ACT Onco® + Report

PATIENT		
Identifier: 游翔育		Patient ID: 42744810
Date of Birth: Apr 13, 1976		Gender: Female
Diagnosis: Lung adenocarcinoma		
ORDERING PHYSICIAN		
Name: 趙恒勝醫師		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: S11221175A	Collection site: Neck	Type: FFPE tissue
Date received: May 16, 2023	Lab ID: AA-23-03061	D/ID: NA

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 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_T751delinsP (Exon 19 deletion)	Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib	-	-

VARIANTS/BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Genomic Alterations/Biomarkers	Possibly Sensitive	Possibly Resistant
EGFR L747_T751delinsP (Exon 19 deletion)	-	Cetuximab
SMAD4 P198fs	-	Cetuximab

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 criterial 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
EGFR	L747_T751delinsP (Exon 19 deletion)	25.8%
SMAD4	P198fs	13.6%
TP53	H193R	15.2%

- Copy Number Alterations

Chromosome	Gene	Variation	Copy Number
Not detected			

- 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 60% tumor purity.
- 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 1		
EGFR L747_T751delinsP (Exon 19 deletion)	Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib	**sensitive**
Level 4		
EGFR L747_T751delinsP (Exon 19 deletion)	Cetuximab	**resistant**
SMAD4 P198fs	Cetuximab	**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

Genomic Alterations	Therapies	Effect	Level of Evidence	Cancer Type
SMAD4 P198fs	Fluorouracil	**Resistant**	Clinical	Colorectal cancer

HORMONAL THERAPIES

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

OTHERS

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

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

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VARIANT INTERPRETATION

EGFR L747_T751delinsP (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[1]. 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[2]. 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[3]. On the other hand, in the brain and colorectal cancers, the most prevalent EGFR alteration is copy number amplification that results in receptor overexpression[4].

EGFR L747_T751delinsP (exon 19 deletion) lies within the tyrosine kinase domain of the EGFR protein, resulting in a deletion of 5 amino acids from residues 747 to 751 and insertion of a proline residue (UniProtKB). Despite not being previously characterized, L747_T751delinsP is predicted to lead to a gain of function, given other exon 19 deletions and insertions in the EGFR protein are activating[5][6].

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[7].

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[8](Annals of Oncology (2017) 28 (suppl_5): v403-v427. 10.1093/annonc/mdx376).

In a retrospective study, 3 patients with lung adenocarcinoma harboring EGFR L747_T751delinsP had partial responses to gefitinib treatment[9]. In a preclinical study, transformed cells expressing EGFR L747_T751delinsP were sensitive to afatinib, erlotinib, gefitinib and osimertinib, but resistant to cetuximab treatment in vitro[10].

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[11]. 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[12]. 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[13].

SMAD4 P198fs

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[14]. 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[15]. SMAD4 germline mutations are associated with juvenile polyposis syndrome (JPS)[16][17][18][19]. Somatic mutations of SMAD4 are commonly observed in pancreatic cancer[20], colorectal cancer (CRC)[18][21][22], and less frequently seen in other cancers such as lung adenocarcinoma[23], head and neck cancer[24][25], and cutaneous squamous cell carcinoma[26].

P198fs mutation results in a change in the amino acid sequence beginning at 198, likely to cause premature truncation of the functional SMAD4 protein (UniProtKB). This mutation is predicted to lead to a loss of SMAD4 protein function, despite not being characterized in the literature.

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[27]. 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[28].

SMAD4 is also suggested as a predictive marker for 5-fluorouracil-based chemotherapy in colorectal cancer (CRC)[29][30]. 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[31].

Results from clinical and meta-analyses showed that loss of SMAD4 in CRC, pancreatic cancer was correlated with poor prognosis[32][33][34][35][36][37][38][39]. 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[40].

TP53 H193R

Biological Impact

TP53 encodes the p53 protein, a crucial tumor suppressor that orchestrates essential cellular processes including cell cycle arrest, senescence and apoptosis[41]. TP53 is a proto-typical haploinsufficient gene, such that loss of a single copy of TP53 can result in tumor formation[42].

TP53 H193R lies within the DNA-binding domain of the p53 protein (UniProtKB). H193R confers a loss of function to the p53 protein as demonstrated by decreased p53 transactivation activity in vitro[43].

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)[44].

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[45]. Results from another Phase I trial of advanced solid tumors (n=78) demonstrated that TP53 hotspot

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mutations are associated with better clinical response to the combination of pazopanib and vorinostat[46].

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[47][48][49]. 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)[50]. TP53 mutations were correlated with poor survival of advanced breast cancer patients receiving tamoxifen or primary chemotherapy[51][52]. 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[53].

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[54] 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[55] 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[11] 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[56] 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[57] NCT00026338	**Pancreatic cancer** (Approved on 2005/11/02)
	-
	Gemcitabine vs. Placebo [OS(M): 6.4 vs. 6]

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

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[13] 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[59] NCT02151981	**Non-small cell lung carcinoma** (Approved on 2017/03/30)
	EGFR T790M
	Osimertinib vs. Chemotherapy [PFS(M): 10.1 vs. 4.4]
AURA[60] NCT01802632	**Non-small cell lung carcinoma** (Approved on 2015/11/13)
	EGFR T790M
	Osimertinib [ORR(%): 59.0]

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

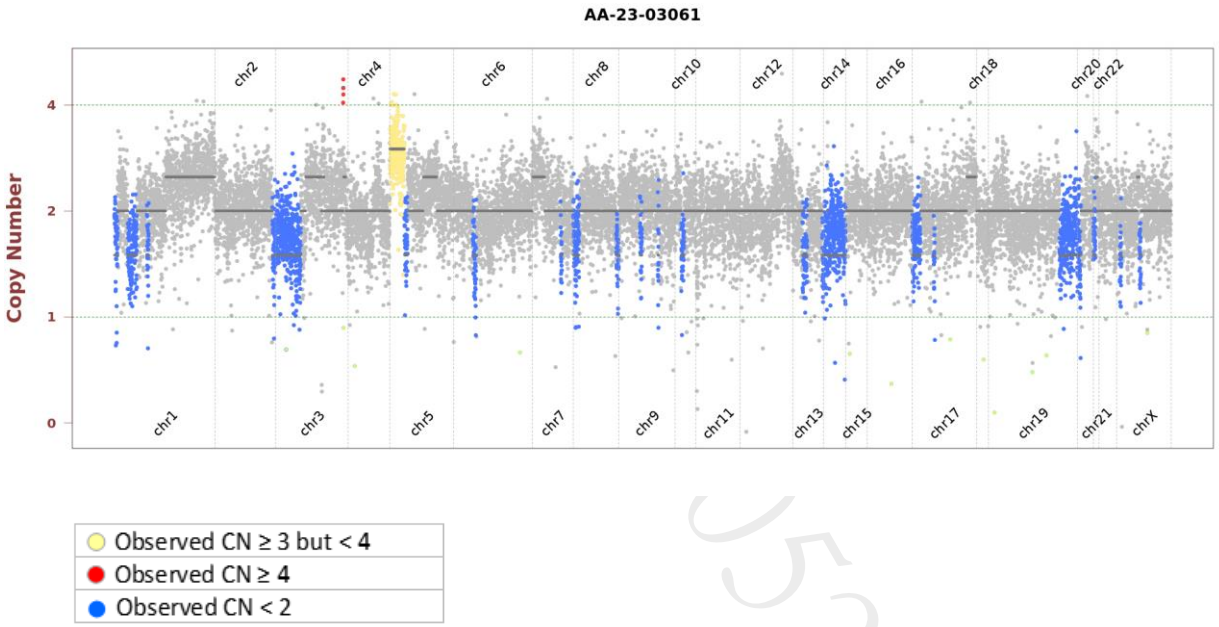
DETAILED INFORMATION OF VARIANTS WITH CLINICAL RELEVANCE

- Single Nucleotide and Small InDel Variants

Gene	Amino Acid Change	Exon	cDNA Change	Accession Number	COSMIC ID	Allele Frequency	Coverage
EGFR	L747_T751delinsP (Exon 19 deletion)	19	c.2239_2251delinsC	NM_005228	COSM12383	25.8%	2394
SMAD4	P198fs	5	c.593del	NM_005359	-	13.6%	2398
TP53	H193R	6	c.578A>G	NM_000546	COSM10742	15.2%	698

- 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
APC	S2469F	16	c.7406C>T	NM_000038	COSM6284583	20.2%	1432
DICER1	R1368H	22	c.4103G>A	NM_177438	COSM8528483	54.8%	881
DOT1L	V467A	15	c.1400T>C	NM_032482	-	47.0%	1527
FGFR3	A429T	10	c.1285G>A	NM_000142	-	58.2%	1172
MET	Splice region	-	c.2637+6A>G	NM_001127500	-	51.7%	908
MSH6	K453R	4	c.1358A>G	NM_000179	-	50.2%	1455
MUTYH	Splice region	-	c.389-7C>T	NM_001128425	-	50.7%	685
PIK3C2B	D1230E	26	c.3690C>A	NM_002646	-	11.0%	2025
PTPRD	L1490V	35	c.4468C>G	NM_002839	-	5.9%	1984
RBM10	N392S	12	c.1175A>G	NM_005676	-	49.5%	705
REL	T433I	11	c.1298C>T	NM_002908	-	43.1%	357
SYNE1	D2068E	42	c.6204T>G	NM_182961	-	47.9%	1222
SYNE1	M5634T	89	c.16901T>C	NM_182961	-	53.0%	589
TET1	E2069K	12	c.6205G>A	NM_030625	-	11.6%	387

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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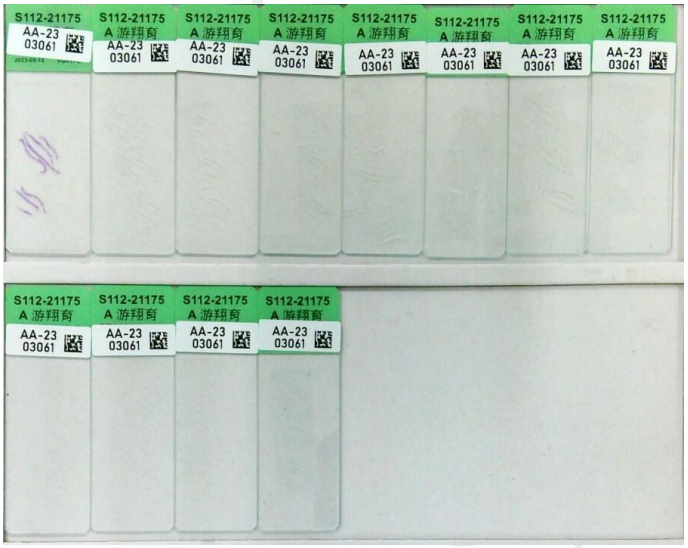
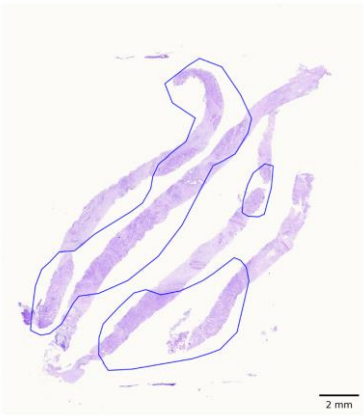
TEST DETAILS

SPECIMEN RECEIVED AND PATHOLOGY REVIEW





- Collection date: May 10, 2023
- Facility retrieved: 臺北榮總
- H&E-stained section No.: S11221175A
- Collection site: Neck
- Examined by: Dr. Chien-Ta Chiang
    1. The percentage of viable tumor cells in total cells in the whole slide (%): 60%
    2. The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 70%
    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: 1049x
- Target Base Coverage at 100x: 95%

RNA test

- Average unique RNA Start Sites per control GSP2: 177

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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 ≥ 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 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).

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.

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

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

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

Yun Yu Chen

Sign Off
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	BMPR1A	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTG2*	BTK	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*	SLCO1B1*
SLCO1B3*	SMAD2	SMAD3	SMAD4	SMARCA4	SMARCB1	SMO	SOCS1*	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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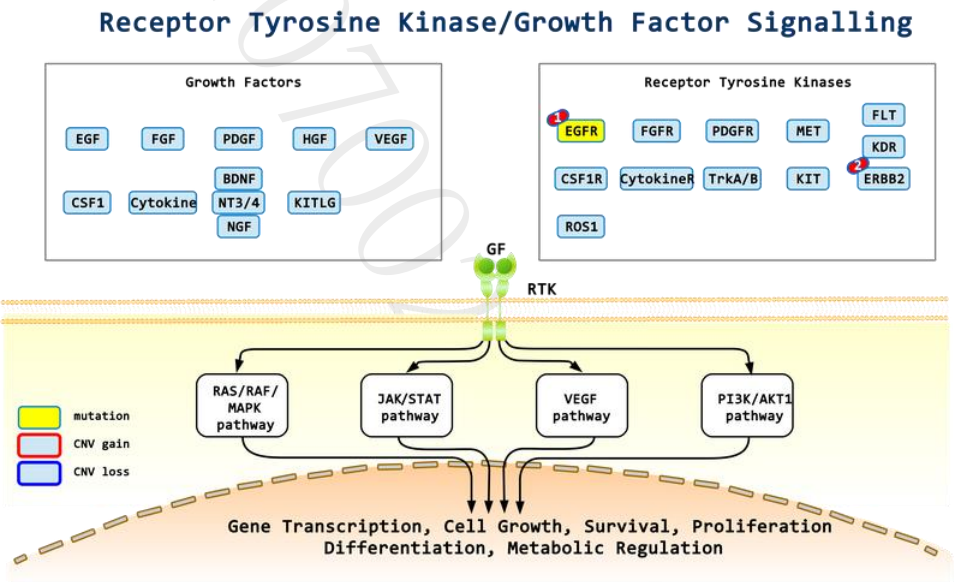
APPENDIX

POSSIBLE THERAPEUTIC IMPLICATIONS FOR HETEROZYGOUS DELETION

Not Applicable.

SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS



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

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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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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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REFERENCE

1. PMID: 18045542; 2007, Cell;131(5):1018
SnapShot: EGFR signaling pathway.

2. PMID: 10880430; 2000, EMBO J;19(13):3159-67
The ErbB signaling network: receptor heterodimerization in development and cancer.

3. PMID: 15329413; 2004, Proc Natl Acad Sci U S A;101(36):13306-11
EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib.

4. PMID: 11426640; 2000, Oncogene;19(56):6550-65
The EGF receptor family as targets for cancer therapy.

5. PMID: 19147750; 2009, Clin Cancer Res;15(2):460-7
Functional analysis of epidermal growth factor receptor (EGFR) mutations and potential implications for EGFR targeted therapy.

6. PMID: 23387505; 2013, Cancer Sci;104(5):584-9
Sensitivity and kinase activity of epidermal growth factor receptor (EGFR) exon 19 and others to EGFR-tyrosine kinase inhibitors.

7. PMID: 22263017; 2010, J Thorac Dis;2(1):48-51
Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update.

8. PMID: 28351930; 2017, Clin Cancer Res;23(15):4242-4250
Hyperprogressors after Immunotherapy: Analysis of Genomic Alterations Associated with Accelerated Growth Rate.

9. PMID: 18981003; 2008, Clin Cancer Res;14(21):7060-7
Effects of erlotinib in EGFR mutated non-small cell lung cancers with resistance to gefitinib.

10. PMID: 29141884; 2017, Sci Transl Med;9(416):
A method of high-throughput functional evaluation of EGFR gene variants of unknown significance in cancer.

11. PMID: 28958502; 2017, Lancet Oncol;18(11):1454-1466
Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial.

12. PMID: 29653820; 2018, Clin Lung Cancer;19(4):e465-e479
Afatinib as First-line Treatment of Older Patients With EGFR Mutation-Positive Non-Small-Cell Lung Cancer: Subgroup Analyses of the LUX-Lung 3, LUX-Lung 6, and LUX-Lung 7 Trials.

13. PMID: 29151359; 2018, N Engl J Med;378(2):113-125
Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer.

14. PMID: 25935112; 2015, Trends Biochem Sci;40(6):296-308
Structural determinants of Smad function in TGF-β signaling.

15. PMID: 19014666; 2008, Pathogenetics;1(1):2
Smad4 haploinsufficiency: a matter of dosage.

16. PMID: 9545410; 1998, Am J Hum Genet;62(5):1129-36
A gene for familial juvenile polyposis maps to chromosome 18q21.1.

17. PMID: 8553070; 1996, Science;271(5247):350-3
DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1.

18. PMID: 8673134; 1996, Nat Genet;13(3):343-6
Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers.

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19. PMID: 18662538; 2008, Cell;134(2):215-30
TGFbeta in Cancer.

20. PMID: 9135016; 1997, Cancer Res;57(9):1731-4
Tumor-suppressive pathways in pancreatic carcinoma.

21. PMID: 23139211; 2013, Cancer Res;73(2):725-35
SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer.

22. PMID: 22810696; 2012, Nature;487(7407):330-7
Comprehensive molecular characterization of human colon and rectal cancer.

23. PMID: 25890228; 2015, World J Surg Oncol;13():128
Clinical outcome and expression of mutant P53, P16, and Smad4 in lung adenocarcinoma: a prospective study.

24. PMID: 19841540; 2009, J Clin Invest;119(11):3208-11
Smad4: gatekeeper gene in head and neck squamous cell carcinoma.

25. PMID: 15867212; 2005, Clin Cancer Res;11(9):3191-7
Differences in Smad4 expression in human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck squamous cell carcinoma.

26. PMID: 25589618; 2015, Clin Cancer Res;21(6):1447-56
Genomic analysis of metastatic cutaneous squamous cell carcinoma.

27. PMID: 29703253; 2018, BMC Cancer;18(1):479
SMAD4 and NF1 mutations as potential biomarkers for poor prognosis to cetuximab-based therapy in Chinese metastatic colorectal cancer patients.

28. PMID: 28522603; 2017, Clin Cancer Res;23(17):5162-5175
SMAD4 Loss Is Associated with Cetuximab Resistance and Induction of MAPK/JNK Activation in Head and Neck Cancer Cells.

29. PMID: 16144935; 2005, Clin Cancer Res;11(17):6311-6
SMAD4 levels and response to 5-fluorouracil in colorectal cancer.

30. PMID: 24384683; 2014, Br J Cancer;110(4):946-57
Loss of Smad4 in colorectal cancer induces resistance to 5-fluorouracil through activating Akt pathway.

31. PMID: 12237773; 2002, Br J Cancer;87(6):630-4
SMAD4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer.

32. PMID: 25749173; 2015, Transl Oncol;8(1):18-24
A Meta-Analysis of SMAD4 Immunohistochemistry as a Prognostic Marker in Colorectal Cancer.

33. PMID: 19478385; 2009, Cell Oncol;31(3):169-78
Presence of a high amount of stroma and downregulation of SMAD4 predict for worse survival for stage I-II colon cancer patients.

34. PMID: 25681512; 2015, J Clin Pathol;68(5):341-5
Smad4 inactivation predicts for worse prognosis and response to fluorouracil-based treatment in colorectal cancer.

35. PMID: 26861460; 2016, Clin Cancer Res;22(12):3037-47
Reduced Expression of SMAD4 Is Associated with Poor Survival in Colon Cancer.

36. PMID: 26947875; 2016, Transl Oncol;9(1):1-7
Prognostic Value of SMAD4 in Pancreatic Cancer: A Meta-Analysis.

37. PMID: 25760429; 2015, Pancreas;44(4):660-4
SMAD4 expression predicts local spread and treatment failure in resected pancreatic cancer.

38. PMID: 22504380; 2012, Pancreas;41(4):541-6

SMAD4 genetic alterations predict a worse prognosis in patients with pancreatic ductal adenocarcinoma.

39. PMID: 19584151; 2009, Clin Cancer Res;15(14):4674-9
SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer.

40. PMID: 18425078; 2008, Mod Pathol;21(7):866-75
Expression of Smad2 and Smad4 in cervical cancer: absent nuclear Smad4 expression correlates with poor survival.

41. PMID: 24739573; 2014, Nat Rev Cancer;14(5):359-70
Unravelling mechanisms of p53-mediated tumour suppression.

42. PMID: 21125671; 2011, J Pathol;223(2):137-46
Haplo-insufficiency: a driving force in cancer.

43. PMID: 9627118; 1998, Oncogene;16(19):2527-39
Human tumor-derived p53 proteins exhibit binding site selectivity and temperature sensitivity for transactivation in a yeast-based assay.

44. PMID: 27998224; 2016, J Clin Oncol;34(36):4354-4361
Phase II Study of WEE1 Inhibitor AZD1775 Plus Carboplatin in Patients With TP53-Mutated Ovarian Cancer Refractory or Resistant to First-Line Therapy Within 3 Months.

45. PMID: 26646755; 2016, Ann Oncol;27(3):539-43
TP53 mutational status is predictive of pazopanib response in advanced sarcomas.

46. PMID: 25669829; 2015, Ann Oncol;26(5):1012-1018
Phase I study of pazopanib and vorinostat: a therapeutic approach for inhibiting mutant p53-mediated angiogenesis and facilitating mutant p53 degradation.

47. PMID: 27466356; 2016, Mol Cancer Ther;15(10):2475-2485
TP53 Alterations Correlate with Response to VEGF/VEGFR Inhibitors: Implications for Targeted Therapeutics.

48. PMID: 23670029; 2013, Oncotarget;4(5):705-14
P53 mutations in advanced cancers: clinical characteristics, outcomes, and correlation between progression-free survival and bevacizumab-containing therapy.

49. PMID: 17145525; 2006, Semin Oncol;33(5 Suppl 10):S8-14
Bevacizumab in combination with chemotherapy: first-line treatment of patients with metastatic colorectal cancer.

50. PMID: 21399868; 2011, Int J Oncol;38(5):1445-52
p53, HER2 and tumor cell apoptosis correlate with clinical outcome after neoadjuvant bevacizumab plus chemotherapy in breast cancer.

51. PMID: 20549698; 2011, Int J Cancer;128(8):1813-21
p53 status influences response to tamoxifen but not to fulvestrant in breast cancer cell lines.

52. PMID: 10786679; 2000, Cancer Res;60(8):2155-62
Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer.

53. PMID: 25672981; 2015, Cancer Res;75(7):1187-90
VEGF-A Expression Correlates with TP53 Mutations in Non-Small Cell Lung Cancer: Implications for Antiangiogenesis Therapy.

54. PMID: 26156651; 2015, Lancet Oncol;16(8):897-907
Afatinib versus erlotinib as second-line treatment of patients with advanced squamous cell carcinoma of the lung (LUX-Lung 8): an open-label randomised controlled phase 3 trial.

55. PMID: 23816960; 2013, J Clin Oncol;31(27):3327-34
Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations.

56. PMID: 22285168; 2012, Lancet Oncol;13(3):239-46
Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial.

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57. PMID: 17452677; 2007, J Clin Oncol;25(15):1960-6
Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group.

58. PMID: 24263064; 2014, Br J Cancer;110(1):55-62
First-line gefitinib in Caucasian EGFR mutation-positive NSCLC patients: a phase-IV, open-label, single-arm study.

59. PMID: 27959700; 2017, N Engl J Med;376(7):629-640
Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer.

60. PMID: 25923549; 2015, N Engl J Med;372(18):1689-99
AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer.