

ACT Onco[®] + Report

| PATIENT | | |
|--------------------------------------|-----------------------------|----------------------|
| Identifier: 葉美智 | | Patient ID: 49135866 |
| Date of Birth: Jan 07, 1959 | | Gender: Female |
| Diagnosis: Lung small cell carcinoma | | |
| ORDERING PHYSICIAN | | |
| Name: 陳育民醫師 | | Tel: 886-228712121 |
| Facility: 臺北榮總 | | |
| Address: 臺北市北投區石牌路二段 201 號 | | |
| SPECIMEN | | |
| Specimen ID: S11225772A | Collection site: Lymph node | Type: FFPE tissue |
| Date received: Jun 07, 2023 | Lab ID: AA-23-03716 | D/ID: NA |

ABOUT ACT Onco[®]+

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 alterations (CNAs). The test also includes an RNA test, detecting fusion transcripts of 13 genes.

SUMMARY FOR ACTIONABLE VARIANTS

VARIANTS/BIOMARKERS WITH EVIDENCE OF CLINICAL SIGNIFICANCE

| Genomic Alterations/Biomarkers | Probable Effects in Patient's Cancer Type | | Probable Sensitive in Other Cancer Types |
|---|---|-----------|--|
| | Sensitive | Resistant | |
| EGFR L747_T751del (Exon 19 deletion) | - | - | Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib |

VARIANTS/BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

| Genomic Alterations/Biomarkers | Possibly Sensitive | Possibly Resistant |
|---|--------------------|--------------------|
| EGFR L747_T751del (Exon 19 deletion) | - | Cetuximab |
| AKT2 Amplification | - | Erlotinib |

Note:

- The above summary tables present genomic variants and biomarkers based on the three-tiered approach proposed by US FDA for reporting tumor profiling NGS testing. "Variants/biomarkers with evidence of clinical significance" refers to mutations that are widely recognized as standard-of-care biomarkers (FDA level 2/AMP tier 1). "Variants/biomarkers with potential clinical significance" refers to mutations that are not included in the standard of care but are informational for clinicians, which are commonly biomarkers used as inclusion criteria for clinical trials (FDA level 3/AMP tier 2).
- The therapeutic agents and possible effects to a given drug are based on mapping the variants/biomarkers with ACT Genomics clinical knowledge database. The mapping results only provide information for reference, but not medical recommendation.
- Please refer to corresponding sections for more detailed information about genomic alteration and clinical relevance listed above.

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TESTING RESULTS

VARIANT(S) WITH CLINICAL RELEVANCE

- Single Nucleotide and Small InDel Variants

| Gene | Amino Acid Change | Allele Frequency |
|-------|---------------------------------|------------------|
| ARID2 | Q1463* | 32.7% |
| EGFR | L747_T751del (Exon 19 deletion) | 54.5% |
| TP53 | E204* | 94.1% |

- Copy Number Alterations

| Chromosome | Gene | Variation | Copy Number |
|------------|------------|-----------------------|-------------|
| Chr13 | BRCA2, RB1 | Heterozygous deletion | 1 |
| Chr16 | TSC2 | Heterozygous deletion | 1 |
| Chr22 | CHEK2, NF2 | Heterozygous deletion | 1 |
| Chr4 | FBXW7 | Heterozygous deletion | 1 |
| Chr5 | RAD50 | Heterozygous deletion | 1 |
| Chr9 | PTCH1 | Heterozygous deletion | 1 |
| Chr5 | RICTOR | Amplification | 14 |
| Chr19 | AKT2 | Amplification | 28 |

- Fusions

| Fusion Gene & Exon | Transcript ID |
|--|---------------|
| No fusion gene detected in this sample | |

- Immune Checkpoint Inhibitor (ICI) Related Biomarkers

| Biomarker | Results |
|----------------------------------|-----------------------------|
| Tumor Mutational Burden (TMB) | 4.4 muts/Mb |
| Microsatellite Instability (MSI) | Microsatellite stable (MSS) |

Note:

- Variant(s) enlisted in the SNV table may currently exhibit no relevance to treatment response prediction. Please refer to INTERPRETATION for more biological information and/or potential clinical impacts of the variants.
- Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on 74% tumor purity.
- For more therapeutic agents which are possibly respond to heterozygous deletion of genes listed above, please refer to APPENDIX for more information.
- TMB was calculated by using the sequenced regions of ACTOnco[®]+ to estimate the number of somatic nonsynonymous mutations per megabase of all protein-coding genes (whole exome). The threshold for high mutation load is set at ≥ 7.5 mutations per megabase. TMB, microsatellite status and gene copy number deletion cannot be determined if calculated tumor purity is $< 30\%$.

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THERAPEUTIC IMPLICATIONS TARGETED THERAPIES

| Genomic Alterations | Therapies | Effect |
|--|--|------------------|
| Level 3A | | |
| EGFR L747_T751del (Exon 19 deletion) | Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib | sensitive |
| Level 4 | | |
| EGFR L747_T751del (Exon 19 deletion) | Cetuximab | resistant |
| AKT2 Amplification | Erlotinib | resistant |

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

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

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IMMUNE CHECKPOINT INHIBITORS (ICIs)

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

- Other Biomarkers with Potential Clinical Effects for ICIs

| Genomic Alterations | Potential Clinical Effects |
|---------------------|---|
| EGFR aberration | Likely associated with WORSE response to ICIs |

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 | Level of Evidence* |
|--------|----------------|----------|---------------------------|--------------------|
| UGT1A1 | rs4148323 | AA | Irinotecan-based regimens | Level 1B |

Clinical Interpretation:

Patients with the AA 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 AG or GG genotype. Other genetic and clinical factors may also influence a patient's risk of diarrhea and neutropenia.

* 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.

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VARIANT INTERPRETATION

ARID2 Q1463*

Biological Impact

ARID2 (AT-rich interactive domain-containing protein 2) encodes a component of the SWI/SNF chromatin remodeling complex, which plays a crucial role in the regulation of gene expression by altering chromatin structure^[1]. Cancer-associated ARID2 mutations are predominantly inactivating as characterized in hepatocellular carcinoma^[2], non-small cell lung cancer^[3], and melanoma^[4]. ARID2's tumor suppressor role is further supported by several functional studies in which artificial suppression of ARID2 led to increased tumorigenicity in vitro and in vivo^{[5][6]}. However, ARID2 knockdown has also been shown to inhibit myeloma cell proliferation, suggesting possible multifaceted function^[7].

Q1463* mutation results in a premature truncation of the ARID2 protein at amino acid 1463 (UniProtKB). This mutation is predicted to lead to a loss of ARID2 function, despite not having characterized in the literature.

Therapeutic and prognostic relevance

Decreased ARID2 expression has been linked to poor prognosis in hepatocellular carcinoma and head and neck cancer^{[8][5]}. An intriguing reverse trend was noted in multiple myeloma, where ARID2 overexpression was associated with inferior overall survival and chemoresistance^[7].

EGFR L747_T751del (Exon 19 deletion)

Biological Impact

The EGFR gene encodes for the Epidermal Growth Factor Receptor, a receptor tyrosine kinase which binds to its ligands, including Epidermal Growth Factor (EGF) and Transforming Growth Factor-alpha (TGF-alpha), activates downstream signaling pathways, including the canonical oncogenic MAPK and PI3K/AKT/mTOR signaling cascades^[9]. Increased EGFR activity by mutations and/or amplification of the EGFR gene has been described in a wide range of cancers, such as lung, brain, colorectal and head and neck cancer^[10]. Mutations in the kinase domain of EGFR are commonly observed in non-small cell lung cancer (NSCLC), resulting in a constitutively activated form of the receptor^[11]. On the other hand, in the brain and colorectal cancers, the most prevalent EGFR alteration is copy number amplification that results in receptor overexpression^[12].

EGFR L747_T751del (exon 19 deletion) lies within the tyrosine kinase domain of EGFR, resulting in a deletion of 5 amino acids from residues 747 to 751 (UniProtKB). This mutation was demonstrated as an activating mutation with an increased EGFR kinase activity in vitro^[13].

EGFR exon 19 deletions are in-frame deletions of 9–24 nucleotides in exon 19 centred around codons 746–750 of the kinase domain of EGFR. The two most common EGFR alterations, L858R mutation and exon 19 deletions can result in constitutive activation of signal transduction pathways, leading to cell proliferation or anti-apoptosis without ligand binding^[14].

Therapeutic and prognostic relevance

There is accumulated clinical evidence suggested that patients with MDM2/MDM4 amplification or EGFR aberrations exhibited poor clinical outcome and demonstrated a significantly increased rate of tumor growth (hyper-progression) after receiving immune checkpoint (PD-1/PD-L1) inhibitors therapies^[15] (Annals of Oncology (2017) 28 (suppl_5): v403-v427. 10.1093/annonc/mdx376).

EGFR mutation has been determined as an inclusion criteria for the trials examining afatinib efficacy in malignant glioma and pediatric tumors (NCT02423525, NCT02372006). In two case reports, two lung adenocarcinoma patients harboring EGFR L747_T751del mutation had partial responses to erlotinib treatment, but were found to have acquired EGFR T790M or MET amplification upon disease progressions^{[16][17]}.

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In a preclinical study, transformed cells expressing EGFR L747_T751del were sensitive to afatinib, erlotinib and gefitinib treatment that reduced cell viability, but resistant to cetuximab in vitro^[18].

The first- and second-generation EGFR-TKIs, including dacomitinib, erlotinib, gefitinib, and afatinib, have been approved by the U.S. FDA as first-line treatments for non-small cell lung cancer patients with EGFR exon 19 deletion or L858R mutation. Osimertinib, a third-generation EGFR-TKI, has also been approved by the U.S. FDA. It is indicated for adjuvant treatment or first-line treatment of metastatic NSCLC patients with EGFR exon 19 deletion or L858R mutation.

A phase III trial (NCT01774721) show that dacomitinib significantly improved PFS over gefitinib in first-line treatment of patients with EGFR-mutation-positive NSCLC^[19]. Another phase III trial (NCT00949650) demonstrated that median PFS among lung cancer patients with exon 19 deletion or L858R EGFR mutation (n=308) was 13.6 months for afatinib and 6.9 months for chemotherapy^[20]. Results from a double-blind, phase 3 trial further showed that osimertinib significantly demonstrated longer PFS than standard EGFR-TKIs (18.9 months vs. 10.2 months) in previously untreated EGFR mutation-positive (exon 19 deletion or L858R) advanced NSCLC^[21].

TP53 E204*

Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis^[22]. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation^[23].

E204* mutation results in a premature truncation of the p53 protein at amino acid 204 (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)^[24].

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^[25]. 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^[26].

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^{[27][28][29]}. 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)^[30]. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy^{[31][32]}. 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^[33].

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AKT2 Amplification

Biological Impact

The v-akt murine thymoma viral oncogene homolog 2 (AKT2, also known as HIHGH, PRKBB, PKKBETA, RAC-BETA, PKBB) gene encodes an AKT family of serine/threonine protein kinases, including AKT1 and AKT3 isoforms, that act as a downstream effector of the pro-oncogenic PI3-kinase signaling pathway^{[34][35][36][37][38]}. Whereas somatic AKT2 mutations have been described rarely in cancer, germline autosomal dominant mutations in AKT2 are associated with familial diabetes mellitus in humans^[39].

Therapeutic and prognostic relevance

A preclinical study demonstrated that an AKT2-amplified pancreatic cancer cell line exhibited resistance to erlotinib. Besides, amplification of AKT2 was predominantly expressed across pancreatic cancer patients in TCGA datasets and correlated with high mRNA expression. Patients with a high AKT2 expression tended to have poor response to erlotinib plus gemcitabine^[40].

BRCA2 Heterozygous deletion

Biological Impact

The BRCA2 gene encodes a tumor suppressor involved in the homologous recombination pathway for double-strand DNA repair^[41]. 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^[42]. 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^[43]. Somatic mutations in BRCA2 are highest in colorectal, non-small cell lung cancer (NSCLC), and ovarian cancers^[44].

Therapeutic and prognostic relevance

Multiple PARP inhibitors, including olaparib, rucaparib, niraparib, and talazoparib, have been approved by the U.S. FDA for the treatment of cancer. Olaparib is approved for multiple settings in advanced ovarian cancer, metastatic breast cancer with BRCA mutations, metastatic pancreatic cancer, and mCRPC with HRR gene mutations, including BRCA mutations. Rucaparib is approved for maintenance treatment of recurrent ovarian cancer with BRCA mutations and mCRPC with BRCA mutations. Niraparib is approved for maintenance treatment of advanced ovarian cancer and recurrent ovarian cancer with BRCA mutations. Talazoparib is approved for locally advanced or metastatic breast cancer with BRCA mutations.

According to the NCCN guidelines, rucaparib is recommended as recurrence therapy for patients with BRCA-mutated ovarian cancer who have been treated with multiple lines of chemotherapy. It is also recommended as maintenance therapy for patients with metastatic pancreatic cancer who have undergone prior platinum-based therapy and harbor germline or somatic BRCA mutations. Additionally, niraparib is recommended as maintenance therapy for ovarian cancer patients with BRCA mutations.

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^[45]. 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^{[46][47]}. CHEK2 aberrations are associated with glioblastoma, breast, ovarian, prostate, colorectal, gastric, thyroid, and lung cancers^{[48][49][50][51][52]}.

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Therapeutic and prognostic relevance

Olaparib is FDA-approved for treating mCRPC patients harboring mutations in homologous recombination repair (HRR) genes, including CHEK2.

CHEK2 mutation has been determined as an inclusion criterion for the trials evaluating olaparib, rucaparib, niraparib, and talazoparib efficacies in various types of solid tumors (NCT03297606, NCT01968213, NCT03840967, NCT02401347, NCT03148795).

In TBCRC 048 trial, olaparib treatment did not show response in 7 metastatic breast cancer patients with germline mutations in CHEK2 (SD: n=3, PD: n=4)^[53]. In TRITON2 trial, rucaparib treatment had limited response in 12 mCRPC patients with CHEK2 alterations^[54].

FBXW7 Heterozygous deletion

Biological Impact

The F-box/WD repeat-containing protein 7 (FBXW7) gene encodes a protein that belongs to the SCF (SKP1-CUL1-F-box protein) E3 ligase complex. FBXW7 is recognized as a tumor suppressor which is involved in the negative regulation of oncogenes such as c-Myc^{[55][56]}, c-Jun^[57], cyclin E^[58], Notch family members^{[59][60]}, Aurora-A^[61], mTOR^[62], KLF5^[63], and MCL-1^[64]. Inactivating FBXW7 mutation or copy number loss may result in the accumulation of oncoproteins and therefore lead to malignant transformation^[65]. FBXW7 is a haploinsufficient tumor suppressor gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions^{[63][64][66]}.

Therapeutic and prognostic relevance

Clinical efficacy of mTOR inhibitors was seen in patients harboring aberrations in the FBXW7 gene (one patient with refractory fibrolamellar hepatocellular carcinoma, and one patient with lung adenocarcinoma)^{[67][68]}. Moreover, in vitro assay also suggested that loss or inactivation of FBXW7 may confer sensitivity to mTOR inhibitor^[62].

Preclinical studies suggested that mutations or loss of FBXW7 were associated with regorafenib and oxaliplatin resistance in CRC cell lines and gefitinib resistance in lung cancer cells^{[69][70][71][72]}.

Retrospective studies have indicated that a relatively low expression level of FBXW7 is an independent prognostic marker of poor survival for patients with hepatocellular carcinoma, lung adenocarcinoma and squamous cell carcinoma^{[73][71]}.

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^{[74][75][76]}. 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^[77]. 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^{[74][78]}. Somatic mutations or deletion of NF2 are frequently observed in human cancers, including 20-50% of pleural mesotheliomas^[79], 6% papillary renal cell carcinoma, 5% pancreas cancer, and 4% melanoma (cbioPortal; June 2015), and less frequently in other cancers^[80].

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^{[81][82][83][84]}. There are at least two case studies indicating the clinical efficacy of everolimus in bladder cancer and urothelial carcinoma^{[85][86]}, both harboring NF2 truncating mutations. Preclinical

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evidence has shown the efficacy of MEK1/2 inhibitor selumetinib in KRAS-mutant thyroid cancer model with NF2 loss^[87].

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^[88].

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^[89]. Inactivation of PTCH1 results in hedgehog ligand-independent activation of SMO, causing a downstream activation of the pathway and lead to the neoplastic growth^{[90][91]}. Recurrent PTCH1 mutations have been reported in sporadic basal cell carcinoma (BCCs) and medulloblastoma^{[92][93][94][95]}. Germline PTCH1 mutations are associated with the nevoid basal cell carcinoma syndrome (NBCCS, Gorlin syndrome), predisposing patients to basal cell carcinoma and medulloblastoma^[93]. PTCH1 is a haploinsufficient tumor suppressor gene with one copy loss may be sufficient to promote tumor development in mice^{[90][96]}.

Therapeutic and prognostic relevance

Vismodegib and sonidegib are small molecule inhibitors of SMO approved by the U.S. FDA for the treatment of patients with basal cell carcinoma^{[97][98][99][100]}. 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^[101]. 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^[102]. In the phase II MyPathway trial, three advanced solid tumors patients harboring PTCH1 loss-of-function mutations had partial responses to vismodegib treatment^[103]. In a clinical study, two patients with Sonic Hedgehog (SHH) activated medulloblastoma harboring PTCH1 loss-of-function mutations demonstrated partial responses to sonidegib treatment^[104].

RAD50 Heterozygous deletion

Biological Impact

The RAD50 gene encodes a highly-conserved DNA double-strand break (DSB) repair factor. It forms MRN complex with NBS1 and MRE11 protein and is involved in sensing and early processing of DSB, cell cycle checkpoints, DNA recombination and maintenance of telomeres^{[105][106]}. Mutations in the components of the MRN complex could increase susceptibility to familial breast cancer^{[107][108]}, gastric cancer^[109], colorectal cancer^[110], and urothelial cancer^[111]. RAD50 has been implicated as a haploinsufficient gene with one copy loss may lead to weak protein expression and is insufficient to execute its original physiological functions^[112]. Besides, RAD50 deletion was also suggested as a marker of BRCAness, a phenotype shared between non-BRCA1/2-mutated ovarian cancers and BRCA1/2-mutated ovarian cancers^[113].

Therapeutic and prognostic relevance

Preclinical data showed that knockdown of the RAD50 gene in ovarian cancer cell lines was significantly associated with better responses to two PARP inhibitors, olaparib and rucaparib^[113]. RAD50 has been selected as an inclusion criterion for the trials examining talazoparib efficacy in HER2-negative breast cancer, olaparib efficacy in breast cancer, rucaparib efficacy in metastatic prostate cancer and niraparib efficacy in any malignancy (except prostate) (NCT02401347, NCT03207347, NCT03344965, NCT03413995).

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RB1 Heterozygous deletion

Biological Impact

The Retinoblastoma (RB1) gene encodes a tumor suppressor that negatively regulates the cell cycle, cell division, and DNA replication^[114]. Loss-of-function RB1 could lead to unregulated cell division and growth, abrogation of multiple mechanisms that safeguard against cellular transformation, and tumorigenesis^[115]. 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^{[116][117][118]}. 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^[119].

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^[120]. High RB loss was found to be associated with improved pathologic clinical response in breast cancer patients treated with 5-fluorouracil/adriamycin/cytosine (FAC), T/FAC, and Taxane/Adriamycin neoadjuvant therapy^[121].

Clinical and experimental data suggested that a non-functional retinoblastoma pathway is associated with resistance to tamoxifen in breast cancer^{[122][123]}.

Acquired RB1 mutations were found in hormone receptor positive breast cancer patients who developed resistance to palbociclib or ribociclib treatment^[124]. Preclinical data also showed that knockdown of RB1 would impair antitumor activity of CDK4/6 inhibitor, abemaciclib^[125].

Two large-scale genome-sequencing projects have identified a high prevalence of mutations in TP53 and RB1 in small cell lung cancer (SCLC)^{[126][127]}. Analyses of repeat biopsy samples from patients with EGFR-mutant adenocarcinoma that had transformed to the SCLC subtype have revealed that 100% of these patients have loss of RB1 and may be the alteration that induces this non-small-cell to small-cell transformation^{[123][128]}.

RICTOR Amplification

Biological Impact

The RICTOR (rapamycin-insensitive companion of mTOR) gene encodes a core component of the mTOR complex-2 (mTOR2) which phosphorylated downstream kinases such as AKT and regulated cell proliferation and survival^{[129][130][131]}. Amplification of the RICTOR locus is observed in melanoma^[132] and lung cancer^[133].

Therapeutic and prognostic relevance

A non-small cell lung cancer (NSCLC) patient harboring RICTOR-amplified tumor achieved stable disease for more than 18 months from the treatment with dual mTOR1/2 inhibitors CC-223 and MLN0128^[133]. Preclinical data showed that a RICTOR-amplified patient-derived cancer cell line is sensitive to mTORC1/2 inhibitor (AZD2014)^[134]. RICTOR amplification and mutation have been determined as an inclusion criterion for the trial examining everolimus efficacy in patients with prostate cancer (NCT03580239).

Several studies have demonstrated that patients with a RICTOR-amplified tumor have lower overall survival in small cell lung cancer, colorectal cancer, and esophageal squamous cell carcinoma^{[135][136][137][138]}. Overexpression of RICTOR is positively associated with tumor progression and poor survival in hepatocellular carcinoma, gastric cancer, pituitary adenoma, and endometrial carcinoma^{[139][140][141][142]}.

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TSC2 Heterozygous deletion

Biological Impact

The tuberous sclerosis complex 2 (TSC2) gene encodes a protein called tuberin, which interact with a protein called hamartin (encoded by the TSC1 gene). This hamartin-tuberin tumor suppressor complex plays a critical role in growth control as a negative regulator of the mammalian target of rapamycin (mTOR) pathway^{[143][144]}. Mutations in TSC1/TSC2 tumor suppressor genes that result in inactivation of the complex are commonly found in patients with tuberous sclerosis complex^{[145][146][147]}, while the loss of heterozygosity (LOH) in TSC1/TSC2 has been identified in head and neck squamous cell carcinoma (HNSCC)^[148] and endometrial cancer^[149]. TSC2 deletion, splicing-mutant, and inactivating mutations such as A1141T, G305V, S1514X, and R1032X, has been identified in TSC2-null hepatocellular carcinoma (HCC) cell lines, patient-derived xenograft, and primary tumors. Mutations in the TSC1 and TSC2 genes cause the autosomal dominant genetic disorder tuberous sclerosis complex (TSC)^[150].

Therapeutic and prognostic relevance

Everolimus is FDA-approved for treating Tuberous Sclerosis Complex (TSC)-associated renal angiomyolipoma and Tuberous Sclerosis Complex (TSC)-associated subependymal giant cell astrocytoma (SEGA).

TSC1/2 mutation/loss has been selected as an inclusion criteria for the trials examining temsirolimus efficacy in multiple cancer types (NCT02693535, NCT03297606).

TSC1/TSC2 genomic alterations activate the mTOR signaling pathway and confer sensitivity to mTOR inhibitors, including everolimus, sirolimus, and temsirolimus. Everolimus is effective in multiple cancers, such as bladder tumors, gastric, sarcoma, thyroid cancer, and HNSCC^{[85][84]}. Sirolimus is effective in treating malignant uterine PEComa with TSC1/TSC2 mutations/deletions^{[151][152][153]}, while temsirolimus is effective in those with hyperactivated mTOR pathway^[154]. In advanced endometrial cancer, TSC1, and TSC2 mutations may predict clinical benefits from temsirolimus with or without megestrol acetate and tamoxifen^[155].

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US FDA-APPROVED DRUG(S)

Afatinib (GILOTRIF)

Afatinib acts as an irreversible covalent inhibitor of the ErbB family of receptor tyrosine kinases, including epidermal growth factor receptor (EGFR) and erbB-2 (HER2). Afatinib is developed and marketed by Boehringer Ingelheim under the trade name GILOTRIF (United States) and GIOTRIF (Europe).

- FDA Approval Summary of Afatinib (GILOTRIF)

| | |
|--|--|
| LUX-Lung 8 ^[156] NCT01523587 | Non-small cell lung carcinoma (Approved on 2016/04/15) |
| | EGFR ex19del or L858R |
| | Afatinib vs. Erlotinib [PFS(M): 2.4 vs. 1.9] |
| LUX-Lung 3 ^[157] NCT00949650 | Non-small cell lung carcinoma (Approved on 2013/07/13) |
| | EGFR ex19del or L858R |
| | Afatinib vs. Pemetrexed + cisplatin [PFS(M): 11.1 vs. 6.9] |

Dacomitinib (VIZIMPRO)

Dacomitinib is an oral kinase inhibitor that targets EGFR. Dacomitinib is developed and marketed by Pfizer under the trade name VIZIMPRO.

- FDA Approval Summary of Dacomitinib (VIZIMPRO)

| | |
|--|--|
| ARCHER 1050 ^[19] NCT01774721 | Non-small cell lung carcinoma (Approved on 2018/09/27) |
| | EGFR ex19del or L858R |
| | Dacomitinib vs. Gefitinib [PFS(M): 14.7 vs. 9.2] |

Erlotinib (TARCEVA)

Erlotinib is a small molecule, reversible inhibitor of epidermal growth factor receptor (EGFR), a receptor tyrosine kinase. Erlotinib is developed by OSI Pharmaceuticals, Genentech and Roche, and marketed by Astellas Pharm Global Development under the trade name TARCEVA.

- FDA Approval Summary of Erlotinib (TARCEVA)

| | |
|--|---|
| RELAY NCT02411448 | Non-small cell lung carcinoma (Approved on 2020/05/29) |
| | EGFR ex19del or L858R |
| | Erlotinib + ramucirumab vs. Erlotinib + placebo [PFS(M): 19.4 vs. 12.4] |
| EURTAC ^[158] NCT00446225 | Non-small cell lung carcinoma (Approved on 2013/05/14) |
| | EGFR ex19del or L858R |
| | Erlotinib vs. Cisplatin + gemcitabine or cisplatin + docetaxel or carboplatin + gemcitabine or carboplatin + docetaxel [PFS(M): 10.4 vs. 5.2] |
| PA.3 ^[159] NCT00026338 | Pancreatic cancer (Approved on 2005/11/02) |
| | - |
| | Gemcitabine vs. Placebo [OS(M): 6.4 vs. 6] |

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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 ^[160] NCT01524783 | Lung or gastrointestinal neuroendocrine tumor (Approved on 2016/02/26) |
| | - Everolimus vs. Placebo [PFS(M): 11 vs. 3.9] |
| BOLERO-2 ^[161] NCT00863655 | Breast cancer (Approved on 2012/07/20) |
| | ER+/HER2- Everolimus + exemestane vs. Placebo + exemestane [PFS(M): 7.8 vs. 3.2] |
| EXIST-2 NCT00790400 | Tuberous sclerosis complex (tsc)-associated renal angiomyolipoma (Approved on 2012/04/26) |
| | - Everolimus vs. Placebo [ORR(%): 41.8 vs. 0] |
| RADIANT-3 ^[162] NCT00510068 | Pancreatic neuroendocrine tumor (Approved on 2011/05/05) |
| | - Everolimus vs. Placebo [PFS(M): 11 vs. 4.6] |
| EXIST-1 ^[163] NCT00789828 | Subependymal giant cell astrocytoma (Approved on 2010/10/29) |
| | - Everolimus vs. Placebo [ORR(%): 35.0] |
| RECORD-1 ^[164] NCT00410124 | Renal cell carcinoma (Approved on 2009/05/30) |
| | - Everolimus vs. Placebo [PFS(M): 4.9 vs. 1.9] |

Gefitinib (IRESSA)

Gefitinib is a small molecule inhibitor of epidermal growth factor receptor (EGFR), a receptor tyrosine kinase. Gefitinib is developed and marketed by AstraZeneca under the trade name IRESSA.

- FDA Approval Summary of Gefitinib (IRESSA)

| | |
|---|---|
| IFUM ^[165] NCT01203917 | Non-small cell lung carcinoma (Approved on 2015/07/13) |
| | EGFR ex19del or L858R |
| | Gefitinib [ORR(%): 50.0] |

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)

| | |
|-----------------------------|---|
| PRIMA NCT02655016 | Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma (Approved on 2020/04/29) |
| | - Niraparib vs. Placebo [PFS (overall population)(M): 13.8 vs. 8.2] |

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| | |
|--------------------------------------|--|
| NOVA ^[166] NCT01847274 | Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma (Approved on 2017/03/27) |
| | - |
| | Niraparib vs. Placebo [PFS (overall population)(M): 11.3 vs. 4.7] |

Olaparib (LYNPARZA)

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

- FDA Approval Summary of Olaparib (LYNPARZA)

| | |
|---|---|
| PROpel NCT03732820 | Prostate cancer (Approved on 2023/05/31) |
| | BRCA mutation |
| | Olaparib + abiraterone + prednisone vs. Placebo + abiraterone + prednisone [rPFS(M): not reached vs. 8] |
| OlympiA NCT02032823 | Her2-negative high-risk early breast cancer (Approved on 2022/03/11) |
| | HER2-/gBRCA mutation |
| | Olaparib vs. Placebo [invasive disease-free survival (IDFS)(M):] |
| PROfound ^[167] NCT02987543 | Prostate cancer (Approved on 2020/05/19) |
| | HRR genes mutation |
| | Olaparib vs. Enzalutamide or abiraterone acetate [PFS(M): 5.8 vs. 3.5] |
| PAOLA-1 ^[168] NCT02477644 | Ovarian cancer (Approved on 2020/05/08) |
| | HRD+ |
| | Olaparib + bevacizumab vs. Placebo + bevacizumab [PFS(M): 37.2 vs. 17.7] |
| POLO ^[169] NCT02184195 | Pancreatic adenocarcinoma (Approved on 2019/12/27) |
| | gBRCA mutation |
| | Olaparib vs. Placebo [ORR(%): 23.0 vs. 12.0, PFS(M): 7.4 vs. 3.8] |
| SOLO-1 ^[170] NCT01844986 | Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma (Approved on 2018/12/19) |
| | gBRCA mutation or sBRCA mutation |
| | Olaparib vs. Placebo [PFS(M): NR vs. 13.8] |
| OlympiAD ^[171] NCT02000622 | Breast cancer (Approved on 2018/02/06) |
| | HER2-/gBRCA mutation |
| | Olaparib vs. Chemotherapy [PFS(M): 7 vs. 4.2] |
| SOLO-2/ENGOT-Ov21 ^[172] NCT01874353 | Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma (Approved on 2017/08/17) |
| | gBRCA mutation |
| | Olaparib vs. Placebo [PFS(M): 19.1 vs. 5.5] |
| Study19 ^[173] NCT00753545 | Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma (Approved on 2017/08/17) |
| | - |
| | Olaparib vs. Placebo [PFS(M): 8.4 vs. 4.8] |

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Osimertinib (TAGRISSO)

Osimertinib is a third-generation tyrosine kinase inhibitor (TKI) for patients with tumors harboring EGFR T790M mutation. Osimertinib is developed and marketed by AstraZeneca under the trade name TAGRISSO.

- FDA Approval Summary of Osimertinib (TAGRISSO)

| | |
|---|---|
| ADAURA NCT02511106 | Non-small cell lung carcinoma (Approved on 2020/12/18) |
| | EGFR ex19del or L858R |
| | Osimertinib vs. Placebo + adjuvant chemotherapy [DFS(M): NR vs. 19.6] |
| FLAURA^[21] NCT02296125 | Non-small cell lung carcinoma (Approved on 2018/04/18) |
| | EGFR ex19del or L858R |
| | Osimertinib vs. Gefitinib or erlotinib [PFS(M): 18.9 vs. 10.2] |
| AURA3^[174] NCT02151981 | Non-small cell lung carcinoma (Approved on 2017/03/30) |
| | EGFR T790M |
| | Osimertinib vs. Chemotherapy [PFS(M): 10.1 vs. 4.4] |
| AURA^[175] NCT01802632 | Non-small cell lung carcinoma (Approved on 2015/11/13) |
| | EGFR T790M |
| | Osimertinib [ORR(%): 59.0] |

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 mutation or sBRCA mutation |
| | Rucaparib [ORR(%): 44.0, DOR(M): NE] |
| ARIEL3^[176] NCT01968213 | Ovarian cancer, Fallopian tube cancer, Peritoneal carcinoma (Approved on 2018/04/06) |
| | - |
| | Rucaparib vs. Placebo [PFS (All)(M): 10.8 vs. 5.4, PFS (HRD)(M): 13.6 vs. 5.4, PFS (tBRCA)(M): 16.6 vs. 5.4] |

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^[99] NCT01327053 | Basal cell carcinoma (Approved on 2015/07/24) |
| | - |
| | Sonidegib [ORR(%): 58.0] |

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Talazoparib (TALZENNA)

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

- FDA Approval Summary of Talazoparib (TALZENNA)

| | |
|---|--|
| EMBRACA ^[177] NCT01945775 | Breast cancer (Approved on 2018/10/16) |
| | HER2-/gBRCA mutation |
| | Talazoparib vs. Chemotherapy [PFS(M): 8.6 vs. 5.6] |

Temsirolimus (TORISEL)

Temsirolimus is a soluble ester of sirolimus (rapamycin, brand-name drug Rapamune) and functions as an inhibitor of mammalian target of rapamycin complex (mTORC). The inhibitory molecular mechanism is similar to Everolimus. Temsirolimus is developed by Wyeth Pharmaceuticals and marketed by Pfizer under the trade name TORISEL.

- FDA Approval Summary of Temsirolimus (TORISEL)

| | |
|----------------------|--|
| [178] NCT00065468 | Renal cell carcinoma (Approved on 2007/05/30) |
| | - |
| | Temsirolimus vs. Ifn- α [OS(M): 10.9 vs. 7.3] |

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 ^[97] NCT00833417 | Basal cell carcinoma (Approved on 2012/01/30) |
| | - |
| | Vismodegib [ORR (mBCC)(%): 30.3, ORR (laBCC)(%): 42.9] |

D=day; W=week; M=month

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ONGOING CLINICAL TRIALS

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

No trial has been found.

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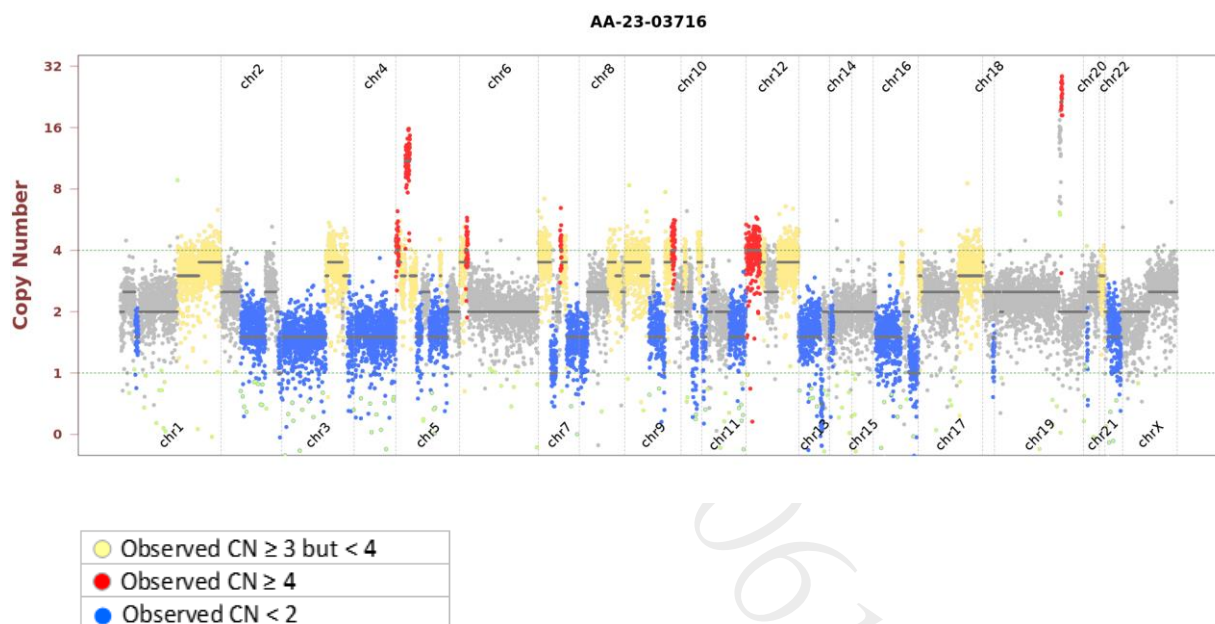
SUPPLEMENTARY INFORMATION OF TESTING RESULTS DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

- Single Nucleotide and Small InDel Variants

| Gene | Amino Acid Change | Exon | cDNA Change | Accession Number | COSMIC ID | Allele Frequency | Coverage |
|-------|------------------------------------|------|----------------|------------------|-----------|------------------|----------|
| ARID2 | Q1463* | 15 | c.4387C>T | NM_152641 | - | 32.7% | 2117 |
| EGFR | L747_T751del (Exon 19 deletion) | 19 | c.2240_2254del | NM_005228 | COSM12369 | 54.5% | 3287 |
| TP53 | E204* | 6 | c.610G>T | NM_000546 | COSM10804 | 94.1% | 1076 |

- Copy Number Alterations

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



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OTHER DETECTED VARIANTS

| Gene | Amino Acid Change | Exon | cDNA Change | Accession Number | COSMIC ID | Allele Frequency | Coverage |
|----------|-------------------|------|----------------------|------------------|-------------|------------------|----------|
| ABL1 | A945T | 11 | c.2833G>A | NM_005157 | - | 12.5% | 537 |
| ADAMTS16 | R820Q | 16 | c.2459G>A | NM_139056 | COSM8832362 | 40.0% | 873 |
| ATRX | S1697R | 19 | c.5091T>A | NM_000489 | - | 26.4% | 1567 |
| AXL | W448L | 11 | c.1343G>T | NM_021913 | - | 57.4% | 747 |
| BLM | M348I | 5 | c.1044G>A | NM_000057 | COSM1580597 | 95.4% | 974 |
| BRIP1 | N196S | 6 | c.587A>G | NM_032043 | COSM249456 | 96.5% | 1273 |
| CCNE1 | Splice region | - | c.1110+10_1110+12del | NM_001238 | - | 76.8% | 164 |
| CIC | Splice region | - | c.3175+3G>A | NM_015125 | - | 50.3% | 451 |
| DDR2 | T681I | 15 | c.2042C>T | NM_006182 | COSM2088236 | 55.4% | 1931 |
| ERCC2 | G36S | 3 | c.106G>A | NM_000400 | - | 58.4% | 793 |
| MSH2 | L719F | 13 | c.2157G>C | NM_000251 | - | 33.6% | 1164 |
| MUC16 | G4979R | 3 | c.14935G>C | NM_024690 | COSM9264561 | 15.2% | 1576 |
| NOTCH2 | Splice region | - | c.2480-4C>G | NM_024408 | - | 57.6% | 794 |
| NOTCH2 | L744V | 14 | c.2230C>G | NM_024408 | - | 42.0% | 721 |
| PALB2 | F164C | 4 | c.491T>G | NM_024675 | - | 64.9% | 362 |
| PRDM1 | S367F | 5 | c.1100C>T | NM_001198 | - | 94.7% | 678 |
| PRKDC | S3365N | 72 | c.10094G>A | NM_006904 | - | 41.8% | 1324 |
| PTCH1 | N584Y | 13 | c.1750A>T | NM_000264 | - | 5.6% | 944 |
| PTPN11 | R527C | 13 | c.1579C>T | NM_002834 | COSM935284 | 36.2% | 1485 |
| SMO | T179M | 2 | c.536C>T | NM_005631 | COSM6927006 | 65.0% | 1498 |
| VEGFA | N321S | 5 | c.962A>G | NM_001025368 | COSM6935311 | 61.5% | 546 |

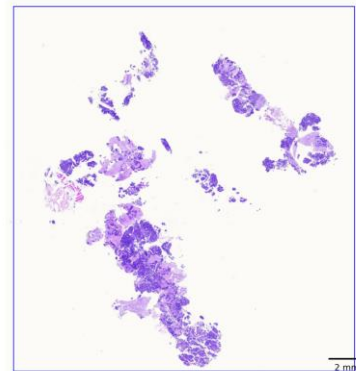
Note:

- This table enlists variants detected by the panel other than those with clinical relevance (reported in Testing Result section). The clinical impact of a genetic variant is determined according to ACT Genomics in-house clinical knowledge database. A negative result does not necessarily indicate absence of biological effect on the tumor. Some variants listed here may possibly have preclinical data or may show potential clinical relevance in the future.

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TEST DETAILS

SPECIMEN RECEIVED AND PATHOLOGY REVIEW



- Collection date: Jun 01, 2023
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11225772A
- Collection site: Lymph node
- Examined by: Dr. Yun-An Chen
 1. The percentage of viable tumor cells in total cells in the whole slide (%): 80%
 2. The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 80%
 3. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
 4. The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
 5. Additional comment: NA
- Manual macrodissection: Not performed
- The outline highlights the area of malignant neoplasm annotated by a pathologist.

RUN QC

- Panel: ACTOnco[®]+

DNA test

- Mean Depth: 972x
- Target Base Coverage at 100x: 95%

RNA test

- Average unique RNA Start Sites per control GSP2: 193

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LIMITATIONS

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

NEXT-GENERATION SEQUENCING (NGS) METHODS

DNA test

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

Raw reads generated by the sequencer were mapped to the hg19 reference genome using the Ion Torrent Suite. Coverage depth was calculated using Torrent Coverage Analysis plug-in. Single nucleotide variants (SNVs) and short insertions/deletions (InDels) were identified using the Torrent Variant Caller plug-in. VEP (Variant Effect Predictor) was used to annotate every variant using databases from Clinvar, COSMIC and Genome Aggregation database. Variants with coverage ≥ 20 , allele frequency $\geq 5\%$ and actionable variants with allele frequency $\geq 2\%$ were retained. This test provides uniform coverage of the targeted regions, enabling target base coverage at $100\times \geq 85\%$ with a mean coverage $\geq 500\times$.

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

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

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

Tumor mutational burden (TMB) was calculated by using the sequenced regions of ACTOnco[®] 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).

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RNA test

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

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

DATABASE USED

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

Variant Analysis:

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



Sign Off

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



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GENE LIST SNV & CNV

| | | | | | | | | | | | |
|----------|---------|---------|----------|----------|---------|-----------|-----------|----------|----------|----------|----------|
| ABCB1* | ABCC2* | ABCG2* | ABL1 | ABL2 | ADAMTS1 | ADAMTS13 | ADAMTS15 | ADAMTS16 | ADAMTS18 | ADAMTS6 | ADAMTS9 |
| ADAMTSL1 | ADGRA2 | ADH1C* | AKT1 | AKT2 | AKT3 | ALDH1A1* | ALK | AMER1 | APC | AR | ARAF |
| ARID1A | ARID1B | ARID2 | ASXL1 | ATM | ATR | ATRX | AURKA | AURKB | AXIN1 | AXIN2 | AXL |
| B2M | BAP1 | BARD1 | BCL10 | BCL2* | BCL2L1 | BCL2L2* | BCL6 | BCL9 | BCOR | BIRC2 | BIRC3 |
| BLM | BMP1R1A | BRAF | BRCA1 | BRCA2 | BRD4 | BRIP1 | BTG1 | BTG2* | BTX | BUB1B | CALR |
| CANX | CARD11 | CASP8 | CBFB | CBL | CCNA1 | CCNA | CCNB1 | CCNB2 | CCNB3 | CCND1 | CCND2 |
| CCND3 | CCNE1 | CCNE2 | CCNH | CD19 | CD274 | CD58 | CD70* | CD79A | CD79B | CDC73 | CDH1 |
| CDK1 | CDK12 | CDK2 | CDK4 | CDK5 | CDK6 | CDK7 | CDK8 | CDK9 | CDKN1A | CDKN1B | CDKN2A |
| CDKN2B | CDKN2C | CEBPA* | CHEK1 | CHEK2 | CIC | CREBBP | CRKL | CRLF2 | CSF1R | CTCF | CTLA4 |
| CTNNA1 | CTNNB1 | CUL3 | CYLD | CYP1A1* | CYP2B6* | CYP2C19* | CYP2C8* | CYP2D6 | CYP2E1* | CYP3A4* | CYP3A5* |
| DAXX | DCUN1D1 | DDR2 | DICER1 | DNMT3A | DOT1L | DPYD | DTX1 | E2F3 | EGFR | EP300 | EPCAM |
| EPHA2 | EPHA3 | EPHA5 | EPHA7 | EPHB1 | ERBB2 | ERBB3 | ERBB4 | ERCC1 | ERCC2 | ERCC3 | ERCC4 |
| ERCC5 | ERG | ESR1 | ESR2 | ETV1 | ETV4 | EZH2 | FAM46C | FANCA | FANCC | FANCD2 | FANCE |
| FANCF | FANCG | FANCL | FAS | FAT1 | FBXW7 | FCGR2B | FGF1* | FGF10 | FGF14 | FGF19* | FGF23 |
| FGF3 | FGF4* | FGF6 | FGFR1 | FGFR2 | FGFR3 | FGFR4 | FH | FLCN | FLT1 | FLT3 | FLT4 |
| FOXL2* | FOXP1 | FRG1 | FUBP1 | GATA1 | GATA2 | GATA3 | GNA11 | GNA13 | GNAQ | GNAS | GREM1 |
| GRIN2A | GSK3B | GSTP1* | GSTT1* | HGF | HIF1A | HIST1H1C* | HIST1H1E* | HNF1A | HR | HRAS* | HSP90AA1 |
| HSP90AB1 | HSPA4 | HSPA5 | IDH1 | IDH2 | IFNL3* | IGF1 | IGF1R | IGF2 | IKBKB | IKBKE | IKZF1 |
| IL6 | IL7R | INPP4B | INSR | IRF4 | IRS1 | IRS2* | JAK1 | JAK2 | JAK3 | JUN* | KAT6A |
| KDM5A | KDM5C | KDM6A | KDR | KEAP1 | KIT | KMT2A | KMT2C | KMT2D | KRAS | LCK | LIG1 |
| LIG3 | LMO1 | LRP1B | LYN | MALT1 | MAP2K1 | MAP2K2 | MAP2K4 | MAP3K1 | MAP3K7 | MAPK1 | MAPK3 |
| MAX | MCL1 | MDM2 | MDM4 | MED12 | MEF2B | MEN1 | MET | MITF | MLH1 | MPL | MRE11 |
| MSH2 | MSH6 | MTHFR* | MTOR | MUC16 | MUC4 | MUC6 | MUTYH | MYC | MYCL | MYCN | MYD88 |
| NAT2* | NBN | NEFH | NF1 | NF2 | NFE2L2 | NFKB1 | NFKBIA | NKX2-1* | NOTCH1 | NOTCH2 | NOTCH3 |
| NOTCH4 | NPM1 | NQO1* | NRAS | NSD1 | NTRK1 | NTRK2 | NTRK3 | PAK3 | PALB2 | PARP1 | PAX5 |
| PAX8 | PBRM1 | PDCD1 | PDCD1LG2 | PDGFRA | PDGFRB | PDIA3 | PGF | PHOX2B* | PIK3C2B | PIK3C2G | PIK3C3 |
| PIK3CA | PIK3CB | PIK3CD | PIK3CG | PIK3R1 | PIK3R2 | PIK3R3 | PIM1 | PMS1 | PMS2 | POLB | POLD1 |
| POLE | PPARG | PPP2R1A | PRDM1 | PRKAR1A | PRKCA | PRKCB | PRKCG | PRKCI | PRKCQ | PRKDC | PRKN |
| PSMB8 | PSMB9 | PSME1 | PSME2 | PSME3 | PTCH1 | PTEN | PTGS2 | PTPN11 | PTPRD | PTPRT | RAC1 |
| RAD50 | RAD51 | RAD51B | RAD51C | RAD51D | RAD52 | RAD54L | RAF1 | RARA | RB1 | RBM10 | RECQL4 |
| REL | RET | RHOA | RICTOR | RNF43 | ROS1 | RPPH1 | RPTOR | RUNX1 | RUNX1T1 | RXRA | SDHA |
| SDHB | SDHC | SDHD | SERPINB3 | SERPINB4 | SETD2 | SF3B1 | SGK1 | SH2D1A* | SLC19A1* | SLC22A2* | SLC1B1* |
| SLC1B3* | SMAD2 | SMAD3 | SMAD4 | SMARCA4 | SMARCB1 | SMO | SOC1* | SOX2* | SOX9 | SPEN | SPOP |
| SRC | STAG2 | STAT3 | STK11 | SUFU | SYK | SYNE1 | TAF1 | TAP1 | TAP2 | TAPBP | TBX3 |
| TEK | TERT | TET1 | TET2 | TGFBR2 | TMSB4X* | TNF | TNFAIP3 | TNFRSF14 | TNFSF11 | TOP1 | TP53 |
| TPMT* | TSC1 | TSC2 | TSHR | TYMS | U2AF1 | UBE2A* | UBE2K | UBR5 | UGT1A1* | USH2A | VDR* |
| VEGFA | VEGFB | VHL | WT1 | XIAP | XPO1 | XRCC2 | ZNF217 | | | | |

*Analysis of copy number alterations NOT available.

FUSION

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

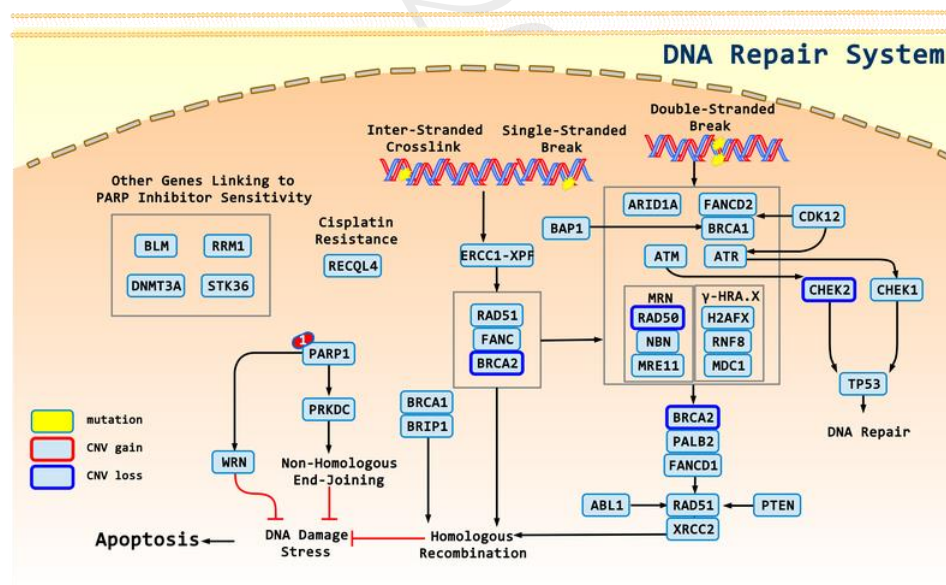
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APPENDIX

POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

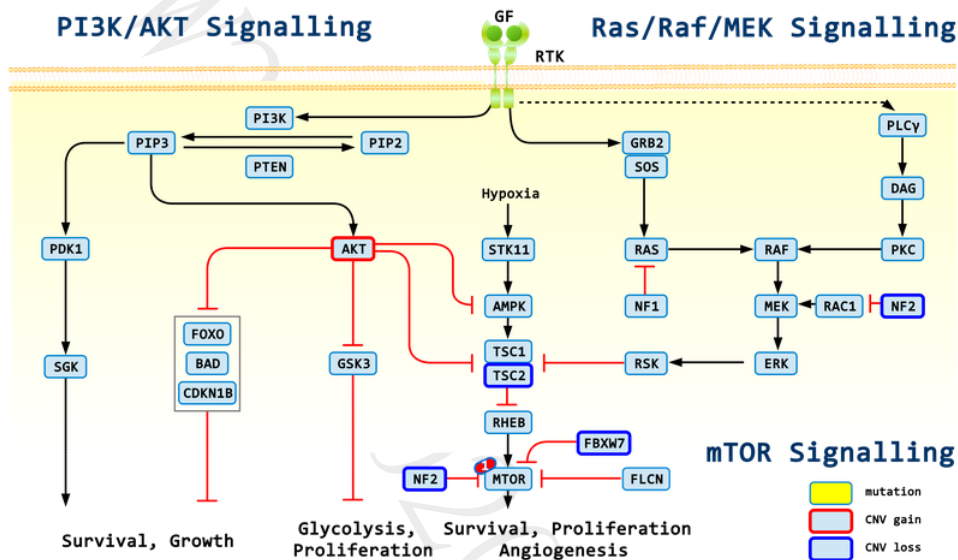
| Gene | Therapies | Possible effect |
|--------------|---|------------------|
| <i>FBXW7</i> | Everolimus, Temsirolimus | sensitive |
| <i>NF2</i> | Everolimus, Temsirolimus | sensitive |
| <i>TSC2</i> | Everolimus, Temsirolimus | sensitive |
| <i>BRCA2</i> | Niraparib, Olaparib, Rucaparib, Talazoparib | sensitive |
| <i>CHEK2</i> | Niraparib, Olaparib, Rucaparib, Talazoparib | sensitive |
| <i>RAD50</i> | Niraparib, Olaparib, Rucaparib, Talazoparib | sensitive |
| <i>PTCH1</i> | Sonidegib, Vismodegib | sensitive |
| <i>RB1</i> | Abemaciclib, Palbociclib, Ribociclib | resistant |
| <i>FBXW7</i> | Gefitinib, Regorafenib | resistant |

SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS

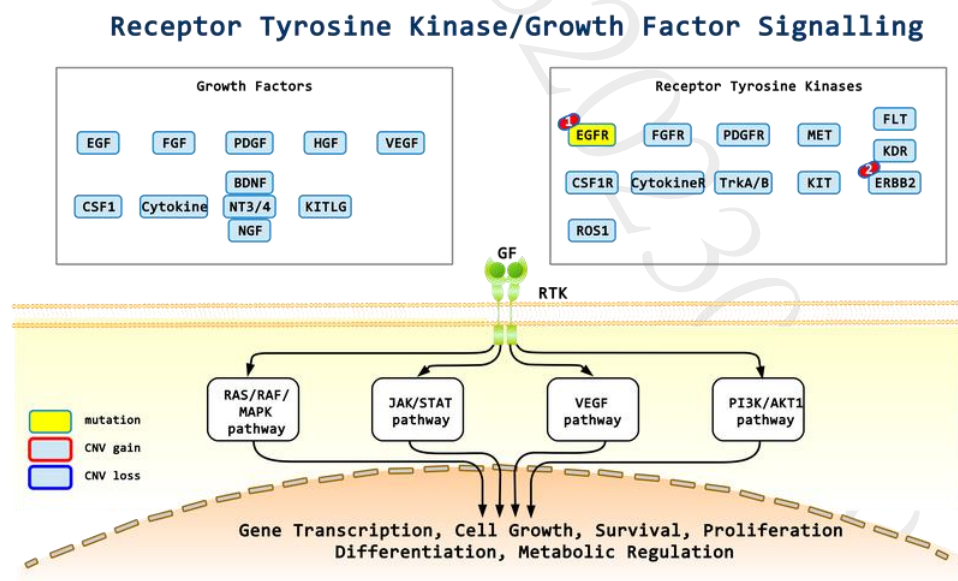


1: Olaparib, Niraparib, Rucaparib, Talazoparib

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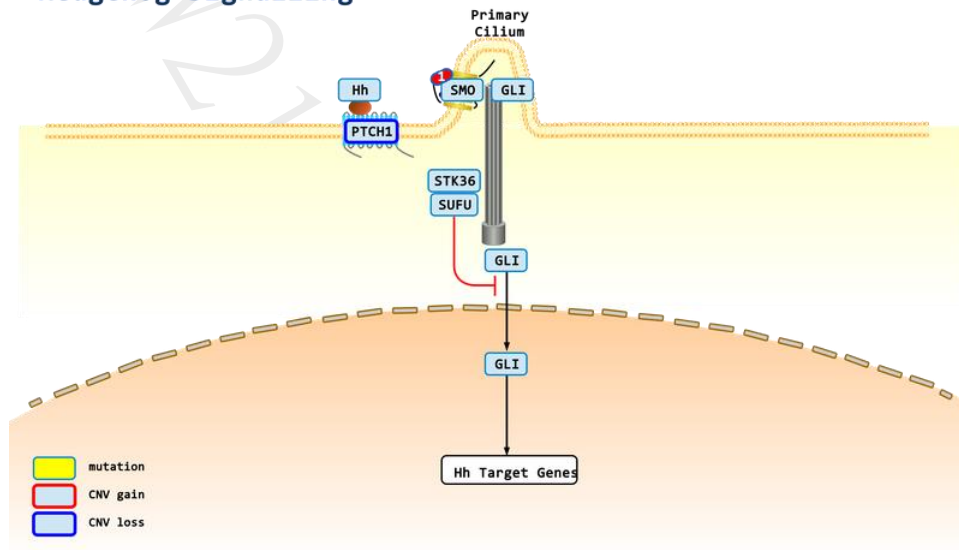
1: Everolimus, Temsirolimus



1: Gefitinib, Afatinib, Erlotinib, Osimertinib, Dacomitinib; 2: Afatinib

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Hedgehog Signalling



1: Sonidegib, Vismodegib

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DISCLAIMER

法律聲明

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

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

醫療決策需由醫師決定

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

基因突變與用藥資訊並非依照有效性排序

本報告中列出之生物標記變異與藥物資訊並非依照潛在治療有效性排序。

證據等級

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

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