陳亭臻女士 您好:

感謝您選用行動基因所提供的檢測服務。行動基因經您的同意，於西元 2021 年 08 月 09 日取得您的檢體，進行 ACTOnco®+癌安克™癌症基因檢測與 ACTFusion™癌融克™癌症融合基因檢測。行動基因實驗室為通過美國病理學會 (The College of American Pathologists，CAP) (CAP#：9028096) 與臺灣衛生福利部食品藥物管理署「精準醫療分子檢測列冊登錄實驗室」(Laboratory Developed Tests and Services, LDTS) (列冊登錄編號：LDTS0001) 的認證機構。

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

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

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

本次檢測於腫瘤檢體未偵測到與標靶用藥相關之基因變異。

腫瘤突變負荷量 (TMB): < 1 mutations/megabase

微小衛星體不穩定性 (MSI): 穩定(stable)

融合基因: 未測得基因融合

詳細變異基因描述與用藥建議，請參閱以下完整基因檢測報告內容。

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

若您對本檢測報告有任何疑問，請隨時與我們聯繫。

行動基因 敬上

ACTOnco® + Report

REPORT SUMMARY

REPORT DETAILS

APPENDIX

陳亭臻
Project ID: C21-M001-00408
Report No.: AA-21-03248_ONC
Date Reported: Aug 20, 2021

PATIENT AND SAMPLE INFORMATION

PATIENT	SPECIMEN	ORDERING PHYSICIAN
Name: 陳亭臻	Type: FFPE tissue	Name: 洪君儀醫師
Gender: Female	Date received: Aug 09, 2021	Facility: 臺北榮總
Date of Birth: Aug 31, 1991	Collection site: Soft tissue	Tel: 886-228712121
Patient ID: 46254119	Specimen ID: S11016643	Address: 臺北市北投區石牌路二段 201 號
Diagnosis: Osteosarcoma	Lab ID: AA-21-03248	Cooperative ID: NA
	D/ID: NA	

VARIANT(S) WITH CLINICAL RELEVANCE

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

SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Not detected.

COPY NUMBER VARIANTS (CNVS)

Loss of heterozygosity (LOH) information was used to infer tumor cellularity. Copy number alteration in the tumor was determined based on **30%** tumor purity.

Amplification (Copy number ≥ 8)

Chr	Gene	Copy Number
chr8	*MYC*	12

Homozygous deletion (Copy number=0)

Chr	Gene
ND	ND

Heterozygous deletion (Copy number=1)

Chr	Gene
ND	ND

ND, Not Detected

TUMOR MUTATIONAL BURDEN (TMB)

< 1 muts/Mb

Muts/Mb, mutations per megabase

MICROSATELLITE INSTABILITY (MSI)

Microsatellite stable (MSS)

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

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

Yun Yu Chen

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

Yun Yu Chen

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THERAPEUTIC IMPLICATIONS

TARGETED THERAPIES

No genomic alterations predicted to confer sensitivity or lack of benefit to targeted therapy approved in this tumor type.

IMMUNE CHECKPOINT INHIBITORS (ICI) THERAPIES

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

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

MMR, mismatch repair; ND, not detected

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

CHEMOTHERAPIES

Therapies	Genomic Alterations	Effect	Gene / Variant Level Evidence	Cancer Type
FAC, CMF and P-FEC regimens	***MYC*** Amplification	**Sensitive**	Clinical	Breast cancer
Platinum-based regimens	***MYC*** Amplification	**Sensitive**	Clinical	Ovarian cancer

FAC, 5-fluorouracil, doxorubicin and cyclophosphamide; CMF, cyclophosphamide, methotrexate and 5-fluorouracil; P-FEC, paclitaxel followed by 5-fluorouracil, epirubicin and cyclophosphamide

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

MYC Amplification

Biological Impact

The v-myc avian myelocytomatosis viral oncogene homolog, also known as c-myc (MYC) gene encodes a transcription factor involved in cellular proliferation, inhibiting exit from the cell cycle, stimulating vascularization and enhancing genomic instability [1][2][3]. Dysregulated MYC expression is implicated in a wide range of human cancers [4].

Frequency

Amplification of the MYC occurs in approximately (1)26.1% (gain) / (2)5.1% (high level amplification) of sarcoma (TCGA, Provisional) [5][6].

Therapeutic and prognostic relevance

MYC amplification was associated with better clinical outcome in breast cancer patients treated with FAC (5-fluorouracil, doxorubicin, and cyclophosphamide), CMF (cyclophosphamide, methotrexate and 5-fluorouracil) [7] and P-FEC (paclitaxel followed by 5-fluorouracil, epirubicin and cyclophosphamide) [8] and higher expression of MYC was also associated with a better response rate in platinum-treated ovarian cancer patients [9].

CDK inhibition using the dinaciclib, a CDK1, 2, 5 and 9 inhibitors, exerted antitumor activity in triple-negative breast cancer (TNBC) tumor xenograft and cell lines with increased activity of the MYC pathway [10][11].

陳亭臻

US FDA-APPROVED DRUG(S)

No genomic alterations predicted to confer sensitivity or lack of benefit to targeted therapy approved.

ONGOING CLINICAL TRIALS

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

No trial has been found.

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DETAILED TEST RESULTS

SINGLE NUCLEOTIDE AND SMALL INDEL VARIANTS

Gene	Chr	Exon	Accession Number	cDNA Change	Amino Acid Change	Coverage	Allele Frequency	COSMIC ID
ATM	11	45	NM_000051	c.6503C>T	S2168L	465	56.1%	COSM3443163
EPHA2	1	17	NM_004431	c.2845G>C	V949L	1143	45.2%	-
FRG1	4	2	NM_004477	c.112G>A	E38K	182	58.8%	-
MEF2B	19	7	NM_005919	c.496C>T	R166C	620	52.4%	-
MYCL	1	2	NM_005376	c.605C>T	P202L	1824	49.1%	-
PDGFRA	4	22	NM_006206	c.2965A>C	I989L	1958	48.7%	-
ROS1	6	24	NM_002944	c.3716A>T	Y1239F	1132	49.8%	-
USH2A	1	17	NM_206933	c.3665C>T	A1222V	621	47.8%	COSM1626815
ZNF217	20	3	NM_006526	c.1645G>A	A549T	1403	50.6%	COSM3939408

Mutations with clinical relevance are highlighted in red.

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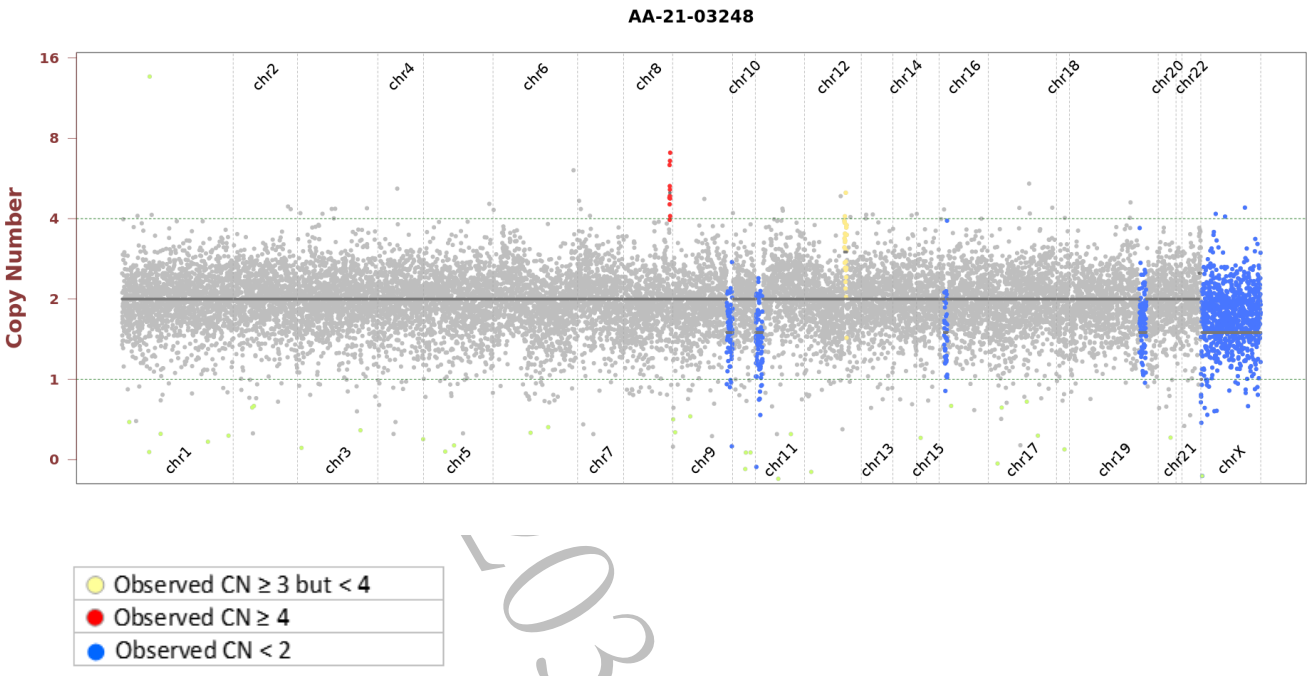
COPY NUMBER VARIANTS (CNVS)

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



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HOTSPOT GENOTYPES

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

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

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

Gene	Copy Number Detected
CDK4	5
EGFR	2
ERBB2	2
MET	2

Copy number ≥ 8 is considered amplification

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

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

Gene	Copy Number Detected
FGFR1	2
MDM2	5
MDM4	2

Copy number ≥ 8 is considered amplification

TEST DETAILS

ABOUT ACTOnco®+

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

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

DATABASE USED

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

NEXT-GENERATION SEQUENCING (NGS) METHODS

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

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

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

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

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

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

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

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

STANDARD OPERATING PROCEDURES (SOPS)

Standard operating procedures (SOPs) are shown below:

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

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

LIMITATIONS

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

NOTES

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

PATHOLOGY EVALUATION

- H&E-stained section No.: S11016643
- Collection site: Soft tissue
- Examined by: Dr. Yeh-Han Wang
- Estimated neoplastic nuclei (whole sample): The percentage of viable tumor cells in total cells in the whole slide (%): 15%
  The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 70%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
  Additional comment: NA
- Manual macrodissection: Performed on the highlighted region

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

SPECIMEN PHOTO(S)

- Collection date: May 2021
- Facility retrieved: 臺北榮總

RUN QC

- Panel: ACTOnco®+
- Mean Depth: 931x
- Target Base Coverage at 100x: 95%

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ACTOnco®+ GENE LIST

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

*Analysis of copy number alteration not available.

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

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SIGNALING PATHWAYS AND MOLECULAR-TARGETED AGENTS

Not Applicable.

REFERENCES

1. PMID: 19029958; 2008, Nat Rev Cancer;8(12):976-90
Reflecting on 25 years with MYC.

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MYC on the path to cancer.

3. PMID: 10378696; 1999, Oncogene;18(19):3004-16
MYC oncogenes and human neoplastic disease.

4. PMID: 16934487; 2006, Semin Cancer Biol;16(4):318-30
The Myc oncoprotein as a therapeutic target for human cancer.

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6. PMID: 22588877; 2012, Cancer Discov;2(5):401-4
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8. PMID: 21741827; 2011, Eur J Cancer;47(12):1779-88
Association between c-myc amplification and pathological complete response to neoadjuvant chemotherapy in breast cancer.

9. PMID: 15132769; 2004, Cancer Sci;95(5):418-23
Expression of the c-myc gene as a predictor of chemotherapy response and a prognostic factor in patients with ovarian cancer.

10. PMID: 22430491; 2012, J Exp Med;209(4):679-96
MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition.

11. PMID: 27486754; 2016, Oncotarget;7(35):56864-56875
Inhibition of cyclin dependent kinase 9 by dinaciclib suppresses cyclin B1 expression and tumor growth in triple negative breast cancer.

ACT GENOMICS®

CAP ACCREDITED COLLEGE of AMERICAN PATHOLOGISTS

ACTFusion™ Report

陳亭臻
Project ID: C21-M001-00408
Report No.: AA-21-03248_FUS
Date Reported: Aug 20, 2021

PATIENT	SPECIMEN	ORDERING PHYSICIAN
Name: 陳亭臻	Type: FFPE tissue	Name: 洪君儀醫師
Gender: Female	Date received: Aug 09, 2021	Facility: 臺北榮總
Date of Birth: Aug 31, 1991	Collection site: Soft tissue	Tel: 886-228712121
Patient ID: 46254119	Specimen ID: S11016643	Address: 臺北市北投區石牌路二段 201 號
Diagnosis: Osteosarcoma	Lab ID: AA-21-03248	Cooperative ID: NA
	D/ID: NA	

ABOUT ACTFusion™

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

VARIANT(S) WITH CLINICAL RELEVANCE

FUSION RESULTS

No fusion gene detected in this sample.

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

Yun Yu Chen

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

Yun Yu Chen

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THERAPEUTIC IMPLICATIONS

TARGETED THERAPIES

Not Applicable.

VARIANT INTERPRETATION

Not Applicable.

US FDA-APPROVED DRUG(S)

Not Applicable.

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ONGOING CLINICAL TRIAL(S)

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

No trial has been found.

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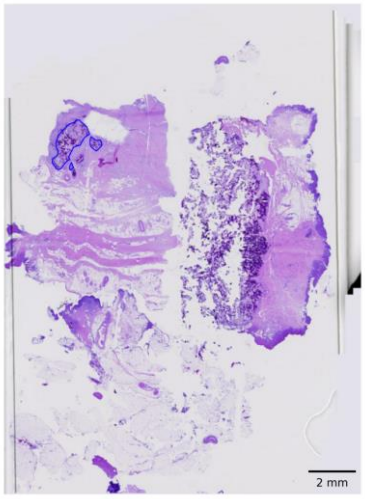
ACTFusion™ GENE LIST

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

TEST DETAILS

SPECIMEN RECEIVED



- H&E-stained section No.: S11016643
- Collection date: May 2021
- Collection site: Soft tissue
- Facility retrieved: 臺北榮總
- Examined by: Dr. Yeh-Han Wang
- Estimated neoplastic nuclei (whole sample): The percentage of viable tumor cells in total cells in the whole slide (%): 15%
  The percentage of viable tumor cells in total cells in the encircled areas in the whole slide (%): 70%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the whole slide (%): 0%
  The percentage of necrotic cells (including necrotic tumor cells) in total cells in the encircled areas in the whole slide (%): 0%
  Additional comment: NA
- Manual macrodissection: Performed on the highlighted region

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

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NEXT-GENERATION SEQUENCING (NGS) METHODS

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

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

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

DATABASE USED

Quiver Gene Fusion Database version 5.1.18

LIMITATIONS

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

STANDARD OPERATING PROCEDURES (SOPs)

Standard operating procedures (SOPs) are shown below:

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

RUN QC

- Panel: ACTFusion™
- Total reads: 1142924
- Average unique RNA Start Sites per control GSP2: 27

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REFERENCES

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