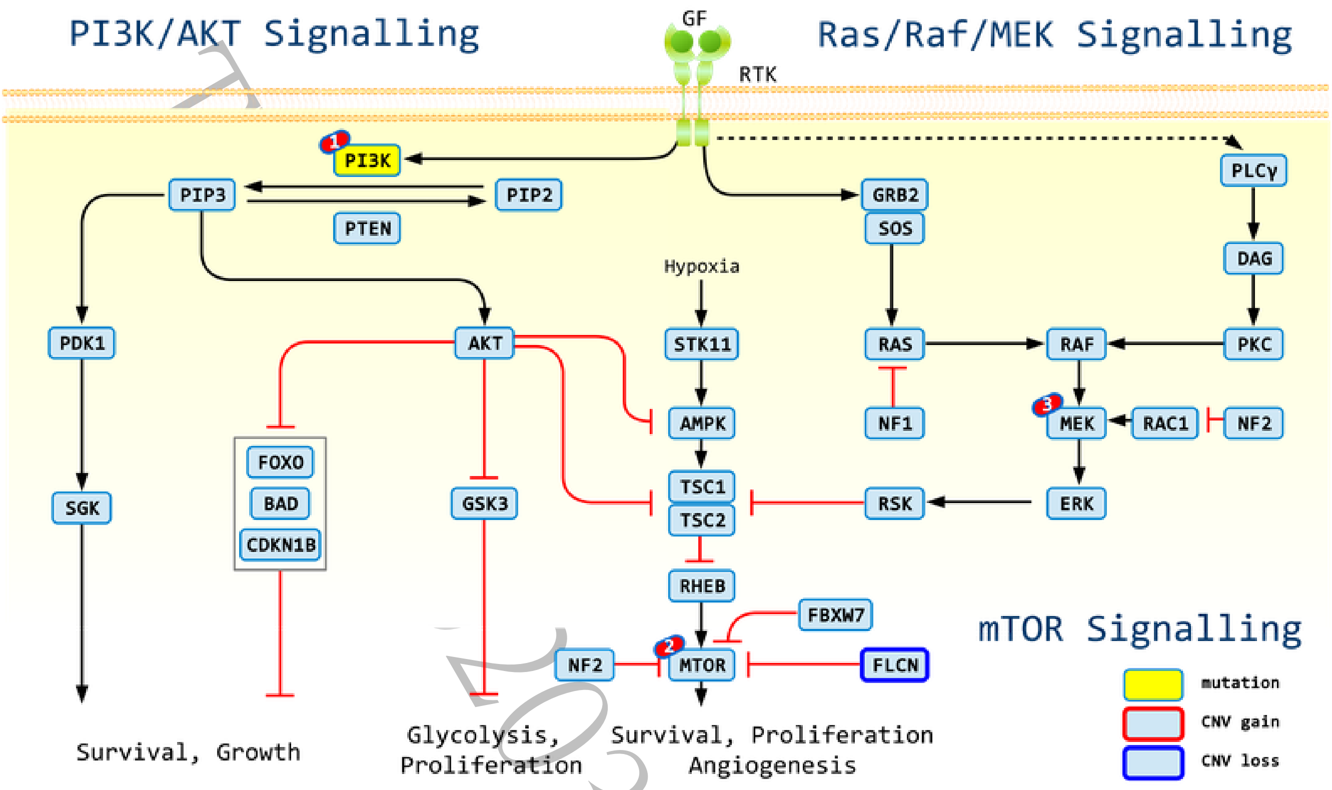
1: Cabozantinib, Crizotinib, Capmatinib, Tepotinib; 2: Cabozantinib; 3: Crizotinib



1: Sonidegib, Vismodegib



1: Olaparib, Niraparib, Rucaparib



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

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張惠昆
Project ID: C21-M001-00882
Report No.: AA-21-04411_FUS
Date Reported: Oct 22, 2021

ACTFusion™ Report

PATIENT	SPECIMEN	ORDERING PHYSICIAN
Name: 張惠昆	Type: FFPE tissue	Name: 江起陸醫師
Gender: Female	Date received: Oct 08, 2021	Facility: 臺北榮總
Date of Birth: Sep 28, 1974	Collection site: Lung	Tel: 886-228712121
Patient ID: 44621365	Specimen ID: S10720970	Address: 臺北市北投區石牌路二段 201 號
Diagnosis: Adenosquamous lung carcinoma	Lab ID: AA-21-04411	
	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

MET(13)-MET(15) fusion (Exon 14 skipping) detected in this sample.

Transcript ID used: MET(NM_000245)

Variant Analysis:
醫檢師陳韻伃 博士
Yun-Yu Chen Ph.D.
檢字第 015647 號

Yun Yu Chen

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

Yun Yu Chen

THERAPEUTIC IMPLICATIONS

TARGETED THERAPIES

Genomic Alterations	Therapies	Effect
Level 1		
MET-MET (Exon 14 skipping)	Capmatinib, Tepotinib	**sensitive**
Level 2		
MET-MET (Exon 14 skipping)	Crizotinib	**sensitive**
Level 3B		
MET-MET (Exon 14 skipping)	Cabozantinib	**sensitive**

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

VARIANT INTERPRETATION

MET(13)-MET(15) fusion (Exon 14 skipping)

Biological Impact

The Mesenchymal-Epithelial Transition (MET) is an oncogene that encodes the MET receptor tyrosine kinase (c-MET, also called HGFR, hepatocyte growth factor receptor). Binding of HGF leads to autophosphorylation and activation of MET and downstream effectors through the PI3K/AKT and RAS/RAF/MEK pathways, which regulates cell growth, proliferation, migration, and angiogenesis[1][2]. Gene amplification or overexpression of the MET occur in a wide range of cancers, including breast cancer[3], non-small cell lung cancer (NSCLC)[4], prostate cancer[5], renal papillary carcinoma[6][7], glioblastoma[8], hepatocellular carcinoma[9], and gastric cancer[10].

MET gene rearrangement, which resulting in constitutively active MET fusion kinase, has been identified in various tumor types (DOI: 10.1200/JCO.2019.37.15_suppl.3078)[11][12].

MET exon 14 skipping alteration (METex14) is introduced by mutations in the MET exon 14 splice acceptor and donor sites which results in delayed receptor turnover upon HGF stimulation, enhanced MET receptor-mediated signaling, and oncogenic transformation[13][14][15][16].

MET exon 14 splice site mutations have been observed in multiple tumor types[14] including non-small cell lung cancer (NSCLC)[16][17], small cell lung cancer[18], glioblastoma multiforme[8] and squamous cell head and neck cancers[19].

Therapeutic and prognostic relevance

MET exon 14 skipping alteration (METex14) is one of the emerging biomarkers that the National Comprehensive Cancer Network (NCCN) guidelines recommend for all patients diagnosed with non-small cell lung cancer, for evaluating the availability of first or subsequent lines of targeted therapy with capmatinib, crizotinib and tepotinib (Version 5. 2021)[20].

In May 2020, the U.S. FDA approved TABRECTA (Capmatinib), a kinase inhibitor, to treat patients with metastatic NSCLC harboring MET exon 14 skipping based on a multicenter, open-label, multi-cohort clinical trial (GEOMETRY mono-1, NCT02414139). In the GEOMETRY mono-1 trial, the ORR of 28 treatment-naïve patients was 68% (95% CI: 48, 84) with a response duration of 12.6 months; the ORR of 69 previously treated patients was 41% with a response duration of 9.7 months.

In February 2021, the U.S. FDA approved Tepmetko (Tepotinib), a kinase inhibitor, to treat patients with metastatic NSCLC harboring MET exon 14 skipping based on a multicenter, non-randomized, open-label, multi-cohort clinical trial (VISION, NCT02864992). In the VISION trial, the ORR of 69 treatment naïve patients was 43% with a median response duration of 10.8 months; the ORR of 83 previously treated patients was 43% with a median response duration of 11.1 months.

Clinical studies of lung adenocarcinoma and histiocytic sarcoma reported that cancer patients harboring oncogenic mutations in the MET exon 14 splice sites responded to MET inhibitors including crizotinib, cabozantinib, and capmatinib[15][21][14][22][23]. A retrospective analysis suggested that treatment with a MET inhibitor crizotinib can prolong survival in patients with advanced non–small cell lung cancer (NSCLC) with a MET exon 14 mutation (DOI: 10.1200/JCO.2017.35.15_suppl.8511). A lung adenocarcinoma patient with concurrent EGFR L858R, METex14 alteration, and MET amplification showed ongoing clinical benefit and stable disease under osimertinib and crizotinib combination treatment. Furthermore, in vitro studies demonstrated that METex14 induces resistance to osimertinib in EGFR-mutant NSCLC cell lines[24].

US FDA-APPROVED DRUG(S)

Cabozantinib (COMETRIQ)

Cabozantinib is a small molecule inhibitors of multiple tyrosine kinases, including RET, MET, VEGFR-1, -2 and -3, KIT, TRKB, FLT-3, AXL, and TIE-2. Cabozantinib is developed and marketed by Exelixis under the trade names COMETRIQ (capsule) and CABOMETYX (tablet).

FDA Approval Summary of Cabozantinib (COMETRIQ)

EXAM[25] NCT00704730	**Thyroid cancer** (Approved on 2012/11/29)
	-
	Cabozantinib vs. Placebo [PFS(M): 11.2 vs. 4]

Cabozantinib (CABOMETYX)

Cabozantinib is a small molecule inhibitors of multiple tyrosine kinases, including RET, MET, VEGFR-1, -2 and -3, KIT, TRKB, FLT-3, AXL, and TIE-2. Cabozantinib is developed and marketed by Exelixis under the trade names COMETRIQ (capsule) and CABOMETYX (tablet).

FDA Approval Summary of Cabozantinib (CABOMETYX)

COSMIC-311 NCT03690388	**Differentiated thyroid cancer (dtc)** (Approved on 2021/09/17)
	-
	Cabozantinib vs. Placebo [PFS(M): 11 vs. 1.9, ORR(%): 18.0 vs. 0]
CHECKMATE-9ER NCT03141177	**Renal cell carcinoma** (Approved on 2021/01/22)
	-
	Nivolumab + cabozantinib vs. Sunitinib [ORR(%): 55.7 vs. 27.1, PFS(M): 16.6 vs. 8.3, OS(M): NR vs. NR]
CELESTIAL [26] NCT01908426	**Hepatocellular carcinoma** (Approved on 2019/01/14)
	-
	Cabozantinib vs. Placebo [OS(M): 10.2 vs. 8]
CABOSUN[27] NCT01835157	**Renal cell carcinoma** (Approved on 2017/12/09)
	-
	Cabozantinib vs. Sunitinib [PFS(M): 8.6 vs. 5.3]

METEOR[28] NCT01865747	**Renal cell carcinoma** (Approved on 2016/04/25)
	-
	Cabozantinib vs. Everolimus [PFS(M): 7.4 vs. 3.8]

Capmatinib (TABRECTA)

Capmatinib is an orally bioavailable inhibitor of the proto-oncogene c-Met (also known as hepatocyte growth factor receptor (HGFR)) with potential antineoplastic activity. Capmatinib is developed and marketed by Novartis under the tradename TABRECTA.

FDA Approval Summary of Capmatinib (TABRECTA)

GEOMETRY mono-1[29] NCT02414139	**Non-small cell lung carcinoma** (Approved on 2020/05/06)
	MET exon 14 skipping
	Capmatinib [ORR (Treatment naive) (%): 68, ORR (Previously treated)(%): 41]

Crizotinib (XALKORI)

Crizotinib is an inhibitor of the tyrosine kinases anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1), by competitively binding with the ATP-binding pocket. Crizotinib is developed and marketed by Pfizer under the trade name XALKORI.

FDA Approval Summary of Crizotinib (XALKORI)

ADVL0912 NCT00939770	**Alk fusion-positive anaplastic large cell lymphoma (alcl)** (Approved on 2021/01/14)
	ALK fusion
	Crizotinib [ORR(%): 88.0, DOR(M): 39 (maintained response for at least 6 months) vs. 22 (maintained response for at least 12 months)]
PROFILE 1001[30] NCT00585195	**Non-small cell lung carcinoma** (Approved on 2016/03/11)
	ROS1-positive
	Crizotinib [ORR(%): 66.0]
PROFILE 1014[31] NCT01154140	**Non-small cell lung carcinoma** (Approved on 2015/03/20)
	ALK-positive
	Crizotinib vs. Pemetrexed + cisplatin or pemetrexed + carboplatin [PFS(M): 10.9 vs. 7]

PROFILE 1007[32] NCT00932893	**Non-small cell lung carcinoma** (Approved on 2013/11/20)
	ALK-positive
	Crizotinib vs. Pemetrexed or docetaxel [PFS(M): 7.7 vs. 3]

Tepotinib (TEPMETKO)

Tepotinib is a potent and selective c-Met inhibitor.Tepotinib is developed and marketed by EMD Serono, Inc. under the trade name TEPMETKO.

FDA Approval Summary of Tepotinib (TEPMETKO)

VISION NCT02864992	**Non-small cell lung carcinoma** (Approved on 2021/02/03)
	MET exon 14 skipping
	Tepotinib [ORR (Treatment naive)(%): 43, ORR (Previously treated)(%): 43]

d=day; w=week; m=month

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.

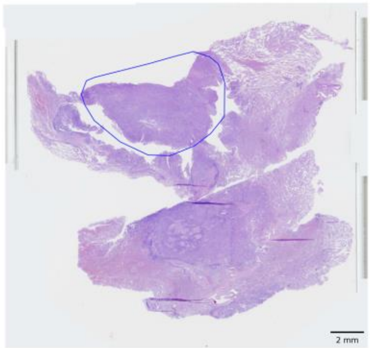
No trial has been found.

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.: S10720970
- Collection date: Jun 2018
- Collection site: Lung
- 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 (%): 30%

  The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%):50%

  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: 944557
- Average unique RNA Start Sites per control GSP2: 27

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.

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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.

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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.

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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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