

<https://medicinaprecisionandalucia.iavante.es> | #PANMEP

PANMEP

PROGRAMA ANDALUZ DE FORMACIÓN EN MEDICINA
PERSONALIZADA Y DE PRECISIÓN

EXPERTO UNIVERSITARIO EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

TÉCNICAS MOLECULARES Y CELULARES EN MEDICINA DE PRECISIÓN

FORMACIÓN
IAVANTE
Fundación
Progreso y Salud

COLABORA

Johnson & Johnson

ORGANIZAN

un
i Universidad
Internacional
de Andalucía
A



Junta de Andalucía
Consejería de Salud y Consumo

Módulo 4: Características y especificidades metodológicas y prácticas de las plataformas asociadas al análisis de NGS

Descripción: Describir las distintas metodologías y plataformas asociadas a la NGS que están actualmente disponibles y aceptadas en la práctica clínica así como las plataformas de NGS de nueva generación.

Dr. Javier Hernández-Losa
Laboratorio Biología Molecular
Servicio Anatomía Patológica
Hospital Universitario Vall d'Hebron

Agenda

- Como funciona la NGS en sistema automatizado.
- ¿Como se analizan los datos y consulta de Bases de Datos?.
- Analisis en linea germinal vs linea somatica
- Casos del comite molecular de tumores

Agenda

- Como funciona la NGS en sistema automatizado.
- **¿Como se analizan los datos y consulta de Bases de Datos?.**
- Analisis en linea germinal vs linea somatica
- Casos del comite molecular de tumores



[1]

Los patólogos hacen el diagnóstico y seleccionan el área tumoral



[2]

Se corta para extracción de ácidos nucleicos



DNA [3]

Extracción manual / automática según tamaño y celularidad de la muestra

RNA [4]

Extracción manual



Manual

[5]

Preparación de librerías

Automática



[6]

Secuenciación

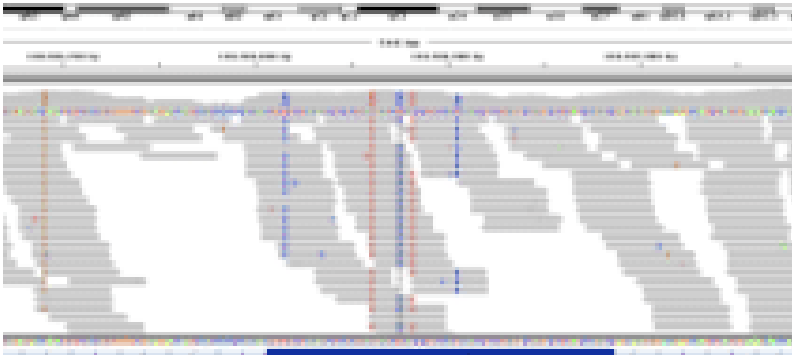
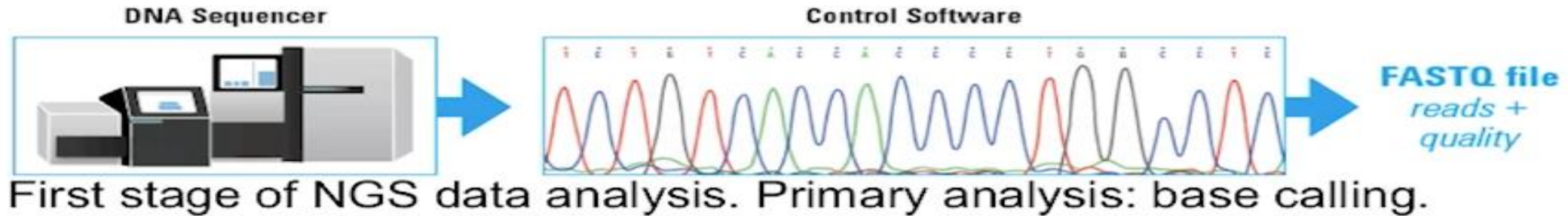


[7]

Análisis e interpretación de variantes

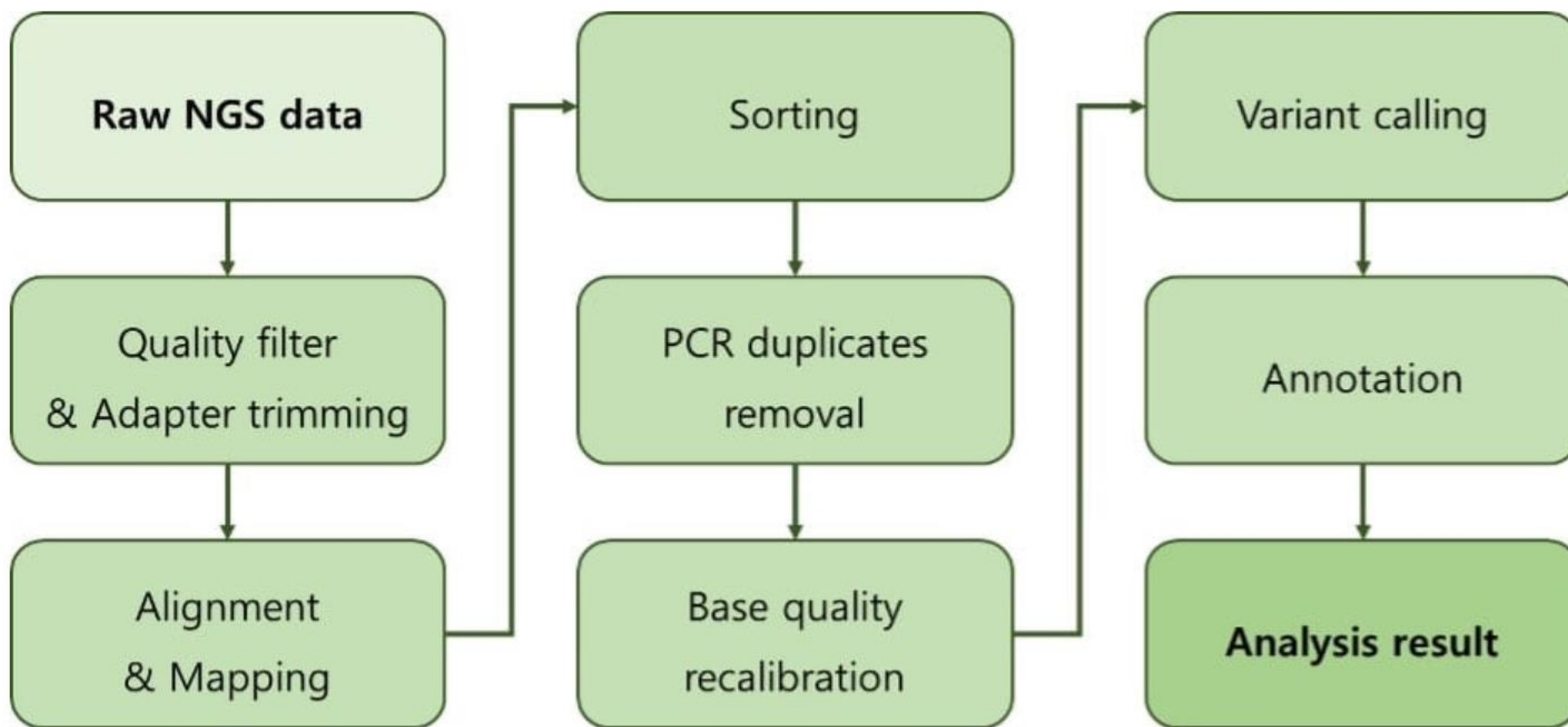
Análisis de Datos primario

Convierten datos crudos a datos de secuenciación.



Phred quality score	Probability of incorrect base	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%
50	1 in 100000	99.999%

@NB502049:122:H5MYCAFXY:1:1101:5654:1036 1:N:0:GAGATTCC+NTTCGCCT
TCCTTNCATGAGACAGGGTTTACTATGTGTGTCAGGCTGGTCTCAAAGTCTGGGCTCAAGTGACTCTCCTGCTCAGCTTCCCAGGTGCTAAGAGTACAGGCACCATGTCCAGCCTCAAAGCTTTAAATAATATATTTTCA
+
AAAA#EEEEEEEEEEEEEEEEEEEEEEEEEEEEEE/ <EEEEEEEEEEEEEEEEEEEEEA<<EEEEEEEEEEEEEEEEEEEE6EEEEAA/<EEAE/E/AEEA/AEEEE//E/AE/AA/</E/A6
@NB502049:122:H5MYCAFXY:1:1101:11190:1037 1:N:0:GAGATTCC+NTTCGCCT
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+
AAAA#EE/EAE/<AAEAE<A/AEEEE<EAE<EEEE/EAA<EEEEEA<A</</E/E/A/EEA///
@NB502049:122:H5MYCAFXY:1:1101:6590:1037 1:N:0:GAGATTCC+NTTCGCCT
CAACGNAGTCTGTGTGAATGGGAAAAGCTTTGGAAAGACAGACATGAGTTCAGTCTGAGTAAGTAACTACTGGATATGAAGAGAAAGACTTGTTCCTCATATAAAATGAGGCTAATGTCTAGCATATCAGATCGGAAGACACACGCTG
+
AAAA#EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE<EEEEEEEE<EEEEEEEE<EEEEEEEEEEEEEEEEEEEE<EAE/E/EEEEEAEEEE/EEAE<AAEEEEEA<A/<AAEA<<6AEA<A<
@NB502049:122:H5MYCAFXY:1:1101:4095:1037 1:N:0:GAGATTCC+NTTCGCCT
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+
AAAA#EEEEEEEE/E/EEEEEEEEEEEEEEEEEAEEEEEEEEEEEE/E/EEEE/E/A/E//EEAEAE/EEE//<6<EEEEEEEE6<EEE<AAEEAE/E/A6<///AEEEAEEEEEA<A6A/<AA/AAAAE//<<///A
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+
AAAA#EE<EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE<AAAAA<<A<6AAA6A</
@NB502049:122:H5MYCAFXY:1:1101:25673:1037 1:N:0:GAGATTCC+NTTCGCCT
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+
AAAA#EEEEEEEEEEEEEEEEEA/E/EEEEEEEEEEEEEEEEEAEE/EEEE/E/EEEE/E6EEE/EEEE//<EE//E/AE/E//<EAE//6EEE<//AA/E/E//EEE/E6//E<EE//<E/</EAEAE<E/EA/
@NB502049:122:H5MYCAFXY:1:1101:15948:1037 1:N:0:GAGATTCC+NTTCGCCT
GGTGANGATCAAGGCCCTTATCAGTTTTGTTATTAGGATAATACTGGACTCACAGAATGAGTTAGGAATCTTCCCTTGGCTCTGTCTTCTGAAAGAGATGATCAAGAATTGGTATAATTTATTTTAAATATTGGTAAATTTACT
+
AAAA#E/EEEEEEEEEEEEEE6EEEEEEEEEEEEEEEEEEEEEEEEEEEEEE//E/EEAEEEEEEEEEEEEEEEE<AAEEEEAE/EEEEEEEEAE<EEEAEEEEEA/EAA<E<EAE/E/AE/<AE<AE/A



Análisis de Datos secundario



Second stage of NGS analysis. Secondary analysis: read alignment and variant calling.

SAM: Sequence Alignment/MAP Format.

BAM: Formato binario de SAM

VCF header

```
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##reference=NCBI36
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality (phred score)">
##FORMAT=<ID=GL,Number=3,Type=Float,Description="Likelihoods for RR,RA,AA genotypes (R=ref,A=alt)">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
```

Mandatory header lines

Optional header lines (meta-data about the annotations in the VCF body)

Body

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	SAMPLE1	SAMPLE2
1	1	.	ACG	A,AT	.	PASS	.	GT:DP	1/2:13	0/0:29
1	2	rs1	C	T,CT	.	PASS	H2;AA=T	GT:GQ	0/1:100	2/2:70
1	5	.	A	G	.	PASS	.	GT:GQ	1/0:77	1/1:95
1	100	.	T		.	PASS	SVTYPE=DEL;END=300	GT:GQ:DP	1/1:12:3	0/0:20

Deletion (points to in ALT)

SNP (points to G in ALT)

Large SV (points to SVTYPE=DEL;END=300 in INFO)

Insertion (points to T,CT in ALT)

Other event (points to H2;AA=T in INFO)

Reference alleles (GT=0) (points to 0/0:29 in SAMPLE2)

Alternate alleles (GT>0 is an index to the ALT column) (points to 1/1:95 in SAMPLE2)

Phased data (G and C above are on the same chromosome) (points to 0/1:100 in SAMPLE1)

Archivo vcf

Anotación de variantes manual

The Journal of Molecular Diagnostics, Vol. 19, No. 1, January 2017



the Journal of
Molecular
Diagnostics

jmd.amjpathol.org

SPECIAL ARTICLE

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer



*A Joint Consensus Recommendation of the Association for
Molecular Pathology, American Society of Clinical Oncology,
and College of American Pathologists*

Marilyn M. Li,^{*†} Michael Datto,^{*‡} Eric J. Duncavage,^{*§} Shashikant Kulkarni,^{*¶} Neal I. Lindeman,^{*||} Somak Roy,^{*,***}
Apostolia M. Tsimberidou,^{*††} Cindy L. Vnencak-Jones,^{*‡‡} Daynna J. Wolff,^{*§§} Anas Younes,^{*¶¶} and Marina N. Nikiforova^{*,**}

Table 1 Databases Relevant to Interpretation of Somatic Sequence Variants

Utility/function	Database	Location (web address)
Population databases to exclude polymorphisms	1000 Genomes Project ¹⁶ Exome Variant Server dbSNP ¹⁷ dbVar ¹⁸ ExAC	http://browser.1000genomes.org http://evs.gs.washington.edu/EVS http://www.ncbi.nlm.nih.gov/snp http://www.ncbi.nlm.nih.gov/dbvar http://exac.broadinstitute.org
Cancer-specific variant databases	Catalog of Somatic Mutations in Cancer ¹⁹ My Cancer Genome Personalized cancer therapy, MD Anderson Cancer Center cBioPortal, Memorial Sloan Kettering Cancer Center ²⁰ Intogen ²¹ ClinicalTrials.gov IARC (WHO) TP53 mutation database ²² Pediatric Cancer Genome Project (St. Jude Children's Research Hospital—Washington University) International Cancer Genome Consortium ²³	http://cancer.sanger.ac.uk/cosmic http://www.mycancergenome.org https://pct.mdanderson.org http://www.cbioportal.org https://www.intogen.org/search https://clinicaltrials.gov http://p53.iarc.fr http://explorecpgp.org https://dcc.icgc.org
Sequence repositories and data hosts	NCBI Genome RefSeqGene ²⁴ Locus Reference Genomic ²⁵ UCSC table browser ²⁶ Ensemble BioMart ²⁷	http://www.ncbi.nlm.nih.gov/genome http://www.ncbi.nlm.nih.gov/refseq/rsg http://www.lrg-sequence.org https://genome.ucsc.edu/cgi-bin/hgTables http://useast.ensembl.org/biomart/martview
Other disease/mutation databases useful in the context of variant interpretation for cancer genomics	ClinVar ²⁸ Human Gene Mutation Database ²⁹ Leiden Open Variation Database ³⁰ dbNSFP (compiled database of precomputed <i>in silico</i> prediction scores for nonsynonymous SNVs) ³¹ Ensemble Variant Effect Predictor ¹⁵	http://www.ncbi.nlm.nih.gov/clinvar http://www.hgmd.org http://www.lovd.nl https://sites.google.com/site/jpopgen/dbNSFP http://www.ensembl.org/info/docs/tools/vep/index.html

Table 2 Algorithms for Computational Prediction of Functional Impact of Sequence Variant/Splice Site Changes

Utility/function	Algorithm/software	Location (web address)
Missense SNV	PolyPhen2 ³⁷	http://genetics.bwh.harvard.edu/pph2
	SIFT ³⁸	http://sift.jcvi.org
	MutationAssessor ³⁹	http://mutationassessor.org
	MutationTaster ⁴¹	http://www.mutationtaster.org
	PROVEAN ⁴⁵	http://provean.jcvi.org/index.php
	Condel ⁴⁶	http://bg.upf.edu/blog/2012/12/condel-for-prioritization-of-variants-involved-in-hereditary-diseases-and-transfic-for-cancer
	CoVEC ⁴⁰	https://sourceforge.net/projects/covec/files
	CADD ⁴⁷	http://cadd.gs.washington.edu
	GERP++ ⁴⁸	http://mendel.stanford.edu/sidowlab/downloads/gerp/index.html
	PhyloP and PhastCons ⁴⁹	http://compugen.bscb.cornell.edu/phast
Splice site prediction	Human Splicing Finder ⁴²	http://www.umd.be/HSF3
	MaxEntScan ⁴³	http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html
	NetGene2 ⁴⁴	http://www.cbs.dtu.dk/services/NetGene2
	NNSplice ⁵⁰	http://www.fruitfly.org/seq_tools/splice.html
	GeneSplicer ⁵¹	http://www.cbcb.umd.edu/software/GeneSplicer/gene_spl.shtml

These are not comprehensive lists, and inclusion does not represent an organizational endorsement of any individual database or product. All websites last accessed June 7, 2016.

SNV, single-nucleotide variant.

Descripción de la variantes

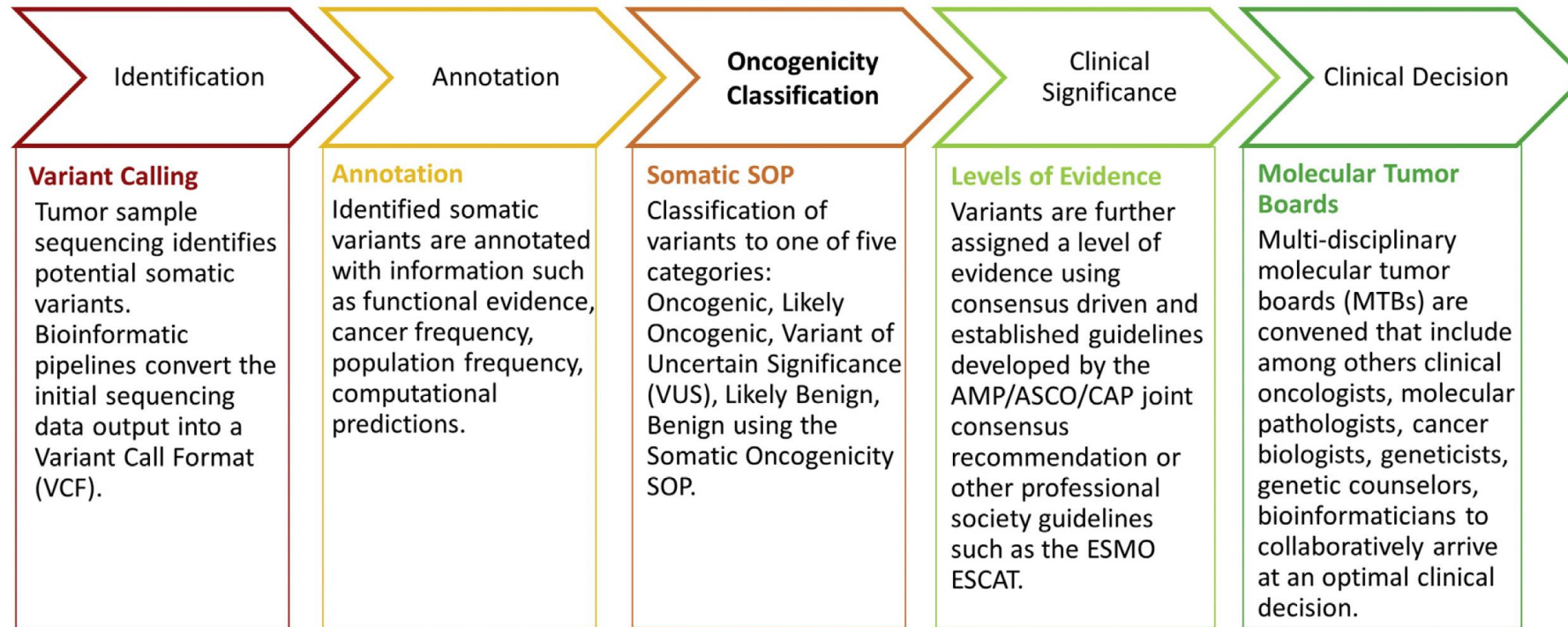


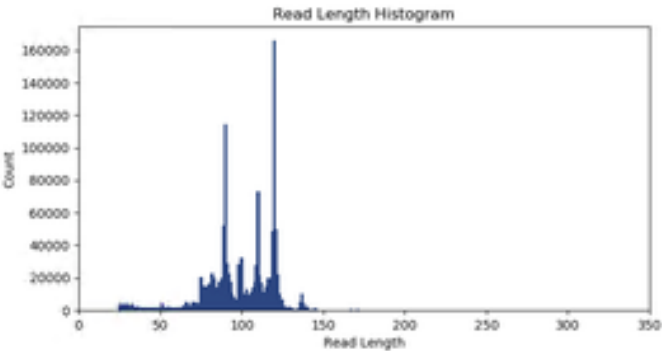
Figure 2 Stepwise process of somatic variant classification and interpretation. AMP, Association of Molecular Pathology; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; ESCAT, European Society for Medical Oncology Scale of Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; SOP, standard operating procedure.

1.1- control de calidad

Sample QC - DNA



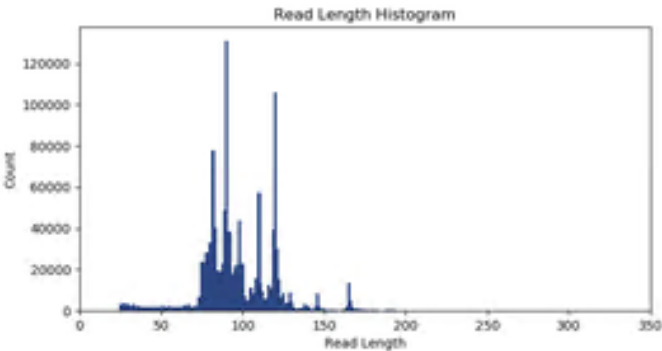
MAPD	✓	0,22
[0-0.5]		
Mapped Reads	✓	1.357.126
>=350000		
Mean AQ20 Read Length (bp)	—	91
Not Set		
Mean Read Length (bp)	✓	98
>=80		
Uniformity Of Base Coverage	✓	99,4
>=94		



Sample QC - RNA



Mapped Reads	✓	179.278
>=0		
Mean Read Length (bp)	✓	97
>=0		
RNA Expression Ctrls Detected	✓	7
>=5		



1.1- control de calidad de la muestra

coverageAnalysis

Library type: AmpliSeq DNA and Fusions
Target regions: Barcode specific

90-95%

800-2.000x

90-100%

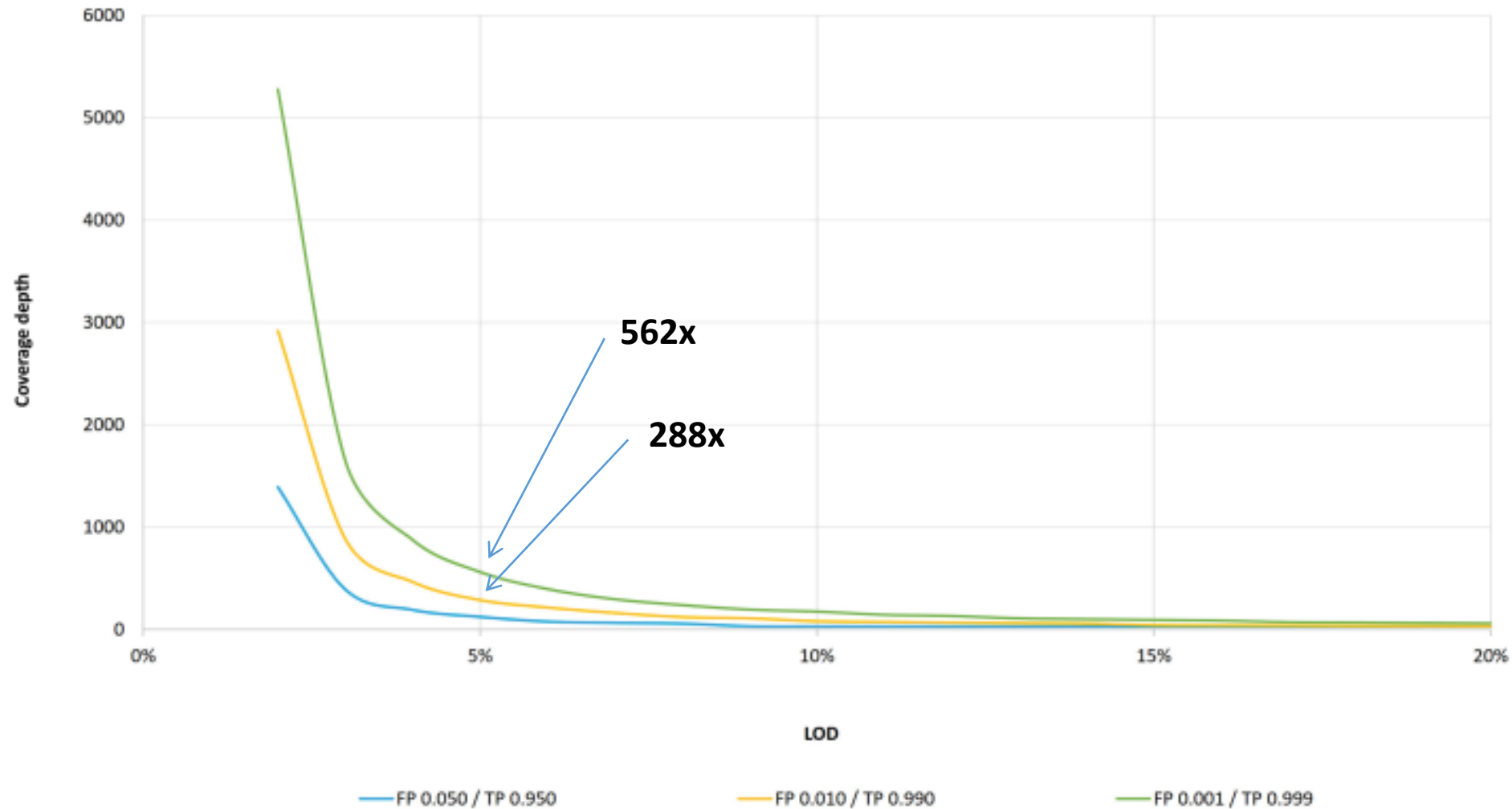
Oncomine Childhood Assay metrics

Barcode Name	Sample		Mapped Reads	On Target	Mean Depth	Uniformity
IonXpress_001	CH_17D	> 3.000.000	7,237,731	95.85%	2,619	96.02%
IonXpress_002	CH_18D		10,491,143	97.93%	3,943	91.00%
IonXpress_003	CH_19D		15,012,548	97.63%	5,595	83.61%
IonXpress_004	CH_20D		20,934,305	98.06%	7,870	90.12%
IonXpress_005	CH_21D		7,360,592	97.63%	2,787	94.67%
IonXpress_006	CH_22D		10,000,444	97.33%	3,752	97.01%
IonXpress_007	CH_23D		8,974,599	95.61%	3,165	94.26%
IonXpress_008	CH_24D		4,928,551	97.05%	1,822	96.97%
IonXpress_009	CH_17R	> 250.000	674,024	94.73%	NA	NA
IonXpress_010	CH_18R		1,052,486	97.38%	NA	NA
IonXpress_011	CH_19R		1,162,224	94.06%	NA	NA
IonXpress_012	CH_20R		989,545	97.38%	NA	NA
IonXpress_013	CH_21R		640,798	80.79%	NA	NA
IonXpress_014	CH_22R		1,276,631	96.48%	NA	NA
IonXpress_015	CH_23R		1,194,653	87.80%	NA	NA
IonXpress_016	CH_24R		872,666	88.83%	NA	NA

DNA

RNA

Cuanta cobertura necesito?



1.1- control de calidad de la variante

Genexus | Ion Torrent

Results / 2022_05_13_OPA ▾ / OPA DNA Fus_V4 ▾ / 22P011036D_22P011036R ▾

22P011036D_22P011036R QC  [Key Findings](#) **[Variants](#)** [Plugins](#) [Reports](#)

All SNVs/Indels Fusions CNVs

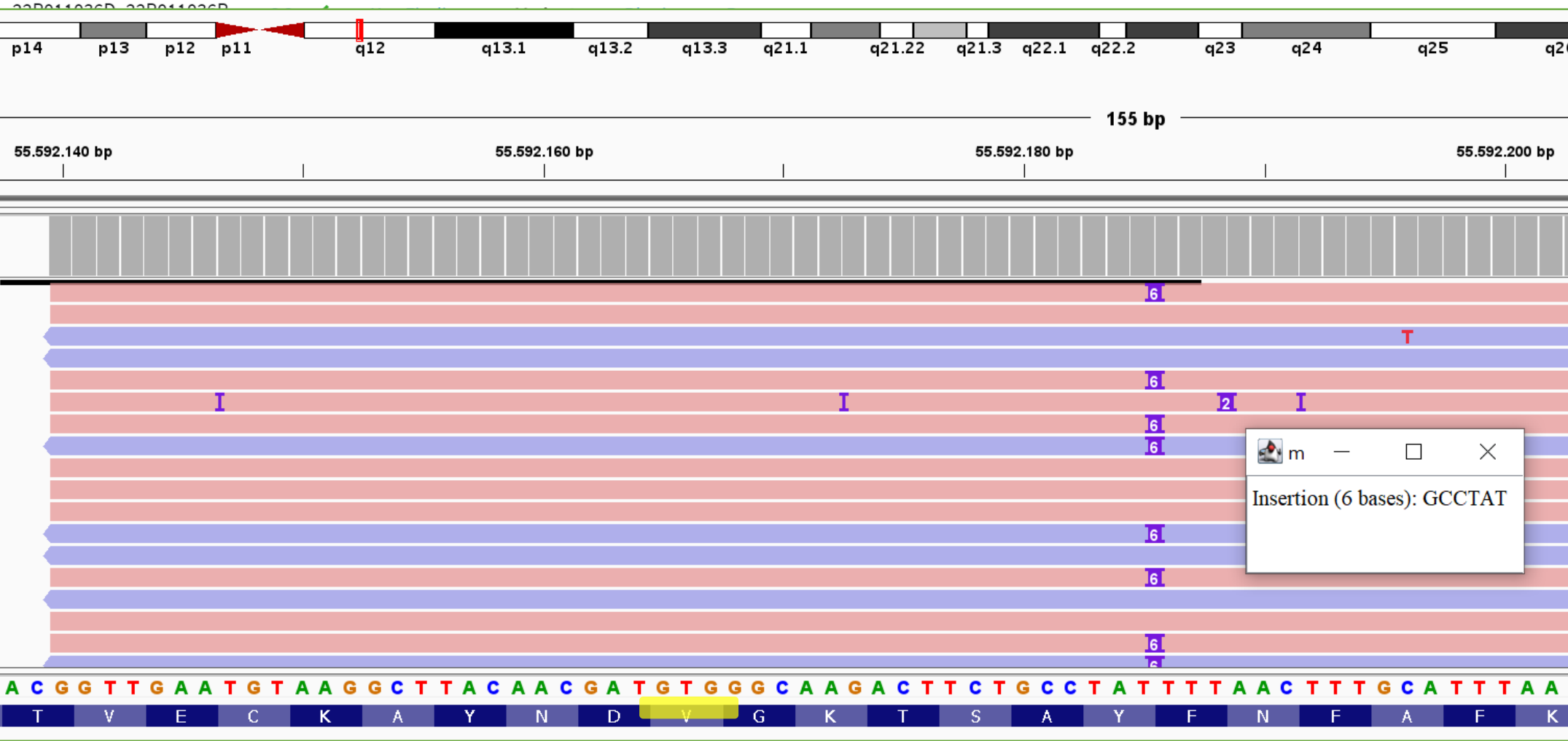
☐ Gene List Only

Type ▾	Oncomine Variant Class ▾	Gene ▾	Locus ▾	AA Change ▾	Allele Frequency (%) ▾	Call ▾	Nuc Change ▾	Effective Read Depth ▾
ins	Hotspot	KIT	chr4:55592185	p.A502_Y503dup	36.7	PRESENT (HETEROZYGOUS)	c.1509_1510insGCCTAT	4551
snp	Hotspot	TP53	chr17:7578413	p.V173L	37.2	PRESENT (HETEROZYGOUS)	c.517G>T	6799
RNAExonTiles	ExpressionImbalance	NTRK2	chr9:87359940			PRESENT		
CNV		AR	chrX:66788672			PRESENT (LOSS)		

1.2 Revisión en IGV

Genexus | Ion Torrent

Results / 2022_05_13_OPA ▾ / OPA DNA Fus_V4 ▾ / 22P011036D_22P011036R ▾



1.3 Anotación de la variante según las guías HGVS

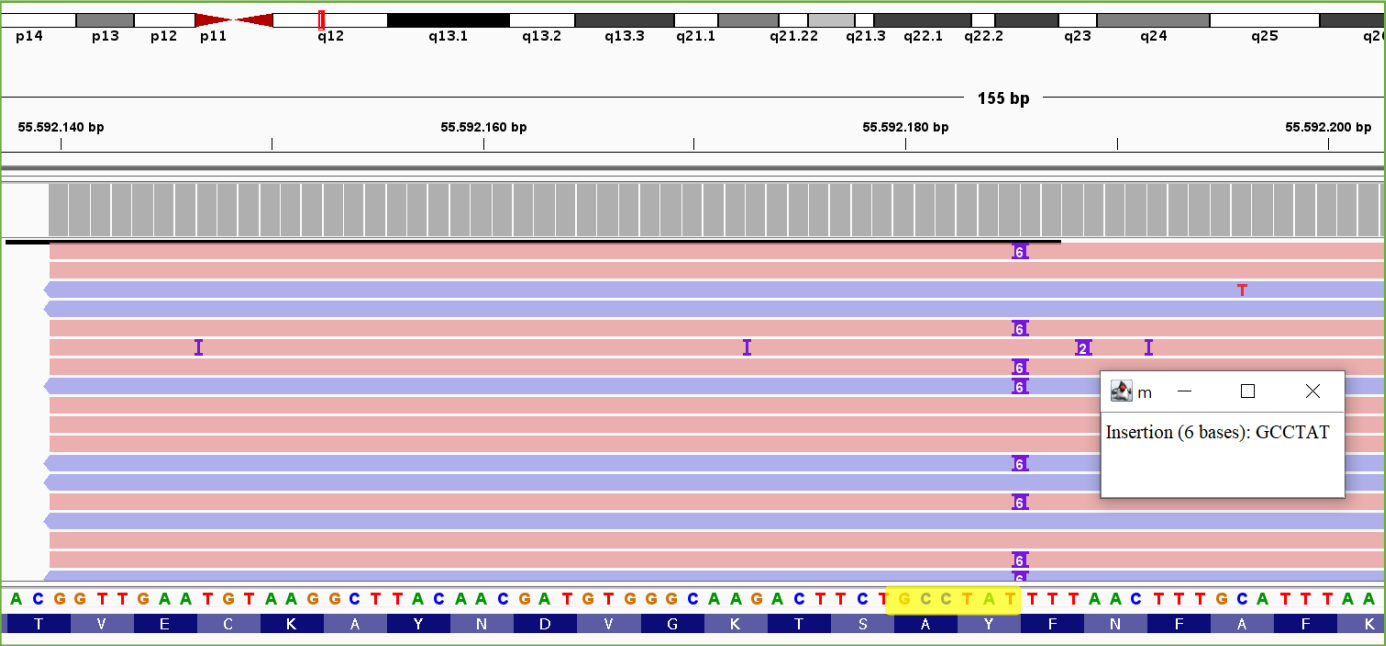


Genexus | Ion Torrent

Results / 2022_05_13_OPA / OPA DNA Fus_V4 / 22P011036D_22P011036R

22P011036D_22P011036R QC Key Findings **Variants** Plugins Reports

Type	Oncomine Variant Class	Gene	Locus	AA Change	Allele Frequency (%)	Call	Nuc Change	Effective Read Depth
ins	Hotspot	KIT	chr4:55592185	p.A502_Y503dup	36.7	PRESENT (HETEROZYGOUS)	c.1509_1510insGCCTAT	4551
snp	Hotspot	TP53	chr17:7578413	p.V173L	37.2	PRESENT (HETEROZYGOUS)	c.517G>T	6799



Según HGVS

KIT NM_000222.3:c.1504_1509dup, p.(Ala502_Tyr503dup)

TP53 NM_000546.5:c.517G>T, p.(Val173Leu)

2. Clasificación de la variante

- 2.1 Clasificación de la patogenicidad
- 2.2 Bases de datos

Genetics in Medicine (2022) ■, 1–13



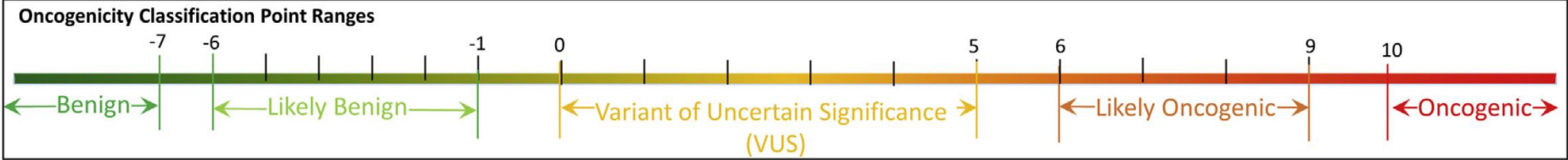
Genetics
in
Medicine

www.journals.elsevier.com/genetics-in-medicine

SPECIAL ARTICLE

Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC)

Genet Med. 2022 May;24(5):986–998. doi: 10.1016/j.gim.2022.01.001.



	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
Predictive Data			Silent mutation (no predicted impact on splicing)		Missense change at an amino acid residue where a different missense change determined to be oncogenic has been documented	Same amino acid change as a previously established oncogenic mutation	Null variant in tumor suppressor
Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			

Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

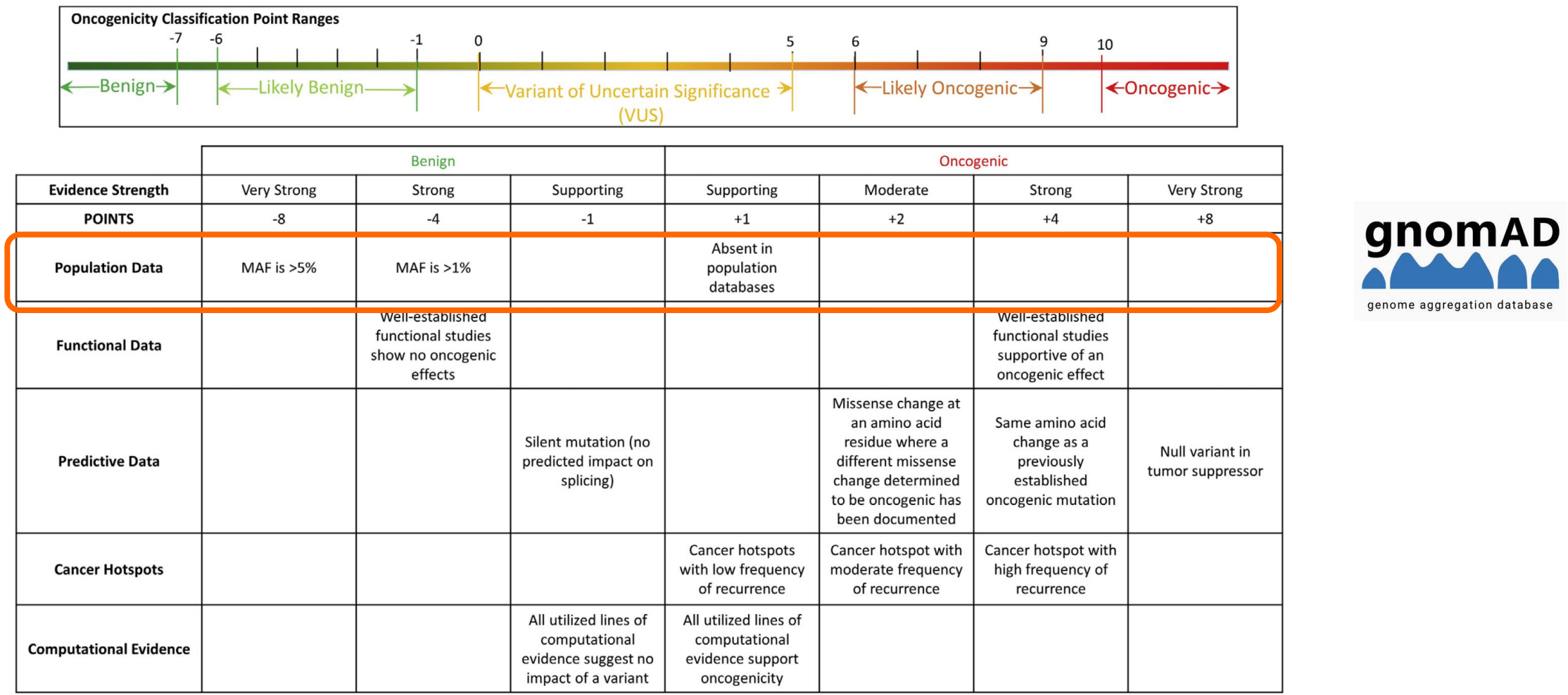


Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

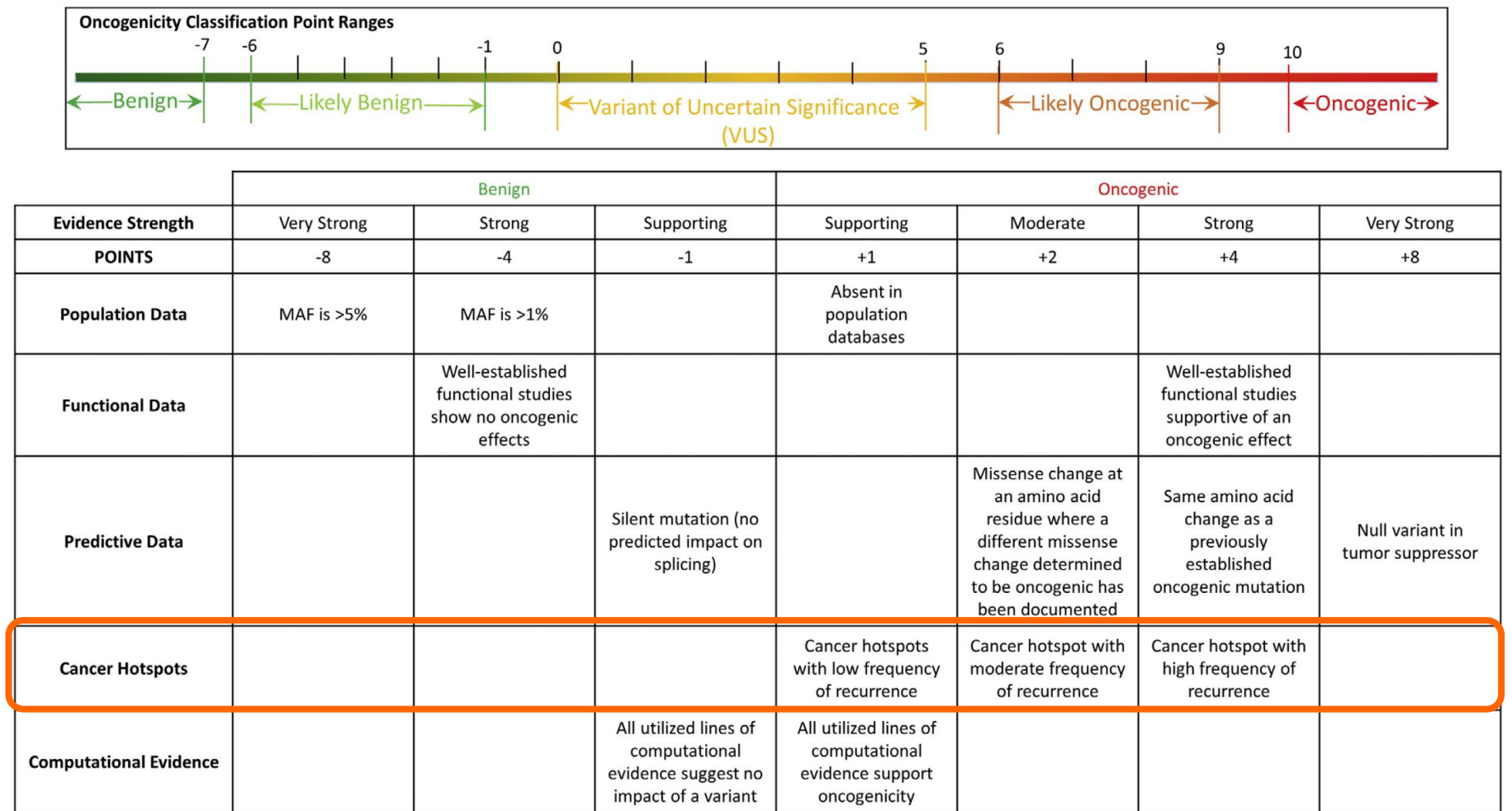
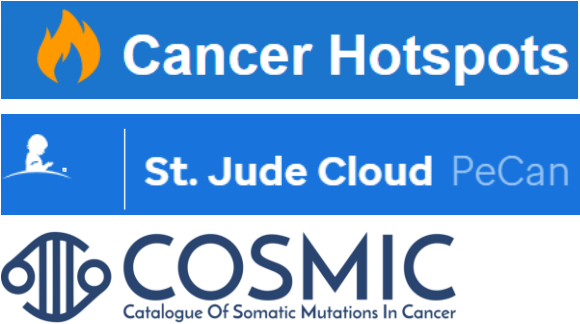


Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

Genet Med. 2022 May;24(5):986-998. doi: 10.1016/j.gim.2022.01.001.



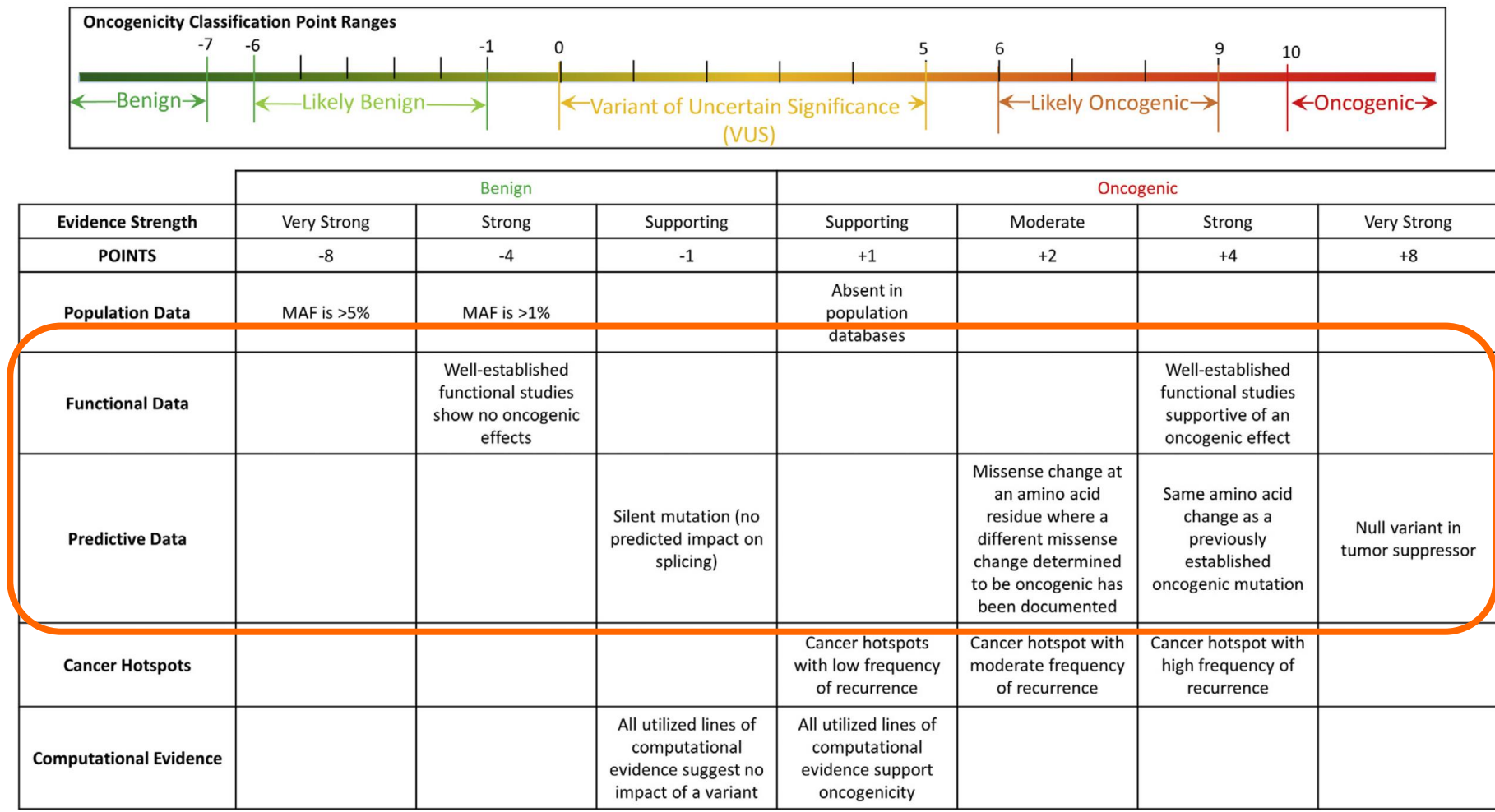
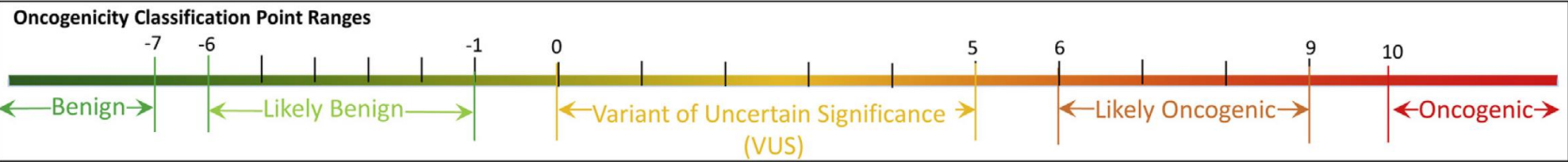


Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

Genet Med. 2022 May;24(5):986-998. doi: 10.1016/j.gim.2022.01.001.



- Bases de Datos específicas de gen:**
- <https://brcaexchange.org/>
 - [https://arup.utah.edu/data base/BRCA/](https://arup.utah.edu/data/base/BRCA/)
 - <https://tp53.isb-cgc.org/>
 - others



	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
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Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			



Predictive algorithms:

Evolutionary conservation: ConSurf, FATHMM, Mutation Assessor, PANTHER, SIFT...

Protein structure: MAPP, AlignGVGD, ...

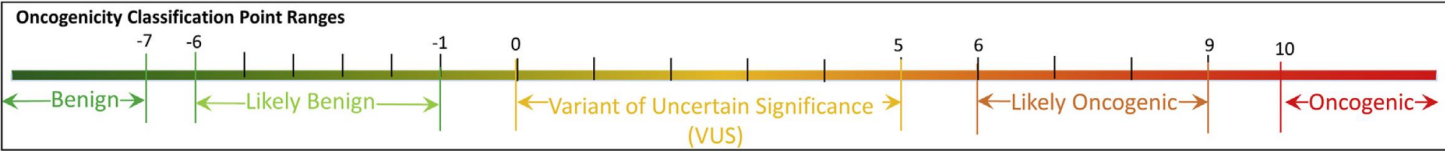
Splice site prediction: GeneSplicer, varSEAK, spliceAI...

Alignment-protein homolog: PROVEAN

Oncogenicity classifications: CADD, VEST-4...

Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

2. Clasificación de la variante (Ejemplo)



Puntuación

	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
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Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			

Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

Genet Med. 2022 May;24(5):986-998. doi: 10.1016/j.gim.2022.01.001.

KIT NM_000222.3:c.1504_1509dup,
p.(Ala502_Tyr503dup)

2. Clasificación de la variante (Ejemplo)

Puntuación

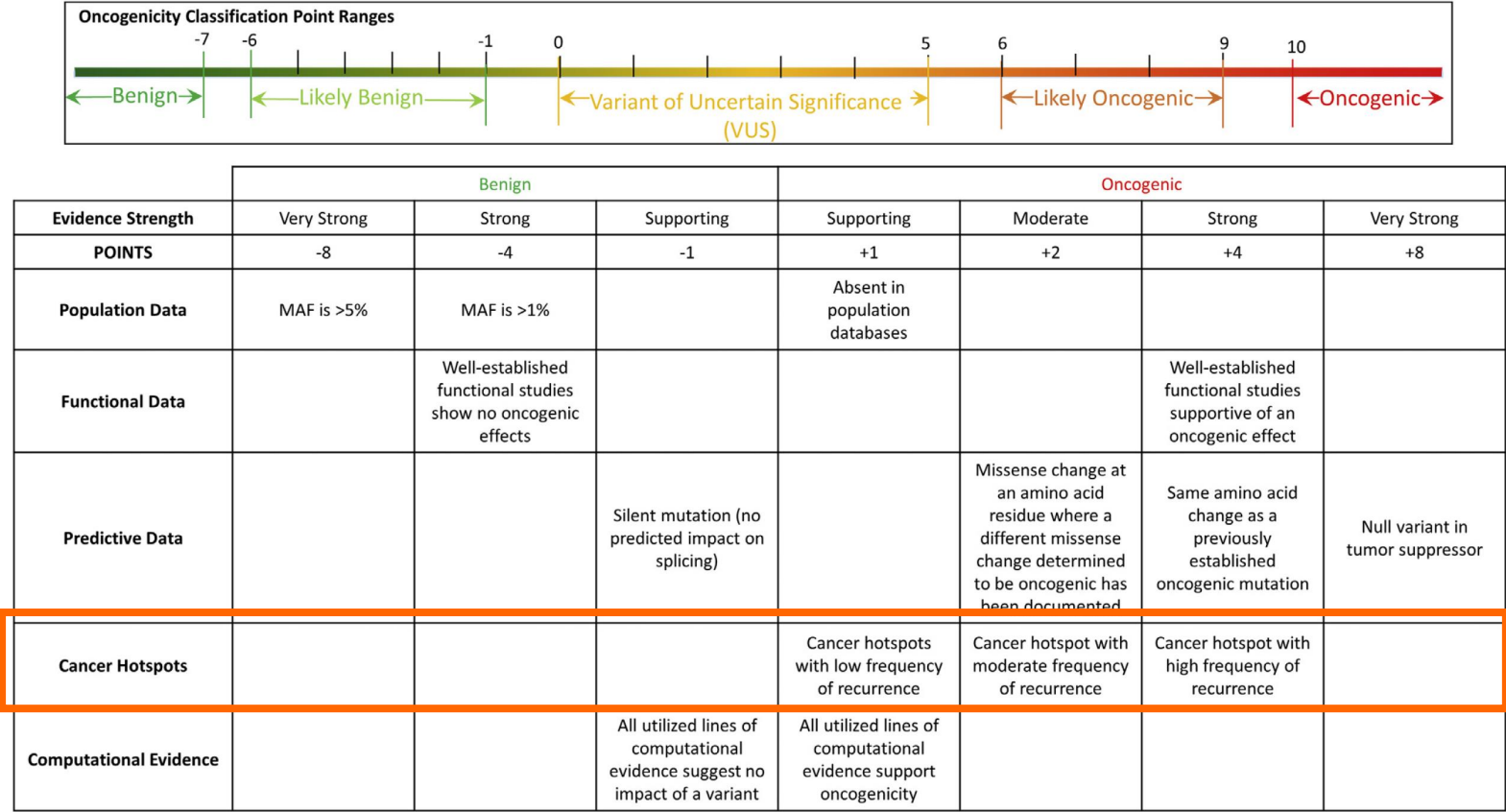


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KIT NM_000222.3:c.1504_1509dup,
p.(Ala502_Tyr503dup)

Base de datos COSMIC

The screenshot shows the COSMIC v98 website. At the top is the COSMIC logo and a navigation bar with links for Projects, Data, Tools, News, Help, About, and Genome Version. A search bar is present with the text 'Search COSMIC...' and a 'SEARCH' button. Below the navigation bar is a red banner with a message about service disruption on September 3rd. The main content area is divided into two columns. The left column features a section titled 'COSMIC v98, released 23-MAY-23' followed by a description of COSMIC as the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer. Below this is a search bar with the text 'eg Braf, COLO-829, Carcinoma, V600E, BRCA-UK, Campbell' and a 'SEARCH' button. The right column is titled 'COSMIC News' and contains three news items, each with a COSMIC logo icon. The first item is 'Topographical features in the genome and how they're helping us understand tumorigenesis', the second is 'Sir Professor Mike Stratton', and the third is 'Mysteries, myths, and malignancy in skin cancers; highlighting three new studies in COSMIC v98'. Below the news section is a 'Tools' section with three links: 'Cancer Browser', 'Genome Browser', and 'GA4GH Beacon'. The bottom of the page features a 'Projects' section with a list of COSMIC projects and their descriptions.

COSMIC
Catalogue Of Somatic Mutations In Cancer

Projects ▾ Data ▾ Tools ▾ News ▾ Help ▾ About ▾ Genome Version ▾ Search COSMIC... **SEARCH** Login ▾

Disruption of COSMIC services from Sunday 3rd September: Please accept our apologies for any inconvenience caused. Our web resources are now back up and running. We will continue to monitor and resolve any subsequent issues over the next few days. Thank you for your patience and support.

COSMIC v98, released 23-MAY-23

COSMIC, the Catalogue Of Somatic Mutations In Cancer, is the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer.

Start using COSMIC by searching for a gene, cancer type, mutation, etc. below.

eg Braf, COLO-829, Carcinoma, V600E, BRCA-UK, Campbell **SEARCH**

Projects

COSMIC is divided into several distinct projects, each presenting a separate dataset or view of our data:

- COSMIC**
The core of COSMIC, an expert-curated database of somatic mutations
- Cell Lines Project**
Mutation profiles of over 1,000 cell lines used in cancer research
- COSMIC-3D**
An interactive view of cancer mutations in the context of 3D structures
- Cancer Gene Census**
A catalogue of genes with mutations that are causally implicated in cancer
- Cancer Mutation Census**
Classification of genetic variants driving cancer
- Actionability**

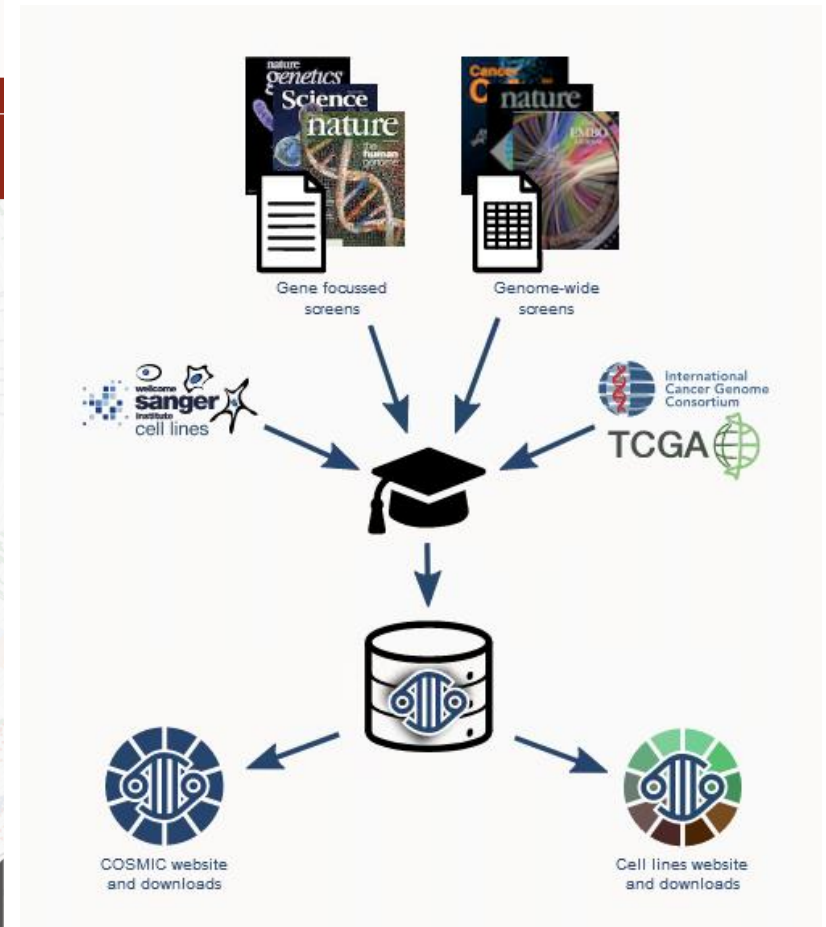
COSMIC News

[Follow @cosmic_sanger](#)

- Topographical features in the genome and how they're helping us understand tumorigenesis**
In a special guest-edition to our blog, Dr Burcak Otlu from the Cancer Grand Challenges Mutographs team talks us through the new topographical analysis data being added to COSMIC following the recent publication of the team's latest paper! [More...](#)
- Sir Professor Mike Stratton**
As he prepared to step down as director of The Sanger Institute we interviewed Sir Professor Mike Stratton. Join us as we reflect on his fascinating career, his role in the early days of COSMIC and his advice for aspiring scientists! [More...](#)
- Mysteries, myths, and malignancy in skin cancers; highlighting three new studies in COSMIC v98**
To celebrate COSMIC v98's release, we took inspiration from our curation team's aim to improve representation of the plethora of rare subtypes of skin cancer and bring you three things you may not know about skin cancer! [More...](#)

Tools

- [Cancer Browser](#) — browse COSMIC data by tissue type and histology
- [Genome Browser](#) — browse the human genome with COSMIC annotations
- [GA4GH Beacon](#) — access COSMIC data through the [GA4GH Beacon Project](#)



2. Clasificación de la variante (Ejemplo)

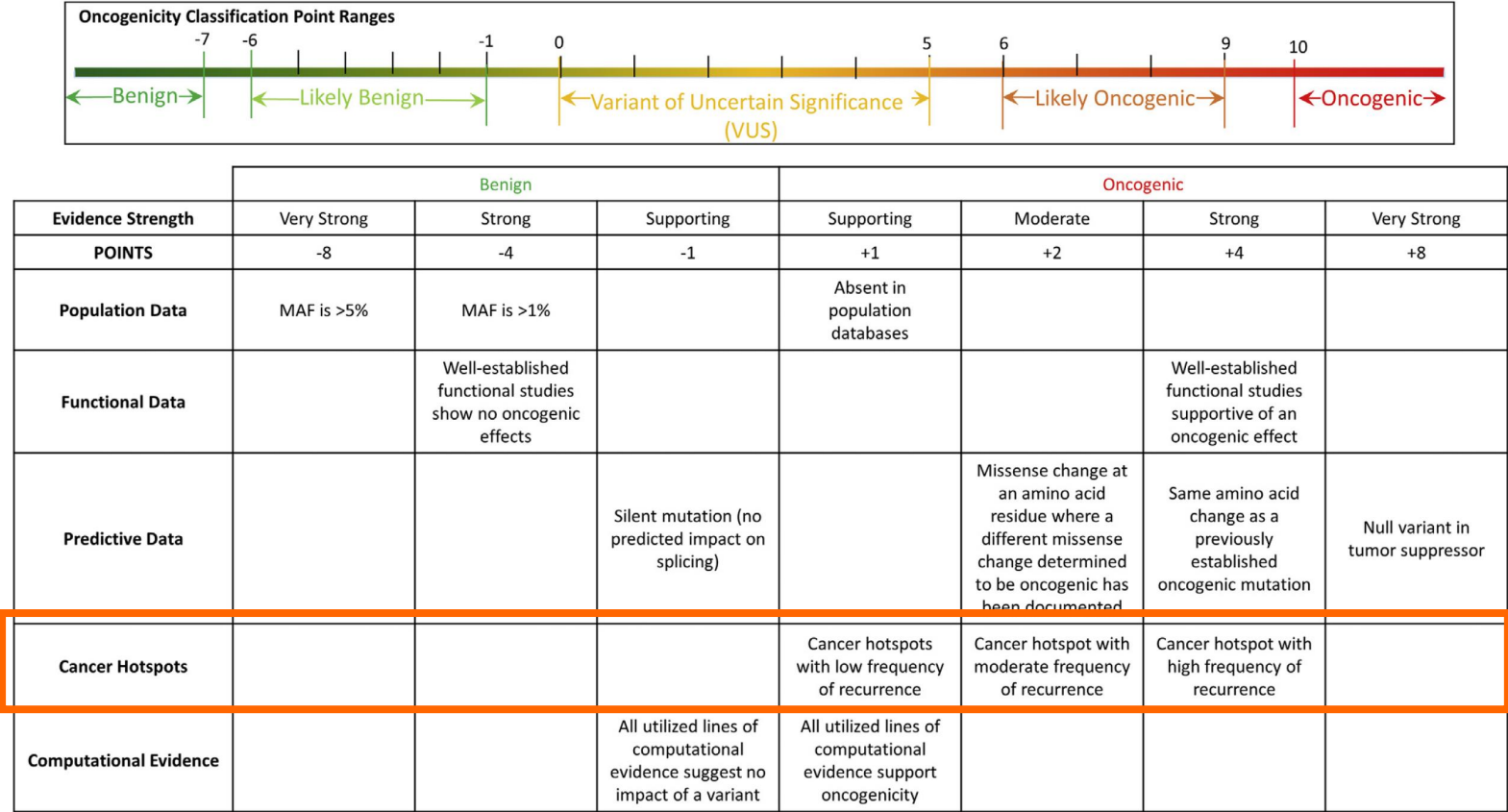
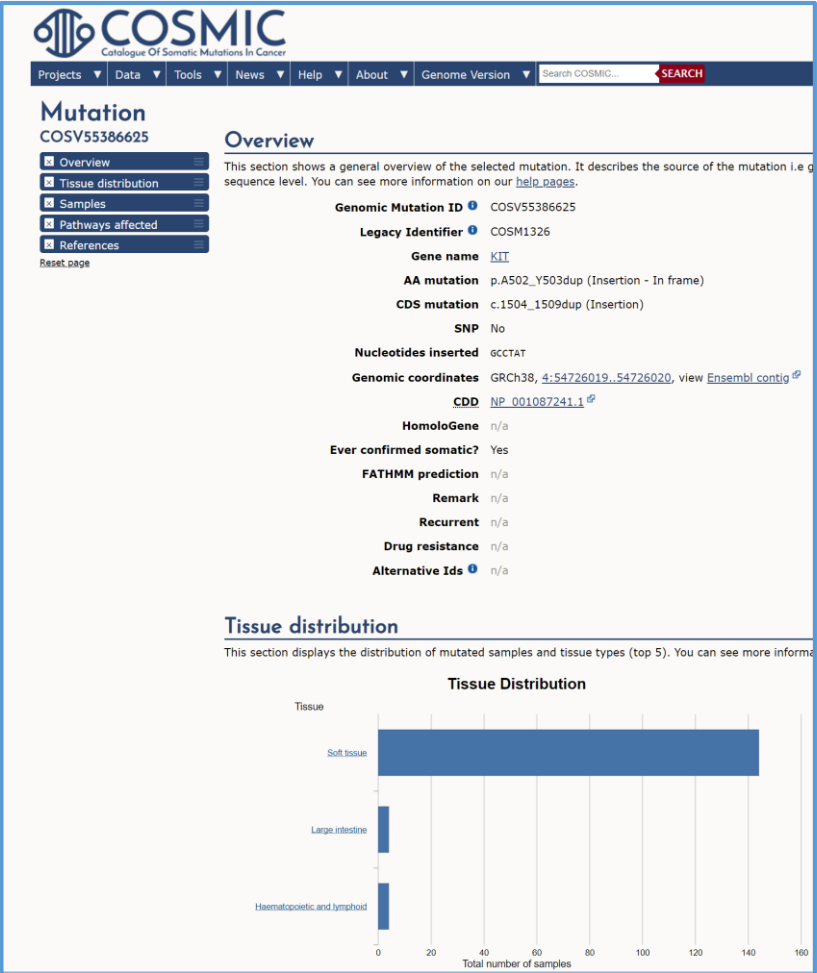


Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

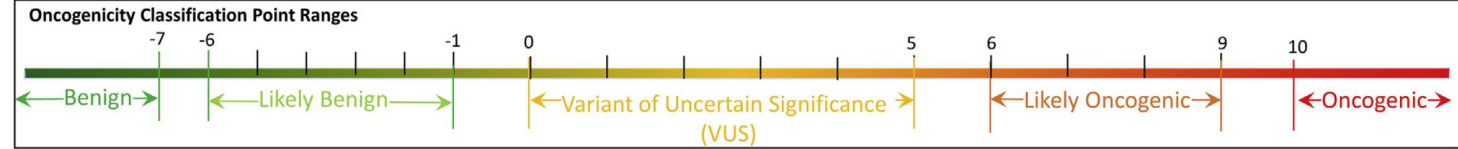
Genet Med. 2022 May;24(5):986-998. doi: 10.1016/j.gim.2022.01.001.

KIT NM_000222.3:c.1504_1509dup,
p.(Ala502_Tyr503dup)



2. Clasificación de la variante (Ejemplo)

Puntuación



	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
Predictive Data			Silent mutation (no predicted impact on splicing)		Missense change at an amino acid residue where a different missense change determined to be oncogenic has been documented	Same amino acid change as a previously established oncogenic mutation	Null variant in tumor suppressor
Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			

+2

Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

Genet Med. 2022 May;24(5):986-998. doi: 10.1016/j.gim.2022.01.001.

KIT NM_000222.3:c.1504_1509dup,
p.(Ala502_Tyr503dup)

2. Clasificación de la variante (Ejemplo)

Oncogene

Evidence

Population

Function

Predictive

Cancer H

Computation

varsome

KIT(NM_000222.3):c.1504_1509dup

hg38

Search

Editions

About

Community

News

Demo

Marta Sese

chr4-54726020--GCCTAT (6bp) (KIT:p.A502_Y503dup)

Link a publication

Classify

☆ Favorites

Copy Shortlink

API Link

Submit to ClinVar

General Information Insertion KIT(NM_000222.3):c.1504_1509dup (p.Ala502_Tyr503dup)	PharmGKB No data available	ACMG Classification Pathogenic	Frequencies Variant not found	Pathogenicity Scores No data available	GWAS No data available	Protein Viewer
Genes KIT	Publications Variant: 3 Gene: 287	ClinVar No data available	MitoMap No data available	Region Browser	Structural Variants	
Community Contributions	Transcripts NM_000222.3 - in frame RefSeq: 8 Ensemble: 17	Uniprot Variants No data available	Conservation Scores phyloP100: 3.372	Expression Data Top: Esophagus - Muscularis Tissues: 51	Beacon Network	

ACMG Classification - Educational use only Version: 11.2.12

Terms of use Documentation Options

Verdict

Pathogenic

NM_000222.3, protein length 977, gene KIT, in frame variant

Users of VarSome Premium benefit from additional data sources included in the automated classification.

Automated criteria

Show summary view

Enable clinical evidence

Pathogenic

PS4 Strong

PM3 Moderate

PP4 Supporting

PP5 Strong

PM1 Moderate

PM2 Moderate

PM4 Moderate

PP3 Supporting

PM5 Moderate

PM6 Moderate

PP1 Supporting

PP2 Supporting

PS1 Strong

PS2 Strong

PS3 Strong

PVS1 Very Strong

Benign

BP2 Supporting

BP5 Supporting

BA1 Stand Alone

BP1 Supporting

BP3 Supporting

BP4 Supporting

BP6 Supporting

BP7 Supporting

BS1 Strong

BS2 Strong

BS3 Strong

BS4 Strong

Rule

Explanation

Show failed criteria

PP5 Strong

Using strength Strong because varSome users have classified this variant as Pathogenic (automatically lifted-over from chr4:55592185_6 insGCCTAT on hg19), citing 22324351, and as Pathogenic (automatically lifted-over from chr4:55592185_6 insGCCTAT on hg19), citing 26158763.

PM1 Moderate

UniProt protein KIT_HUMAN domain 'Ig-like C2-type 5' has 80 missense/in-frame variants (14 pathogenic variants and 66 uncertain variants), pathogenicity = 17.5%, which qualifies as likely pathogenic.

PM2 Moderate

Variant not found in gnomAD exomes (unable to check gnomAD exomes coverage).
Variant not found in gnomAD genomes (with good gnomAD genomes coverage = 31.3).

PM4 Moderate

Protein coding length changes as a result of in frame variant in gene KIT, and is not in a repeat region.

PP3 Supporting

Pathogenic computational verdict based on 1 pathogenic prediction from phyloP vs no benign predictions.

Figure 1
frequency.
Genet Med.

2. Clasificación de la variante (Ejemplo)

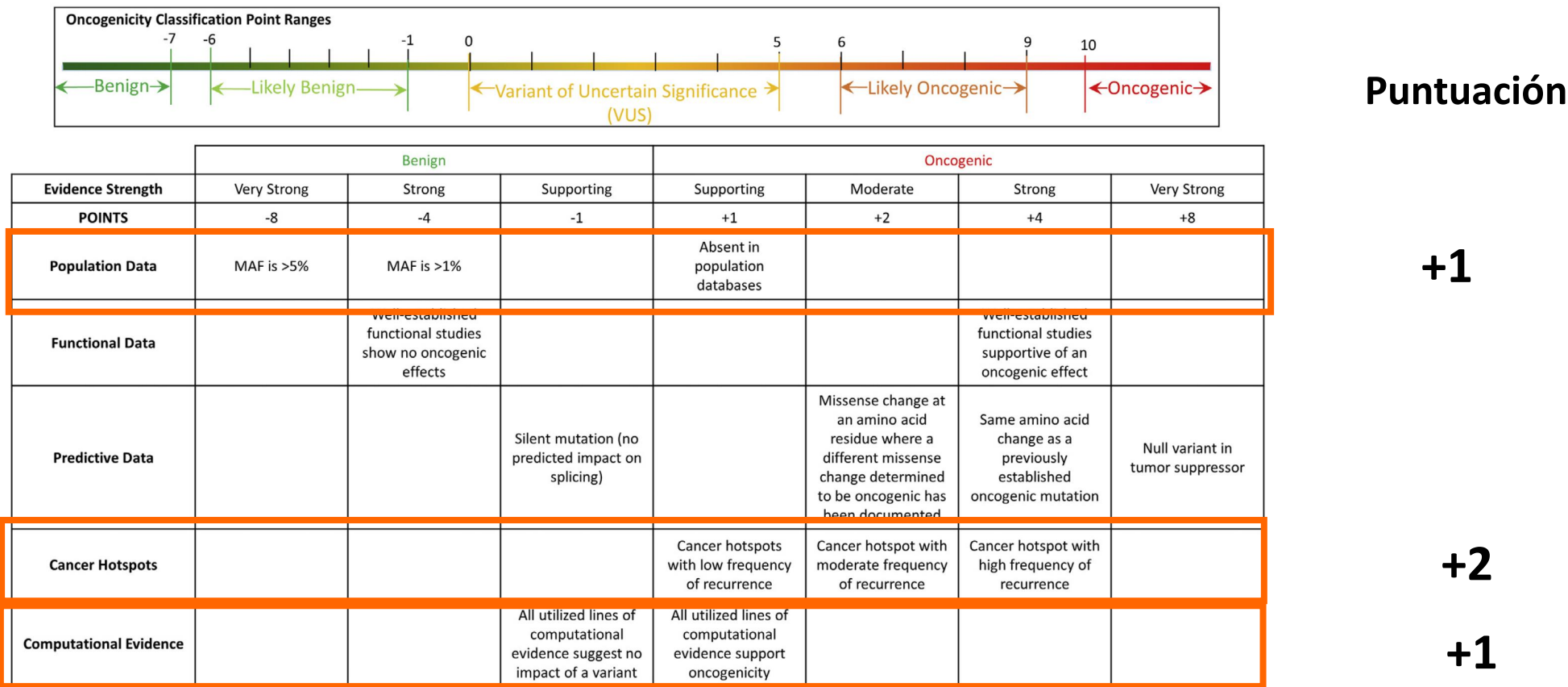


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KIT NM_000222.3:c.1504_1509dup,
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2. Clasificación de la variante (E

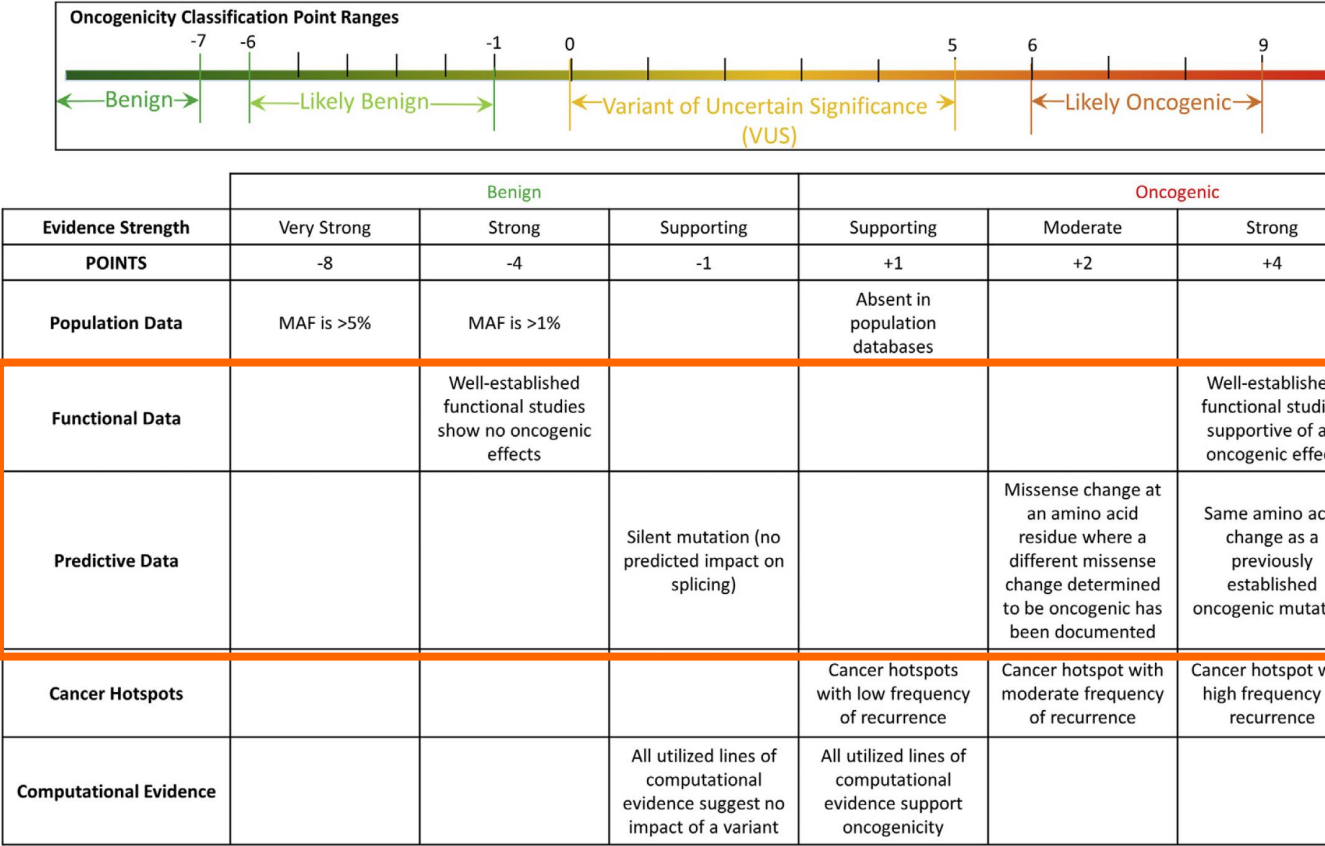


Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed) frequency.

Genet Med. 2022 May;24(5):986-998. doi: 10.1016/j.gim.2022.01.001.

KIT NM_000222.3:c.1504_1509dup,
p.(Ala502_Tyr503dup)

☐

1

[\[Characteristics of Clinicopathology and Genotype in 179 Cases with Gastrointestinal Stromal Tumor\].](#)

Cite

Chen JJ, Shen CY, Chen HJ, Yin Y, Tang SM, Zhang B, Chen ZX.

Share

Sichuan Da Xue Xue Bao Yi Xue Ban. 2016 Mar;47(2):275-8, 282.
PMID: 27263309 Chinese.
Among all the c-**kit** mutation, 92.2% mutation types in exon 11 were deletion mutation, point mutation and hybrid mutations, and in exon 9 the mutation types were just involving **A502_Y503dup** (n = 6) and Y403_F504ins (n = 14), while the mutation type were K642Q ...

☐

2

[Gastrointestinal stromal tumors with **KIT** exon 9 mutations: Update on genotype-phenotype correlation and validation of a high-resolution melting assay for mutational testing.](#)

Cite

Künstlinger H, Huss S, Merkelbach-Bruse S, Binot E, Kleine MA, Loeser H, Mittler J, Hartmann W, Hohenberger P, Reichardt P, Büttner R, Wardelmann E, Schildhaus HU.

Share

Am J Surg Pathol. 2013 Nov;37(11):1648-59. doi: 10.1097/PAS.0b013e3182986b88.
PMID: 24061512
KIT exon 9 mutations in gastrointestinal stromal tumors (GISTs) are highly relevant and have direct therapeutic implications. ...Including these cases and the common p.**A502_Y503dup** mutation, we provide a comprehensive list of all known **KIT** exon 9 mutat ...

☐

3

[Beginning of personalized medicine in Panama: Molecular and pathological characteristics of gastrointestinal stromal tumors from archival paraffin-embedded tissue.](#)

Cite

Mendoza Y, Singh C, Castillo Mewa J, Fonseca E, Smith R, Pascale JM.

Share

Oncol Lett. 2011 Sep 1;2(5):941-947. doi: 10.3892/ol.2011.349. Epub 2011 Jul 5.
PMID: 22866155 Free PMC article.
This is the first study from Central America to analyze genetic mutations and histopathological features associated with gastrointestinal stromal tumors (**GIST**). Mutations found in the tyrosine kinase membrane receptors c-**kit** and pdgfra are associated with clinical a ...

☐

4

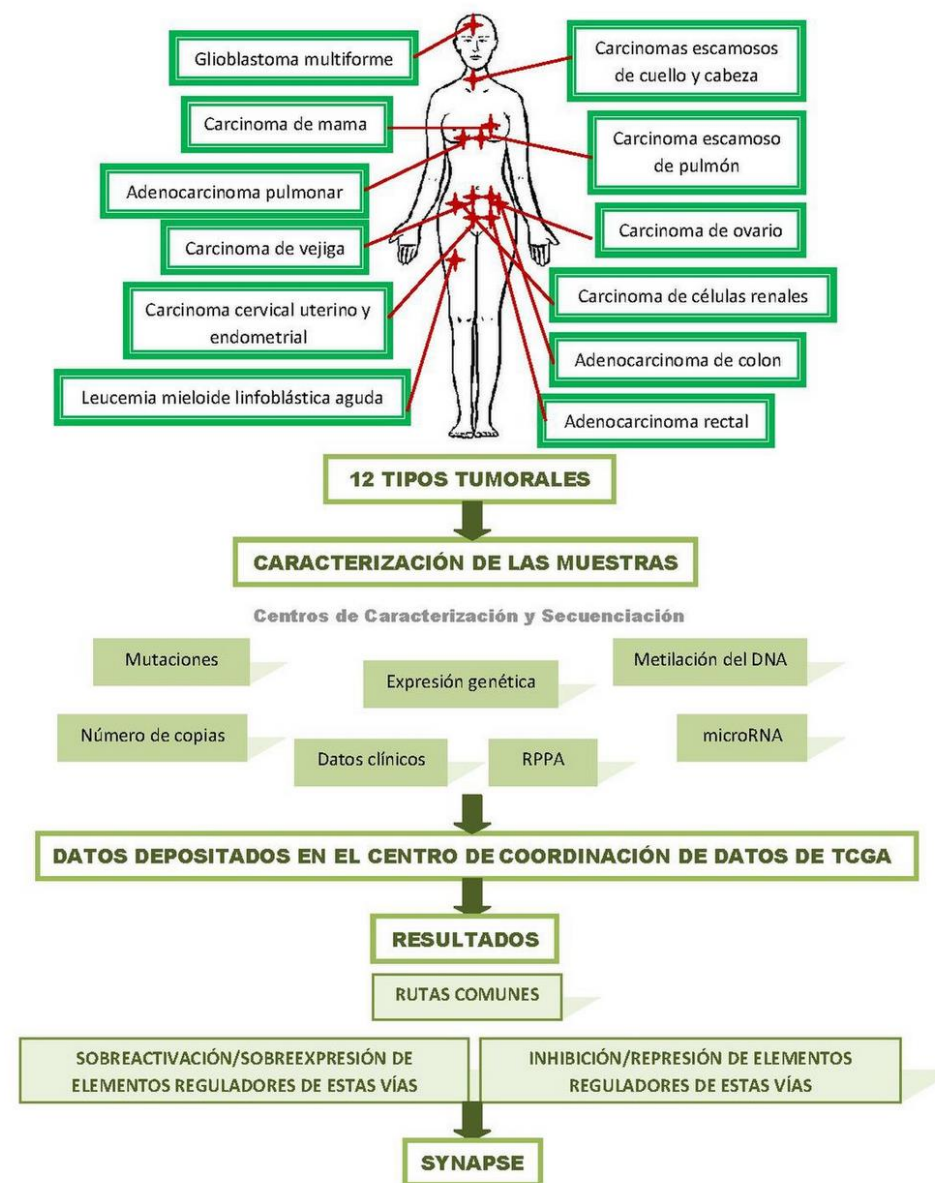
[A case of mast cell leukaemia with exon 9 **KIT** mutation and good response to imatinib.](#)

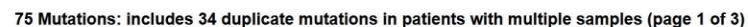
Cite

Mital A, Piskorz A, Lewandowski K, Wasag B, Limon J, Hellmann A.

Share

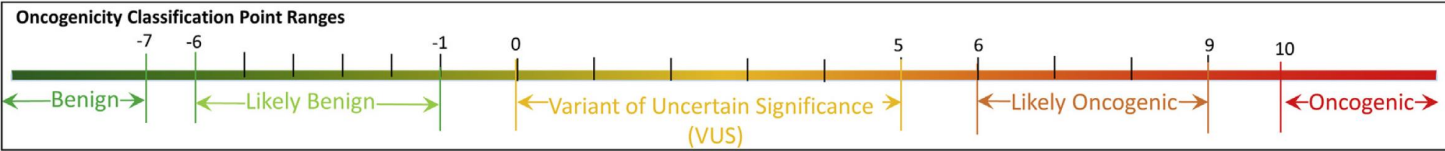
Eur J Haematol. 2011 Jun;86(6):531-5. doi: 10.1111/j.1600-0609.2011.01598.x. Epub 2011 Apr 7.
PMID: 21362052
PATIENT AND METHODS: We present a case of a 56-yr-old female patient with aleukaemic MCL in whom the typical **KIT**-D816V mutation was not detected. Sequencing of the entire coding sequence of **KIT** gene revealed a somatic mutation in exon 9 (p.**A502_Y503dup** ...





Study of Origin	Sample ID	Protein Change	Ann	Biological Effect				Copy #	COSMIC	Allele Freq (T)	# Mut in Sample
				Level	Alteration(s)	Drug(s)	Level-associated cancer type(s)				
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0002680-T01-IM3	A502_Y503dup						Diploid		0.40	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0006901-T01-IM5	A502_Y503dup		1	Oncogenic Mutations	Imatinib	Gastrointestinal Stromal Tumor	Diploid		0.18	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0007230-T01-IM5	A502_Y503dup		1	Oncogenic Mutations	Sunitinib	Gastrointestinal Stromal Tumor	Diploid		0.33	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0007519-T01-IM5	A502_Y503dup		1	Oncogenic Mutations	Regorafenib	Gastrointestinal Stromal Tumor	Diploid		0.33	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0009615-T01-IM5	A502_Y503dup		1	Oncogenic Mutations	Regorafenib	Gastrointestinal Stromal Tumor	Diploid		0.32	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0012459-T02-IM6	A502_Y503dup		1	Oncogenic Mutations	Ripretinib	Gastrointestinal Stromal Tumor	Diploid		0.21	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0013007-T01-IM5	A502_Y503dup						Diploid		0.40	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0013410-T01-IM5	A502_Y503dup			The information above is intended for research purposes only and should not be used as a substitute for professional diagnosis and treatment.			Amp		0.59	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0016848-T01-IM6	A502_Y503dup			Levels of Evidence			Diploid		0.23	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0017729-T02-IM6	A502_Y503dup			OncKB			Diploid		0.35	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0018225-T01-IM6	A502_Y503dup					Feedback	Diploid		0.38	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0018583-T03-IM6	A502_Y503dup						Diploid		0.34	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0018913-T02-IM6	A502_Y503dup						Diploid		0.80	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0020724-T01-IM6	A502_Y503dup						Diploid		0.41	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0022237-T01-IM6	A502_Y503dup						Diploid		0.39	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0023549-T01-IM6	A502_Y503dup						Diploid		0.36	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0027083-T02-IM6	A502_Y503dup						Diploid		0.32	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0028383-T01-IM6	A502_Y503dup						Diploid		0.33	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0029524-T01-IM6	A502_Y503dup						Diploid		0.35	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0031415-T02-IM7	A502_Y503dup						Diploid		0.34	4
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0033352-T01-IM6	A502_Y503dup						Diploid		0.29	1
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0034094-T01-IM6	A502_Y503dup						Diploid		0.41	3
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0034265-T01-IM6	A502_Y503dup						Diploid		0.36	6
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0034856-T01-IM6	A502_Y503dup						Diploid		0.24	2
Gastrointestinal Stromal Tumor (MSK, NPJ Prec...	P-0036219-T01-IM6	A502_Y503dup						Diploid		0.35	

2. Clasificación de la variante (Ejemplo)



Puntuación

	Benign			Oncogenic			
Evidence Strength	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
Predictive Data			Silent mutation (no predicted impact on splicing)		Missense change at an amino acid residue where a different missense change determined to be oncogenic has been documented	Same amino acid change as a previously established oncogenic mutation	Null variant in tumor suppressor
Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			

+1

+4

+4

+2

+1

+12

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KIT NM_000222.3:c.1504_1509dup,
p.(Ala502_Tyr503dup)

SPECIAL ARTICLE

Germline-focused analysis of tumour-detected variants in 49,264 cancer patients: ESMO Precision Medicine Working Group recommendations

Z. Kuzbari^{1†}, C. Bandlamudi^{2†}, C. Loveday¹, A. Garrett¹, M. Mehine², A. George^{1,3}, H. Hanson^{1,4}, K. Snape⁴, A. Kulkarni⁵, S. Allen¹, S. Jezdic⁶, R. Ferrandino⁶, C. B. Westphalen⁷, E. Castro⁸, J. Rodon⁹, J. Mateo^{10,11}, G. J. Burghel¹², M. F. Berger², D. Mandelker^{2†} & C. Turnbull^{1,3*†}

Box 1. Recommendations for genes for inclusion for germline-focused analysis and follow-up

CSG actionability class		All ages			Age <30
Most	BRCA1 BRCA2	MLH1 MSH2	MSH6 PALB2 RET		
High	BRIP1 MUTYH ^c PMS2 RAD51C	RAD51D SDHAF2 ^d SDHB SDHC ^d	SDHD ^d TMEM127 ^d TSC2 ^f VHL ^a	APC PTEN ^{d,f} RB1 TP53 ^{b,f}	
Standard	ATM BAP1 ^f BARD1 CHEK2 DICER1	FH FLCN NF1 ^f PTCH1 ^e POLD1	POLE SDHA SMAD3 ^e SMARCB1 ^{e,f} SUFU ^e	CDKN2A SMARCA4	

Molecular NGS Report

Identification

1. Patients and Doctor who ordered the test
2. Pathological diagnosis
3. Type of specimen (timing, data collection, treatment origin, medium (FFPE, Frozen, fresh...))

Minimal Content

1. List of genes included in the panel used and coverage
2. Panel sensitivity limits
3. Pathological evaluation of sample adequacy and quality
4. Variant allele frequency
5. Human Genome Variation Society nomenclature
6. Results reported accurately, clearly, unambiguously
7. Include interpretation of results, where appropriate.

Additional considerations

1. Include statement around probability of the cancer response to targeted therapy.
2. Provide the report within 5-10 working days
3. Test results should be discussed at the MDTB-MTB
4. Tumor content



Vall d'Hebron
Hospital
Servei d'Anatomia Patològica



Vall
d'Hebron
Barcelona Campus Hospitalari

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS
MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ PACIENT, SOL·LICITANT

Centre sol·licitant:
Metge sol·licitant:

Nom del Pacient:
Data Naixement:
CIP Pacient:
Referència d'origen:
Indicació: Adenocarcinoma metastàtic de pulmó – 2a línia de tractament

S'assumeix la possessió del consentiment informat per part del sol·licitant.

INFORMACIÓ MOSTRA

Numero Biòpsia Vall Hebron:
Origen de la mostra: Pulmó
Tipus de mostra: Bloc de parafina
Referència DNA: Mostra Valorable
Referència RNA: Mostra Valorable
Contingut tumoral: 70%

RESULTATS

1. Variants ACCIONABLES

Mutacions puntuals (SNV)/ Indels					
Gen	regió	Descripció de la variant	Freqüència al·lèlica	Classificació	Accionabilitat (Tier)
EGFR	Exó 21	NM_005228.5:c.2573T>G, p.(Leu858Arg)	27,6%	Patogènica	1

2. ALTRES alteracions

Mutacions puntuals (SNV)/ Indels					
Gen	regió	Descripció de la variant	Freqüència al·lèlica	Classificació	Accionabilitat (Tier)
TP53	Exó 7	NM_000546.5:c.722C>T; p.(Ser241Phe)	43,5%	Patogènica	-

3. Variants DE SIGNIFICAT INCERT

No s'han detectat altres alteracions de significat incert

*Les VUS no estan ben caracteritzades en la literatura científica en la data d'elaboració de l'informe. Aquestes variants s'inclouen al mateix en cas que puguin ser clínicament rellevants en un futur.

ESTUDI DE BIOMARCADORS EN TUMORS PEDIÀTRICS
MITJANÇANT SEQUÈNCIACIÓ MASSIVA (NGS)

INFORMACIÓ PACIENT, SOL-LICITANT

Origen de la mostra: parts toves
Tipus de mostra: FFPE
Indicació: Sarcoma de Ewing
Referència DNA: 21/XXX, Mostra Valorable
Referència RNA: 21/XXX, Mostra Valorable
Contingut tumoral: 60%

S'assumeix la possessió del consentiment informat per part del sol·licitant.

RESULTATS

1. Variants Patogèniques (SNV/Indel)

Gen	regió	Descripció de la variant	Freqüència al·lèlica	Classificació	<u>Accionabilitat (Tier)</u>
KRAS	Exó 2	NM_033360.4; c.35G>C; p.(Gly12Ala)	30.5%	Patogènica	1

2. Alteracions en el Nombre de Còpies (CNV)

Gen	Alteració	Estimació nombre de còpies	Classificació	<u>Accionabilitat (Tier)</u>
ERBB2	Amplificació	11.36	Patogènica	-

3. Fusions i altres variants estructurals

Gen	<u>Reordenament (3'/5'-desequilibri)</u>	Gen de fusió	Locus	<u>Nº de Lectures</u>
EWSR1	-	EWSR1(7) - FLI1(6)	chr22:29683123- chr11:128675261	28.968

COMENTARIS I RECOMANACIONS

[Xxxx](#)

METODOLOGIA

Panell NGS [Oncomine](#) Childhood Research Assay ([ThermoFisher](#) Scientific)

Llistat de gens estudiats

Gens amb cobertura [Hotspot](#): ABL1, ABL2, ALK, ACVR1, AKT1, ASXL1, ASXL2, BRAF, CALR, CBL, CCND1, CCND3, CCR5, CDK4, CIC, CREBBP, CRLF2, CSF1R, CSF3R, CTNNB1, DAXX, DNMT3A, EGFR, EP300, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FASLG, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GATA2, GNA11, GNAQ, H3F3A, HDAC9, HIST1H3B, HRAS, IDH1, IDH2, IL7R, JAK1, JAK2, JAK3, KDM4C, KDR, KIT, KRAS, MAP2K1, MAP2K2, MET, MPL, MSH6, MTOR, MYC, MYCN, NCOR2, NOTCH1,

NPM1, NRAS, NT5C2, PAX5, PDGFRA, PDGFRB, PIK3CA, PIK3R1, PPM1D, PTPN11, RAF1, RET, RHOA, SETBP1, SETD2, SH2B3, SH2D1A, SMO, STAT3, STAT5B, TERT, TPMT, USP7

Gens amb cobertura completa: APC, ARID1A, ARID1B, ATRX, CDKN2A, CDKN2B, CEBPA, CHD7, CRLF1, DDX3X, DICER1, EBF1, EED, FAS, GATA1, GATA3, GNA13, ID3, IKZF1, KDM6A, KMT2D, MYOD1, NF1, NF2, PHF6, PRPS1, PSMB5, PTCH1, PTEN, RB1, RUNX1, SMARCA4, SMARCB1, SOCS2, SUFU, SUZ12, TCF3, TET2, TP53, TSC1, TSC2, WHSC1, WT1, XIAP

CNV (amplificació nombre de còpies): ABL2, ALK, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, ERBB3, FGFR1, FGFR2, FGFR3, FGFR4, GLI1, GLI2, IGF1R, JAK1, JAK2, JAK3, KIT, KRAS, MDM2, MDM4, MET, MYC, MYCN, PDGFRA, PIK3CA

Fusions Gèniques: ABL1, ABL2, AFF3, ALK, BCL11B, BCOR, BCR, BRAF, CAMTA1, CCND1, CIC, CREBBP, CRLF2, CSF1R, DUSP22, EGFR, ETV6, EWSR1, FGFR1, FGFR2, FGFR3, FLT3, FOSB, FUS, GLI1, GLIS2, HMGA2, JAK2, KAT6A, KMT2A, KMT2B, KMT2C, KMT2D, LMO2, MAML2, MAN2B1, MECOM, MEF2D, MET, MKL1, MLLT10, MN1, MYB, MYBL1, MYH11, MYH9, NCOA2, NCOR1, NOTCH1, NOTCH2, NOTCH4, NPM1, NR4A3, NTRK1, NTRK2, NTRK3, NUP214, NUP98, NUTM1, NUTM2B, PAX3, PAX5, PAX7, PDGFB, PDGFRA, PDGFRB, PLAG1, RAF1, RANBP17, RARA, RECK, RELA, RET, ROS1, RUNX1, SS18, SSBP2, STAG2, STAT6, TAL1, TCF3, TFE3, TP63, TSLP, TSPAN4, UBTX, USP6, WHSC1, YAP1, ZMYND11, ZNF384

Genoma de referència Homo [sapiens](#) GRCh37/hg19
Sensibilitat Variants puntuals: freqüència al·lèlica de la variant: $\geq 5\%$
[CNVs](#): > 8 còpies
Gens de fusió: [trànscripts](#) que representin ≥ 100 lectures
Software Torrent Suite 5.12.1 i [Ion](#) Reporter 5.18.0.2
Classificació de les variants No s'informen les variants de significat incert, benignes o probablement benignes.

LIMITACIONS I CONSIDERACIONS

- Existeixen regions del genoma amb característiques que no permeten determinar amb exactitud els canvis en la seva seqüència (com regions repetitives, homopolimers o regions riques en GC).
- La presència de polimorfismes poc freqüents podria produir que alguna regió del DNA no es pogués analitzar correctament.
- Les variants [descrites](#) en població general amb una MAF superior al 1% no es reporten
- Poden existir variants reals en la mostra que no s'hagin informat per estar sota el llindar de sensibilitat establert.
- Poden existir variants amb significat clínic en altres gens no inclosos en aquest estudi. No s'han estudiat variants en el DNA no codificant, pseudogens, expansions repetitives de [triplets](#) o alteracions epigenètiques.
- Aquesta metodologia només permet identificar els gens de fusió inclosos en el panell. No permet detectar grans insercions i delecions.
- No s'han confirmat les variants per metodologies alternatives.
- L'estudi s'ha realitzat a partir de mostra tumoral i no s'han realitzat estudis específics per a descartar o confirmar si les alteracions trobades són somàtiques o germinals.
- La interpretació de variants es realitza en funció del coneixement existent en el moment d'emissió d'aquest informe. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

CLÀUSULA D'EXEMPCIÓ DE RESPONSABILITAT DIAGNÒSTICA

Els estudis genètics de marcadors moleculars mitjançant sequenciació massiva (NGS) estan destinats exclusivament a professionals de la salut qualificats per a la seva interpretació. Els resultats obtinguts mitjançant aquests estudis i la informació que es pugui derivar dels mateixos no poden ser considerats en cap cas com a substitutius del consell diagnòstic o tractament mèdic d'un professional especialitzat, ni constitueixen per si mateixos una consulta mèdica. Els resultats obtinguts han de ser interpretats, juntament amb altres dades clíniques, dins del context general d'una consulta mèdica dirigida per professionals especialistes en diagnòstic genètic i/o clínic. L'Hospital Universitari Vall d'Hebron no es fa responsable de l'ús que es realitzi dels resultats obtinguts mitjançant aquests estudis, així com tampoc de les eventuais conseqüències perjudicials derivades d'aquest ús, fent expressa reserva d'exercir les accions legals oportunes en el supòsit d'un ús indegut dels citats estudis. Les dades obtingudes en aquest estudi són confidencials i es regulen segons la Llei de Protecció de Dades Personals i garantia dels drets digitals (Llei orgànica 3/2018, de 5 de desembre).

Barcelona, a 21 de gener de 2021.

Validació Facultativa

Dra. Marta [Sesé](#)
Dr. Javier Hernández [Losa](#)

Siguientes pasos

- Nueva convocatoria ejercicio 2024
- 52 centros participantes



Centro Participante	Ciudad
BASURTUKO OSPITALEA	Bilbao
CIMA	Pamplona
Complejo Asistencial Universitario de León	León
Complejo Hospitalario Universitario A Coruña (CHUAC)	A Coruña
Consorti Corporació Sanitària Parc Taulí	Barcelona
Consortio Hospital General Universitario de Valencia	Valencia
Fundación Instituto de Investigación Sanitaria de Santiago de Compostela	Santiago Compostela
HCU VIRGE DE LA ARRIXACA	Murcia
Hospital Clinic de Barcelona	Barcelona
Hospital Clínico Universitario de Valencia / INCLIVA	Valencia
Hospital Clinico Universitario Lozano Blesa	Zaragoza
Hospital de Girona	Girona
Hospital de la Santa Creu i Sant Pau	Barcelona
Hospital del mar	Barcelona
Hospital Dr Peset	Valencia
Hospital General Universitario de Albacete	Albacete
Hospital General Universitario de Castellón	Castellón
Hospital Joan XXIII Tarragona	Barcelona
Hospital La Fe de Valencia	Valencia
Hospital Mutua Terrassa	Barcelona
Hospital Regional Universitario de Málaga	Málaga
Hospital San Cecilio de Granada	Granada
Hospital Universitari de Bellvitge	Barcelona
Hospital Universitari Germans Trias i Pujol	Barcelona
Hospital Universitari Vall d'Hebron	Barcelona
Hospital Universitario 12 de Octubre	Madrid
Hospital Universitario de Burgos	Burgos
HOSPITAL UNIVERSITARIO DE CACERES	Caceres
Hospital Universitario de Cruces	Bilbao
Hospital Universitario de Fuenlabrada	Madrid
Hospital Universitario de Gran Canaria Doctor Negrín	Las Palmas Gran Canaria
Hospital Universitario de Jaén	Jaen
Hospital Universitario de Jerez de la Frontera	Cadiz
Hospital Universitario de Toledo	Toledo
Hospital Universitario Fundación Jiménez Díaz	Madrid
Hospital Universitario Marqués de Valdecilla	Santander
Hospital Universitario Puerta del Mar	Cadiz
Hospital Universitario Reina Sofía de Córdoba	Cordoba
Hospital Universitario Río Hortega (Valladolid)	Valladolid
Hospital universitario Son Espases	Mallorca
Hospital Universitario Virgen del Rocío - IBIS, Sevilla	Sevilla
Hospital Universitario Virgen Macarena	Sevilla
Hospital Universitario Ramon y Cajal	Madrid
Laboratorio de Biología Molecular del Cáncer (LBMC). CIMES. Málaga	Malaga

Coordinadores de nodos

Beatriz Bellosillo. Hospital del Mar. Barcelona (Zona 1)

Javier Hernandez Losa. Hospital Univ. Vall d'hebron. Barcelona (Zona 1)

Javier Freire. Hospital Marqués de Valdecilla. Santander. (Zona 2)

Ihab abdulkader. Complejo Hospitalario (CHUS). Santiago (Zona 2)

Federico Rojo. Fundación Jimenez Diaz. Madrid (Zona 3)

Sarai Palanca. Hospital la Fe de Valencia (Zona 3)

Michele Biscuola. Hospital Univ. Virgen del Rocio. Sevilla (Zona 4)

Miguel Carnero (Hospital Universitario Dr. Negrin. Canarias (Zona 4)



Fase 01: Evaluación de casos y reunion local en cada una de las areas .

Fase 02: Reunión de coordinadores y representantes de Oncología Médica de cada región

Análisis de resultados obtenidos en cada una de las zonas

Establecer un consenso de la actividad

Publicar guía consenso en la revista de Anatomía Patológica.



ACTIVIDAD INFORMES NGS-SEAP



15/01/2024

Inscripciones

Preparación de hoja de inscripción a participantes.



14/02/2024

Final Inscripciones

Subida de documentación a la Web
Excel + vcfs



12/03/2024

Envío informes NGS laboratorios

Envío de los informes de los casos por parte de laboratorios.



Abril 2024

Reunión Local de análisis resultados

Presentación de casos y análisis de informes.



Abril-Mayo

Reunión coordinadores

Reunion global de análisis de resultados.
Redacción documento consenso

Agenda

- Como funciona la NGS en sistema automatizado.
- ¿Como se analizan los datos y consulta de Bases de Datos?.
- **Analisis en linea germinal vs linea somatica**
- Casos del comite molecular de tumores



Definición de VAF

Variant allele frequency (VAF)

VAF is the **percentage of sequence reads observed matching a specific DNA variant divided by the overall coverage at that locus**. Because NGS provides a near random sample, VAF is thus a surrogate measure of the proportion of DNA molecules in the original specimen carrying the variant. For constitutional genetic testing, VAF is a measure of diploid zygosity: heterozygous loci should be near 50% VAF, homozygous loci should be near 100%, and reference loci should be near 0%. Deviations from these three expected values should be considered suspicious as potential errors due to incorrect base calls or alignment. For somatic testing, contamination from normal cells and tumor heterogeneity combine to cause unpredictable VAFs. In some clinical scenarios, such as testing a patient for therapeutic resistance mutations, the desired sensitivity dictates that variants with low VAF be included in downstream analysis. This all means that in order to maintain an acceptable level of sensitivity, variant filtering for somatic variant must be highly permissive from a technical validity perspective.

$$\frac{\text{Nº lecturas variante mutante en DNA}}{\text{Nº lecturas totales en la NGS}}$$

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BRAF **V600E**: GTG > G**A**G

$$\frac{\text{Nº reads GAG (2.350)}}{\text{Nº reads GTG (4.550) + Nº reads GAG (2.350) and others}}$$

VAF
34%

Definición de VAF

Variant allele frequency (VAF)

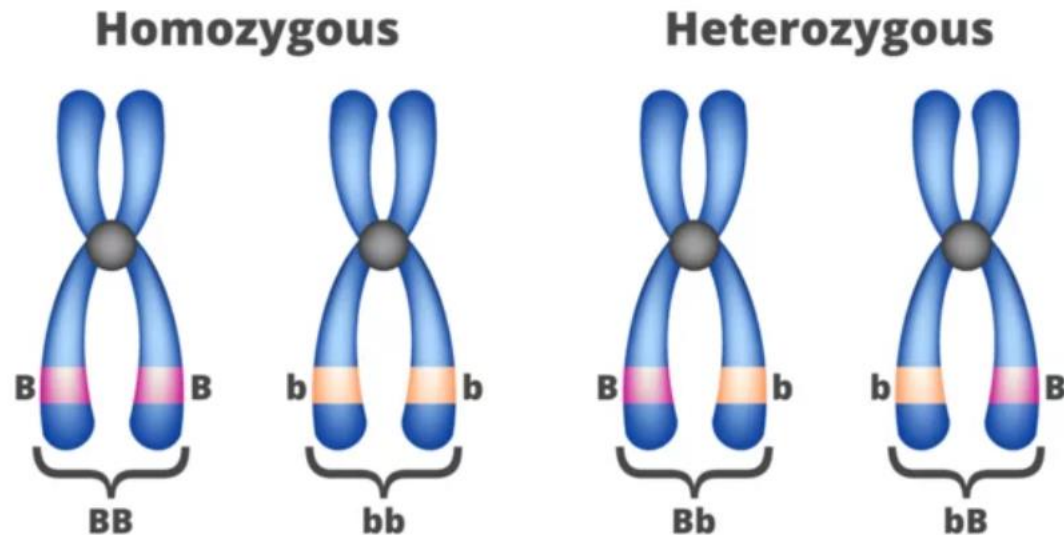
VAF is the percentage of sequence reads observed matching a specific DNA variant divided by the overall coverage at that locus. Because NGS provides a near random sample, VAF is thus a surrogate measure of the proportion of DNA molecules in the original specimen carrying the variant. **For constitutional genetic testing, VAF is a measure of diploid zygosity: heterozygous loci should be near 50% VAF, homozygous loci should be near 100%, and reference loci should be near 0%.** Deviations from these three expected values should be considered suspicious as potential errors due to incorrect base calls or alignment. For somatic testing, contamination from normal cells and tumor heterogeneity combine to cause unpredictable VAFs. In some clinical scenarios, such as testing a patient for therapeutic resistance mutations, the desired sensitivity dictates that variants with low VAF be included in downstream analysis. This all means that in order to maintain an acceptable level of sensitivity, variant filtering for somatic variant must be highly permissive from a technical validity perspective.

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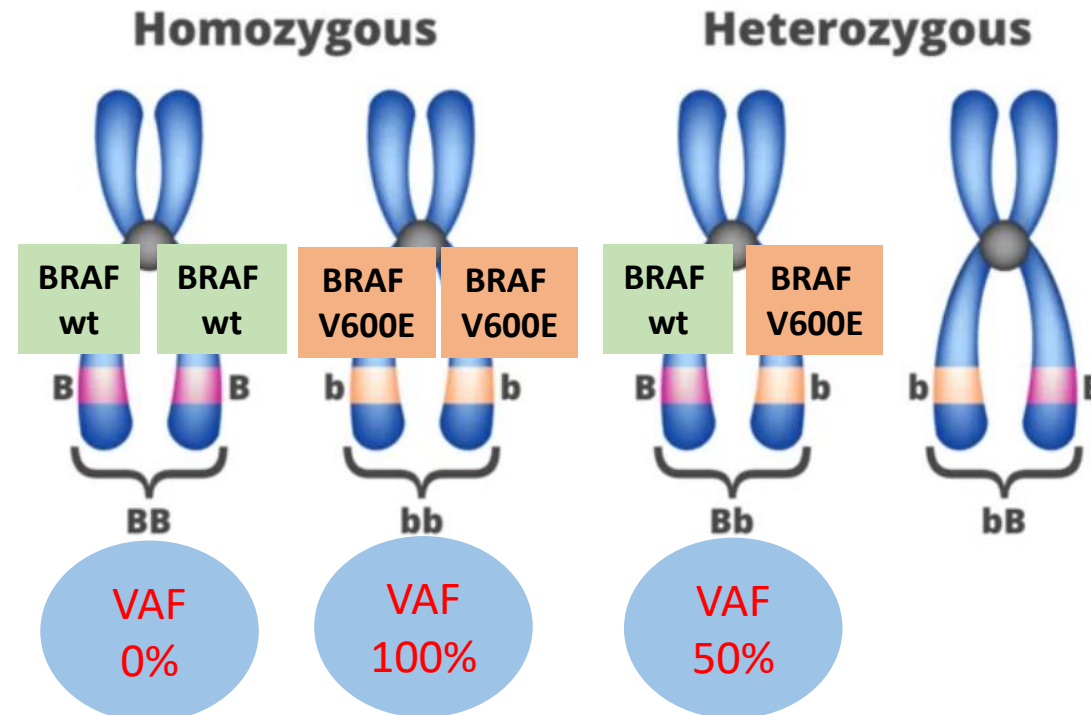
1 GEN → 2 ALELOS



Definición de VAF

Variant allele frequency (VAF)

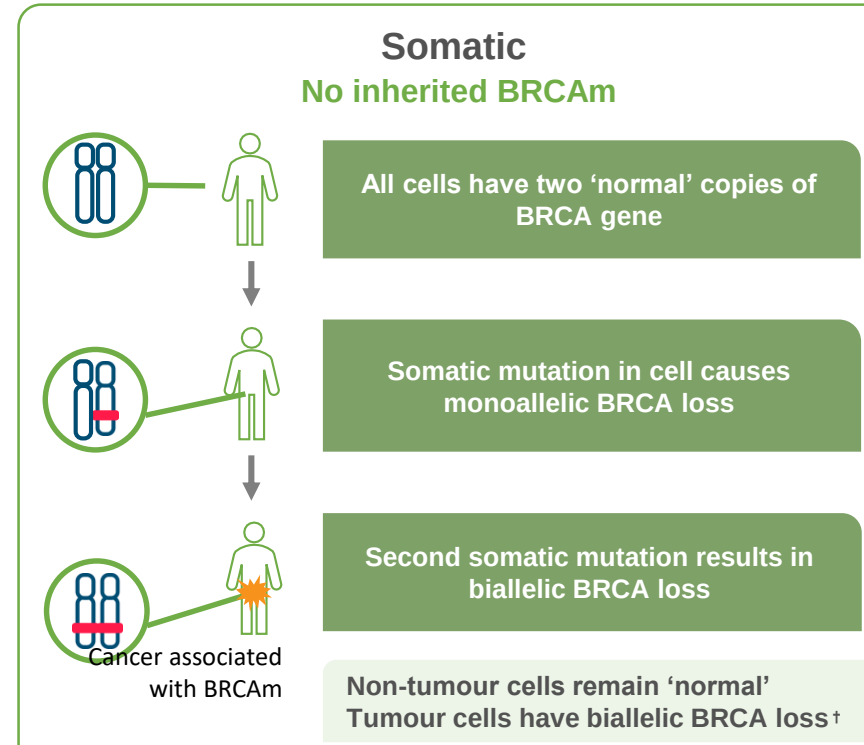
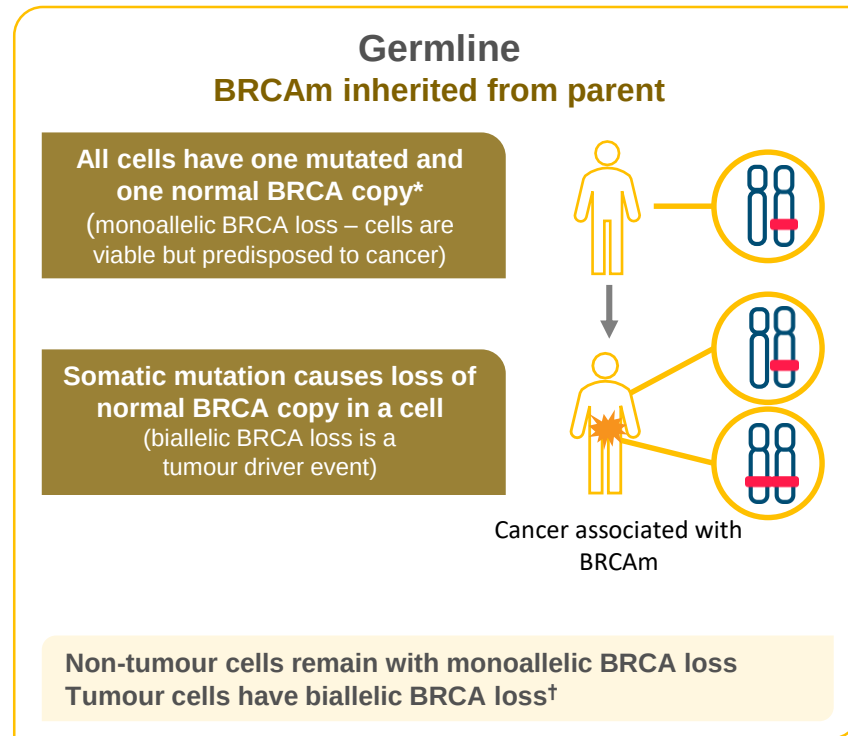
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1 GEN → 2 ALELOS

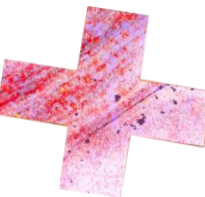
Tumour development can arise from germline or somatic gene alterations¹⁻⁷

BRCA mutations provide a good example of this^{1,2}*



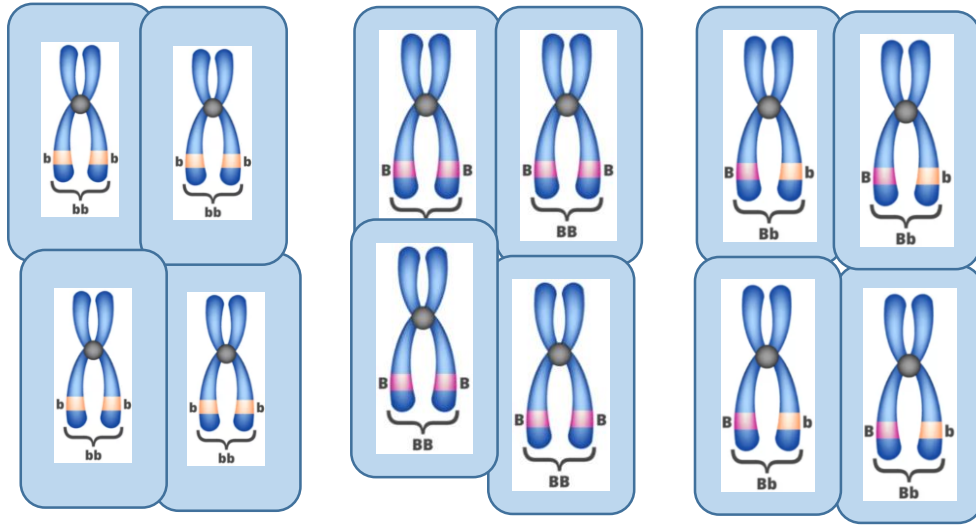
*Can be either *BRCA1* or *BRCA2*. †Loss of function can also result from epigenetic and other non-genomic mechanisms. *BRCA1/2*=breast cancer gene 1/2; BRCAm=breast cancer gene 1/2 mutation

1. Wu H et al. *Gene Ther.* 2017;24:601–609; 2. Castro E et al. *Asian J Androl.* 2012;14:409–414; 3. Macedo GS et al. *Genet Mol Biol.* 2019;42(1suppl):215–231; 4. Ryland G et al. *BMC Medical Genomics.* 2015;8:45; 5. Stoppa-Lyonnet D. *European J Human Genet.* 2016;24:S3–S9; 6. Tucker T & Friedmaenet. 2002;62:345–357; 7. Hunt JL. *Cell & Tissue Based Mol Pathol.* 2009



VAF somáticas vs. VAF germinales

b = wild type
B = mutado



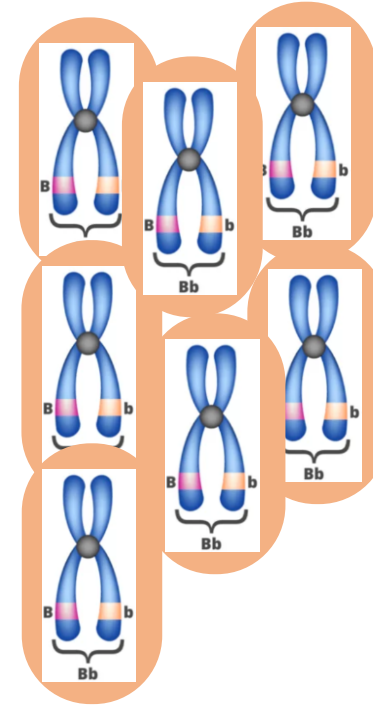
0%

100%

50%

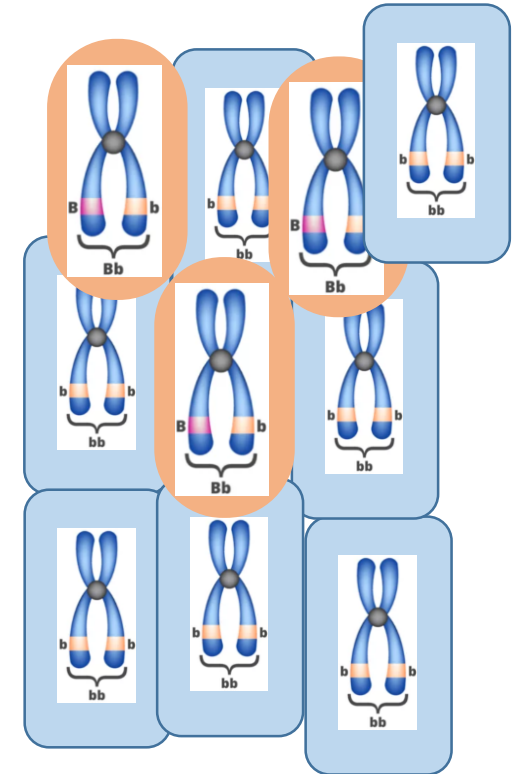
Germline VAF

Contenido tumoral: 100%



50%

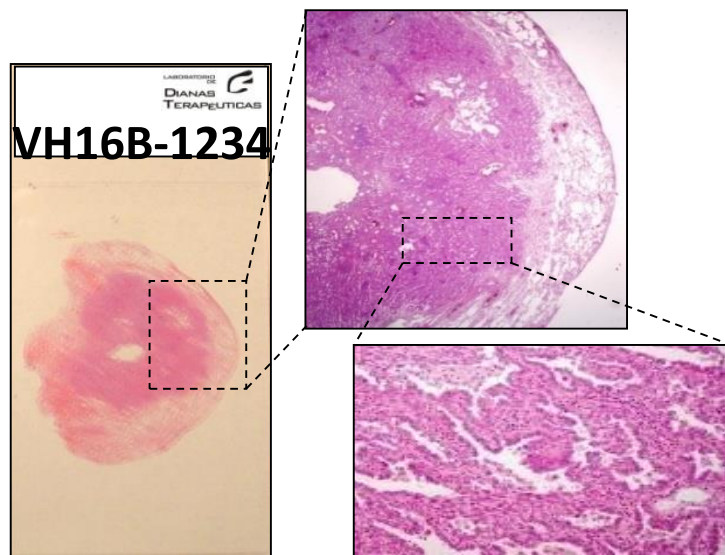
Contenido tumoral: 30%



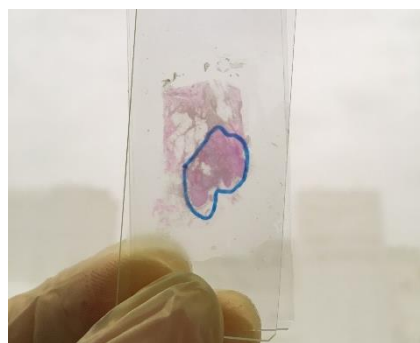
$$\text{VAF} = 50\% \times 30\% = 15\%$$

Somatic VAF

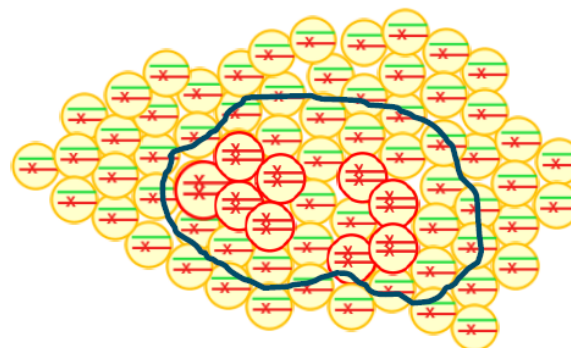
La revisión del tejido tumoral es importante: >30% células tumorales



30% tumor content

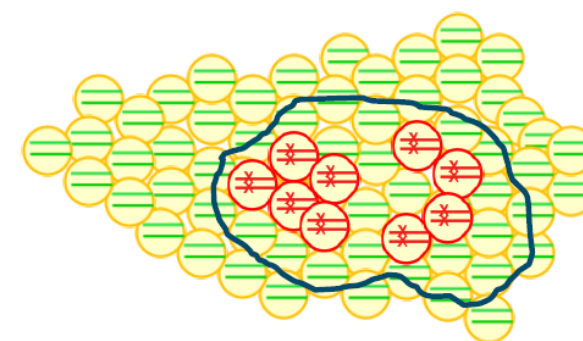


***BRCA* mutation load**



Tumor of *BRCA* mutation carrier:

≥ 50% alleles mutant



Tumor of *wild type* patient:

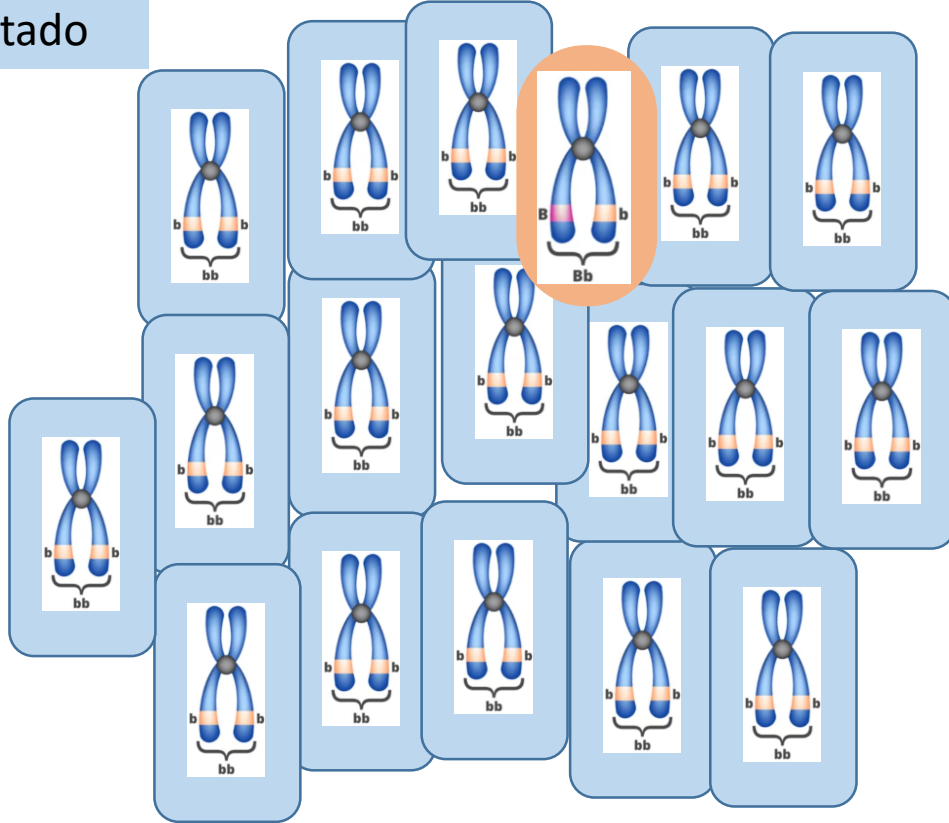
Dependent on neoplastic cell %

Careful selection of tissue
for DNA isolation is essential

VAF somáticas vs. VAF germinales

b = wild type
B = mutado

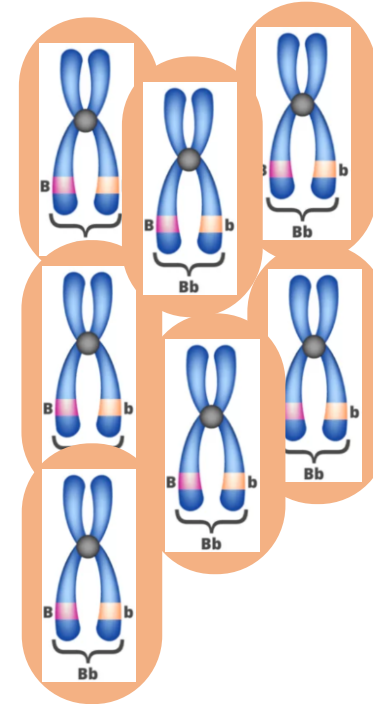
Contenido tumoral: 5%



$$\text{VAF} = 50\% \times 5\% = 2,5\%$$

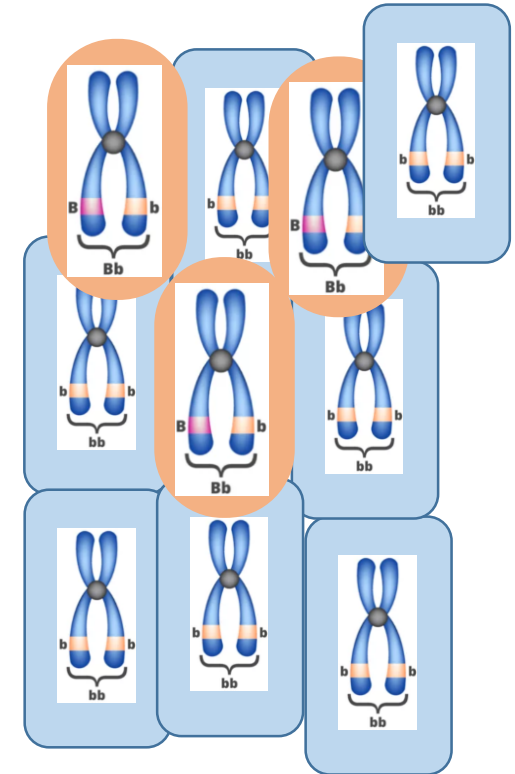
Por debajo de los
límites de sensibilidad de
nuestra NGS

Contenido tumoral: 100%



50%

Contenido tumoral: 30%



$$\text{VAF} = 50\% \times 30\% = 15\%$$

Somatic VAF

Sensibilidad en la NGS

La **realización de técnicas moleculares de alta sensibilidad** requiere como se ha visto anteriormente de un **conocimiento adecuado sobre el material inicial**. Muchos kits diagnósticos marcan su límite de detección vinculado a un porcentaje mínimo de celularidad tumoral como por ejemplo algunos kits de NGS, en los que el límite de detección es de un 5%, siempre sobre una población mínima del 15% de celularidad tumoral. **Sobreestimar el porcentaje celular tumoral implicaría poder cometer errores que puedan llevar a resultados falsos negativos**. Aunque con entrenamiento y práctica el cálculo de celularidad tumoral es una herramienta histopatológica más, **existe una gran variación interpersonal cuando se pregunta a distintos patólogos sobre las mismas muestras⁶⁻⁸**.



NGS: next generation sequencing.

6. Viray H, Li K, Long TA, Vasalos P, Bridge JA, Jennings LJ, et al. A prospective, multi-institutional diagnostic trial to determine pathologist accuracy in estimation of percentage of malignant cells. Arch Pathol Lab Med. 2013 Nov;137(11):1545-9. doi: 10.5858/arpa.2012-0561-CP. PMID: 24168492. 7. Lhermitte B, Egele C, Weingertner N, Ambrosetti D, Dadone B, Kubiniek V, et al. Adequately defining tumor cell proportion in tissue samples for molecular testing improves interobserver reproducibility of its assessment. Virchows Arch. 2017 Jan;470(1):21-27. doi: 10.1007/s00428-016-2042-6. Epub 2016 Nov 16. PMID: 27853865. 8. Smits AJ, Kummer JA, de Bruin PC, Bol M, van den Tweel JG, Seldenrijk KA, et al. The estimation of tumor cell percentage for molecular testing by pathologists is not accurate. Mod Pathol. 2014 Feb;27(2):168-74. doi: 10.1038/modpathol.2013.134. Epub 2013 Jul 26. PMID: 23887293.

VAF somáticas vs. VAF germinales

Normal cells

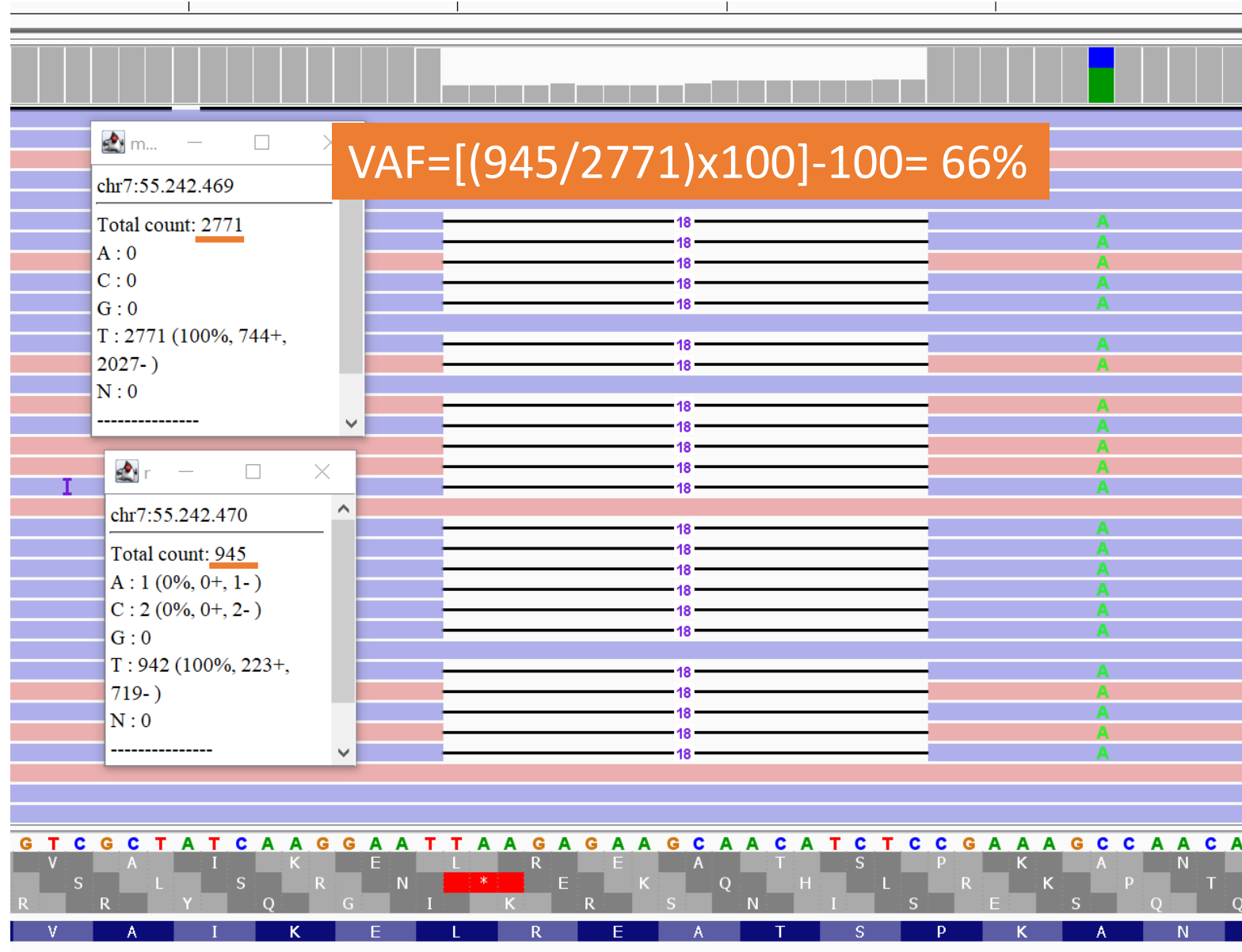
Tumor cells

Tumor 95%

Mutación Delección
en el exon 19 de EGFR

VAF de la mutación?

$$\text{VAF} = [(945/2771) \times 100] - 100 = 66\%$$



VAF somáticas vs. VAF germinales

EGFR

Exon 20

Normal cells

Tumor cells

Tumor 95%

Mutación Deleción
en el exon 19 de EGFR

Mutaciones de
resistencia a TKIs

VAF de las mutaciones?

chr7:55.249.071
Total count: 1585
A : 1 (0%, 0+, 1-)
C : 1302 (82%, 718+, 584-)
G : 0
T : 282 (18%, 145+, 137-)
N : 0

T790M
VAF 17,9%

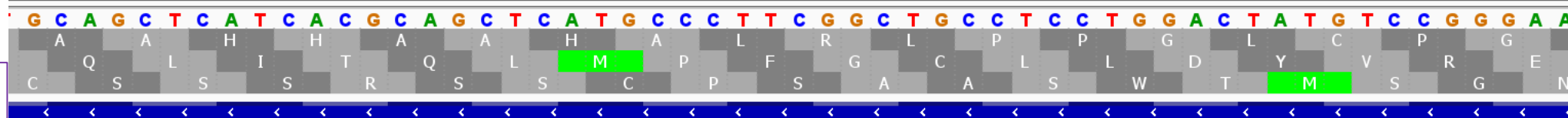
chr7:55.249.091
Total count: 1585
A : 136 (9%, 73+, 63-)
C : 1 (0%, 0+, 1-)
G : 0
T : 1448 (91%, 786+, 662-)
N : 0

C797S
VAF 8,6%

chr7:55.249.092
Total count: 1582
A : 6 (0%, 2+, 4-)
C : 528 (33%, 303+, 225-)
G : 1044 (66%, 550+, 494-)
T : 4 (0%, 1+, 3-)
N : 0

C797S
VAF 32,4%

chr7:55.249.093
Total count: 1571
A : 0
C : 1060 (67%, 558+, 502-)
G : 1 (0%, 1+, 0-)
T : 510 (32%, 295+, 215-)
N : 0

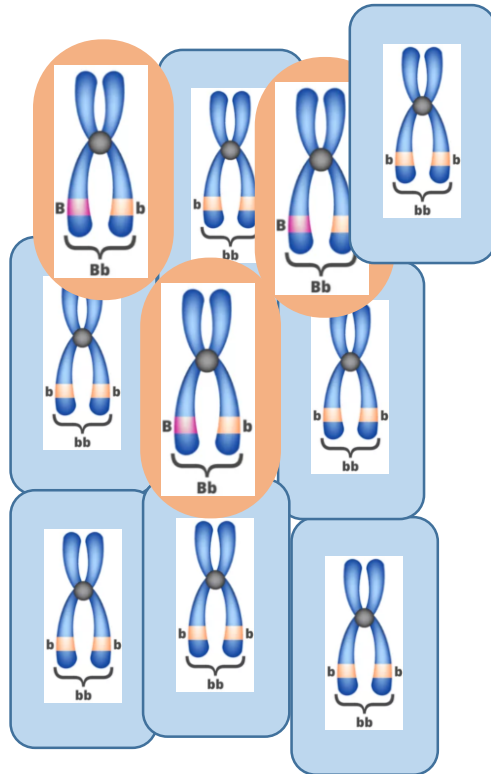




VAF somáticas vs. VAF germinales

b = wild type
B = mutado

Contenido tumoral: 30%



$$\text{VAF} = 50\% \times 30\% = 15\%$$

Si VAF < 15%

- Mutaciones secundarias
- Heterogeneidad tumoral

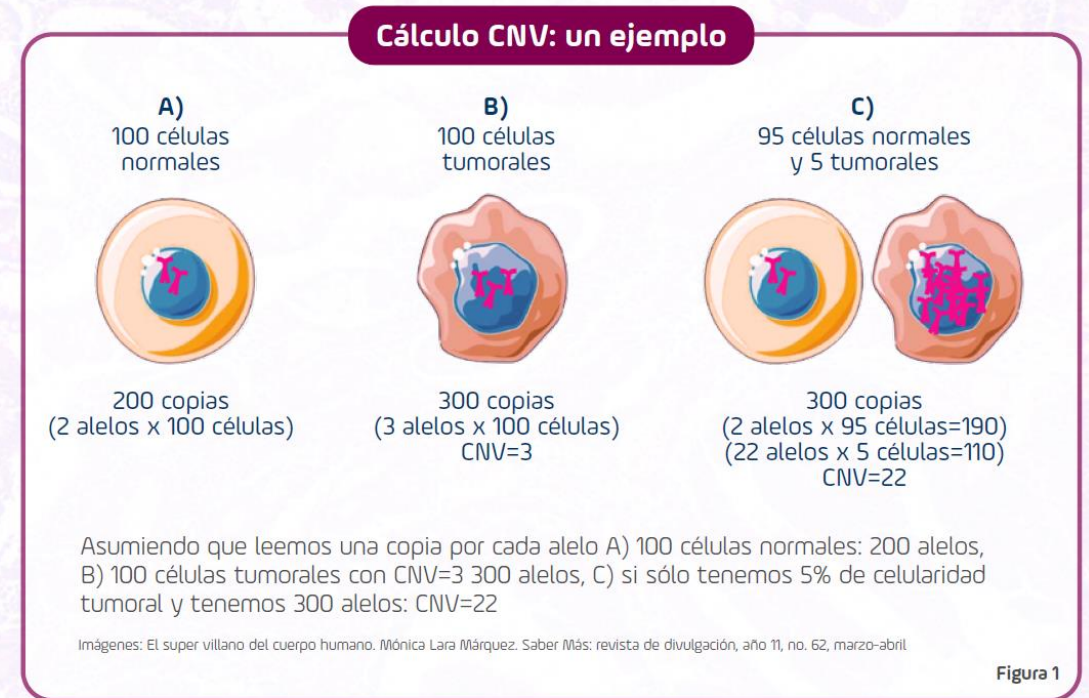
Si VAF > 15%

- Si >30% - sospecha de variante germinal
- Amplificación o ganancia de copias del gen analizado
- LOH o delección (pérdida de una copia del gen analizado)



Cálculo de CNVs en la NGS

Es importante conocer por qué ha de calcularse con precisión el **porcentaje de celularidad tumoral** a la hora de realizar un análisis molecular. Conocer con exactitud este parámetro nos permite **aproximar la sensibilidad analítica de una técnica**, es fácil entender que en ningún caso vamos a poder llegar a una **sensibilidad de un 15% si no tenemos más del 10% de celularidad tumoral**. Además, y sobre todo para tecnologías como la secuenciación masiva, **saber la cantidad de células tumorales de la que partimos es imprescindible a la hora de calcular las alteraciones en el número de copias (CNV)** ya que, en esta técnica, **el cálculo de CNV se basa en la probabilidad de obtener un mayor o menor número de lecturas**, al tener una amplificación o deleción con respecto a un control normal (figura 1).



Cálculo de CNVs en la NGS

OPA

CNV
ALK
AR
CD274
CDKN2A
EGFR
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
KRAS
MET
PIK3CA
PTEN

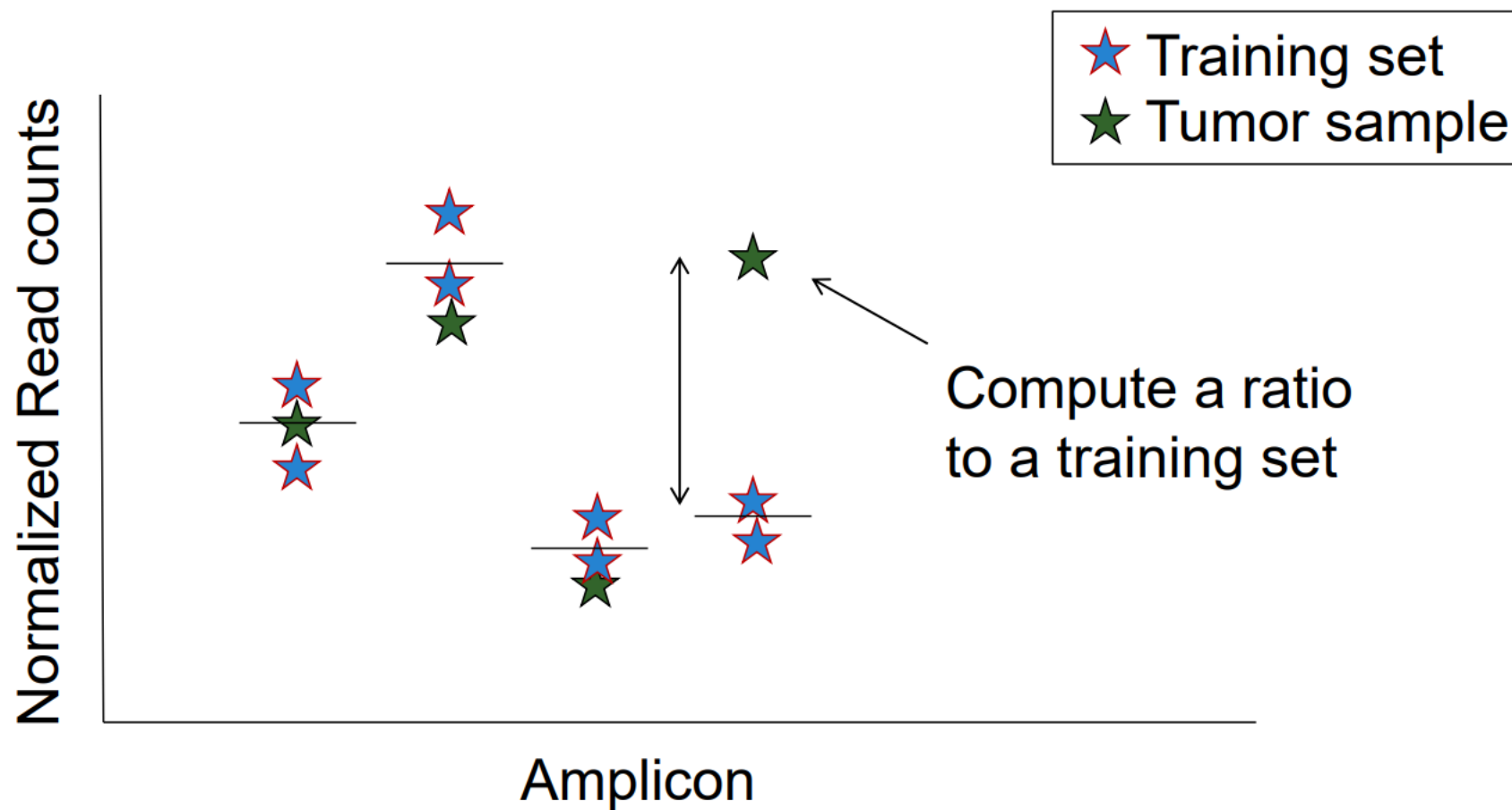
OCAv3

Copy Number Genes	
AKT1	AKT2
AR	AKT3
CCND1	ALK
CCNE1	AXL
CDK4	BRAF
CDK6	CCND2
EGFR	CCND3
ERBB2	CDK2
FGFR1	ESR1
FGFR2	FGF19
FGFR3	FGF3
FGFR4	NTRK1
FLT3	NTRK2
IGF1R	NTRK3
KIT	PDGFRB
KRAS	PIK3CB
MDM2	RICTOR
MDM4	
MET	
MYC	
MYCL	
MYCN	
PDGFRA	
PIK3CA	
PPARG	
TERT	

Childhood

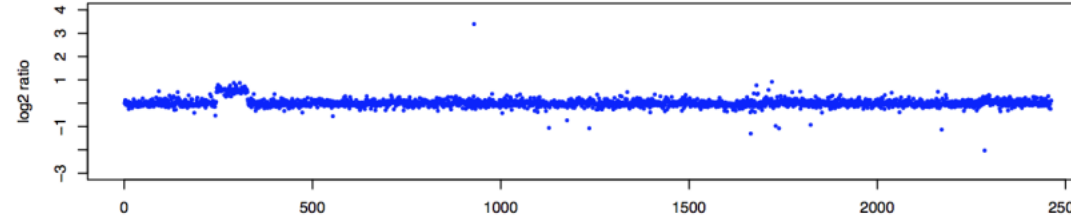
CNV (28)	
ALK	IGF1R
BRAF	JAK1
CCND1	JAK2
CDK4	JAK3
CDK6	KIT
EGFR	KRAS
ERBB2	MDM2
ERBB3	MDM4
FGFR1	MET
FGFR2	MYC
FGFR3	MYCN
FGFR4	PDGFRA
GLI1	PIK3CA
GLI2	

- New CNV algorithm: **Oncomine Focus Panel v2.0 Baseline**
- CNV algorithm in Ion Reporter is used with the following features:
 - New Algorithm adjusts CN estimates based on known sources of variability as detected by prior runs
 - Gender Attribute and % of Tumor Cellularity plays a major role in the algorithm
 - Default % Cellularity is 100% if left unknown
 - Minimum for accurate CNV calling is 50%
 - Median Absolute Pairwise Difference (MAPD) – the higher the value, data introduces more noise

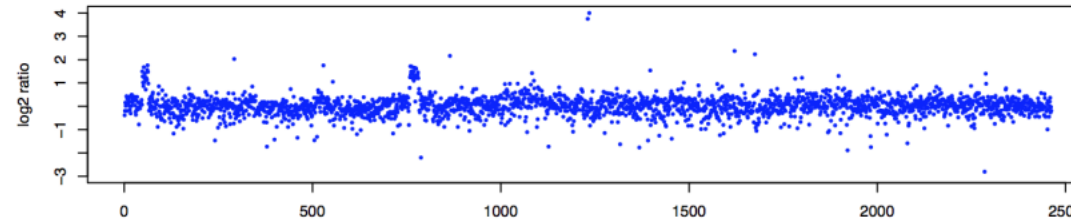


- Amplicons amplify similarly across samples though with different efficiencies
 - Observed differences are due to Copy number changes or noise
 - But not constant over time, even with the same sample!

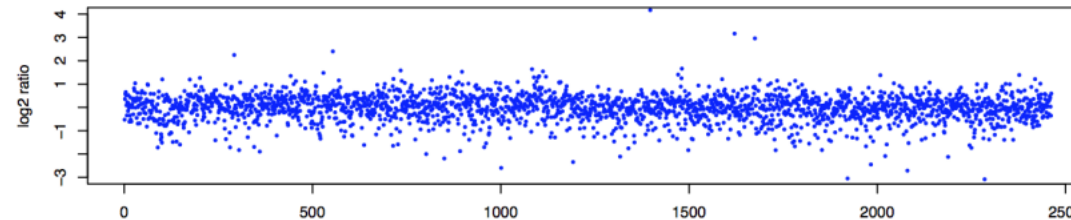
0.11



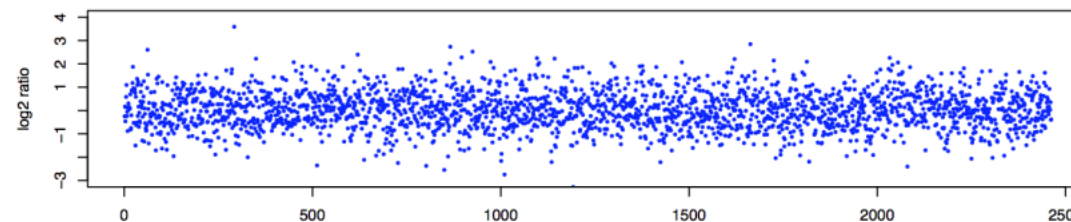
0.29



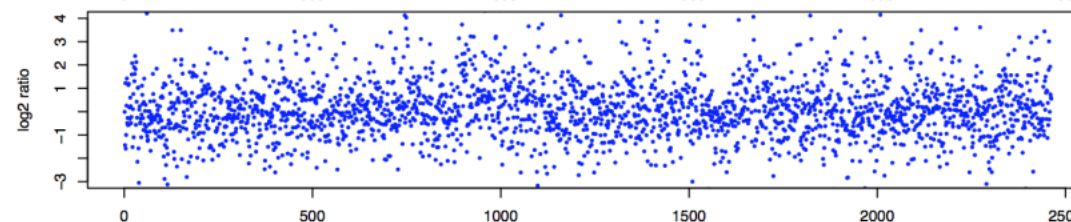
0.47



0.74



1.06



Agenda

- Como funciona la NGS en sistema automatizado.
- ¿Como se analizan los datos y consulta de Bases de Datos?.
- Analisis en linea germinal vs linea somatica
- **Casos del comite molecular de tumores**

Precision Oncology Program (2021)

- Germline
- Hematologic tumors
- **Solid Tumours**
- Pediatric Tumors

Population 7.750.000



Germans Trias i Pujol
Hospital



HOSPITAL DE LA
SANTA CREU I
SANT PAU
UNIVERSITAT AUTÒNOMA DE BARCELONA



Vall d'Hebron
Hospital



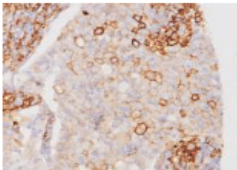
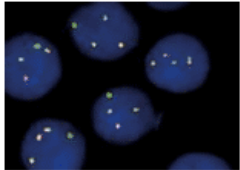
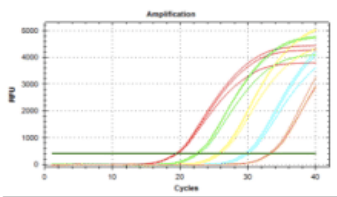
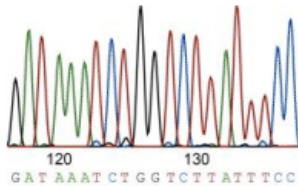
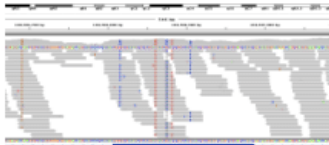
Bellvitge
Hospital Universitari
Institut Català de la Salut

CLÍNICA
BARCELONA
Hospital Universitari

Enfoques diferentes

1. Valor Terapéutico
2. Valor Diagnóstico
3. Valor Pronóstico y predictiva

Different techniques for Molecular Alterations in NSCLC

Immunohistochemistry (IHC) ¹	Fluorescence <i>in situ</i> hybridization (FISH) ¹	Polymerase Chain Reaction (PCR) ¹	Sanger Sequencing ²	Next Generation Sequencing (NGS) ¹
				
IHC is a microscopy-based technique for visualizing and localizing cellular protein in the tissue samples	A fluorescent microscopy-based technique to visualize specific genes within the tissue sample at a high-level (unable to determine sequence of genes or all types of alterations)	A lab process that allows the synthesis of an unlimited number of copies of DNA from a single molecular of DNA or RNA	A lab method used to determine the sequence of a DNA molecule in which the DNA to be sequenced serves as a template for DNA synthesis	A high-capacity, multiplex molecular method that can be designed to detect all classes of genomic alterations but not protein overexpression
Detection Target(s)				
• Protein	• DNA	• DNA • RNA	• DNA	• DNA • RNA

Oncomine Precision Assay (OPA)

Alteraciones hotspots: 45 genes

CNVs: 14 genes

Fusiones génicas: 19

Tipo Tumoral: tumor sólido como NSCLC, cancer de colon, melanoma



Oncomine Focus Assay (OFA)

Alteraciones hotspots : 35 genes

CNVs: 19 genes

Fusiones génicas: 23

Tipo Tumoral: tumor sólido como NSCLC, cancer de colon, melanoma



Fusion Plex Sarcomas

Fusiones génicas: 56

Tipo Tumoral: sarcomas



Oncomine Childhood

Alteraciones hotspots : 86 genes

Genes cobertura completa: 44 genes

CNVs: 28 genes

Fusiones génicas: 91

Tipo Tumoral: Tumores pediátricos



TruSight™ Oncology 500

A comprehensive next-generation sequencing assay that targets somatic variants, TMB, and MSI status from the same FFPE tumor only sample.

Biología Molecular. Vall d'Hebron

Flujo de trabajo del laboratorio 2022

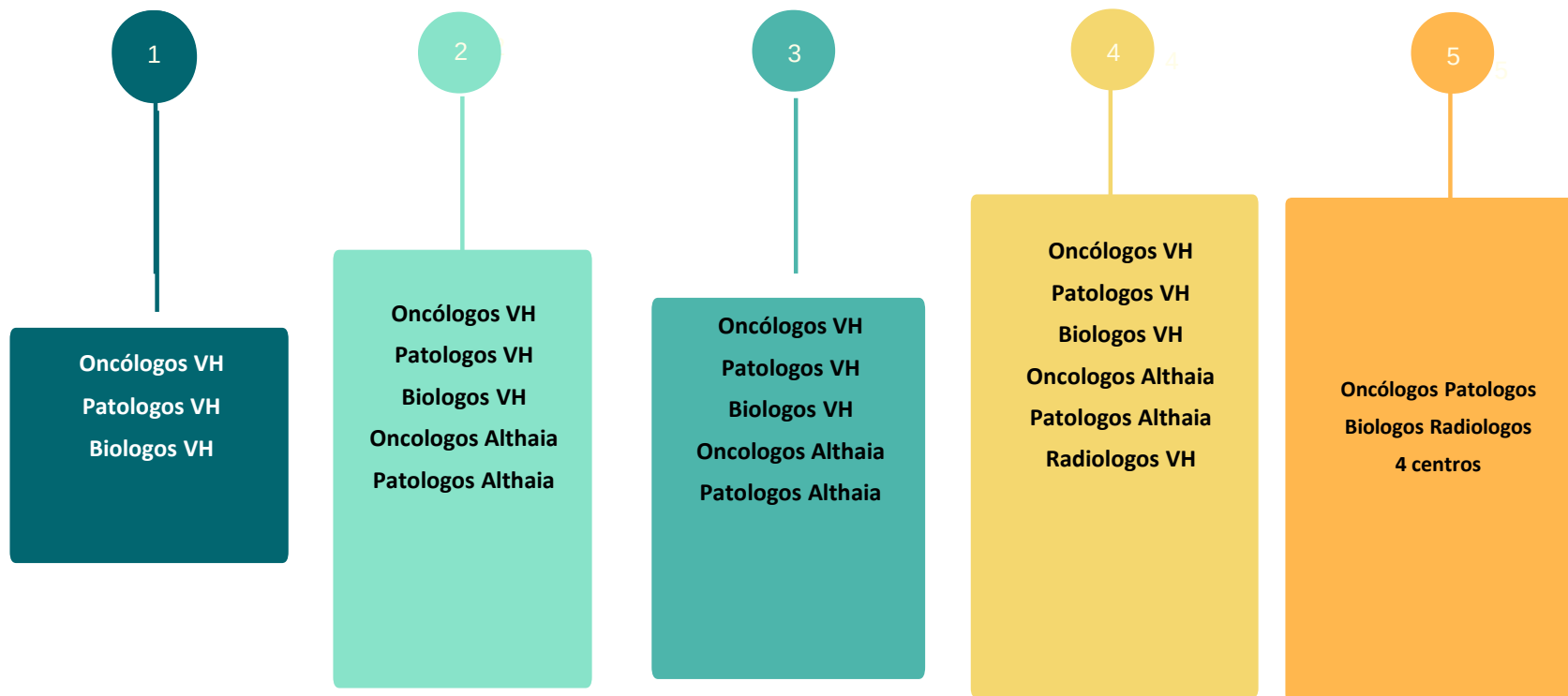
<i>Lunes</i>	<i>Martes</i>	<i>Miércoles</i>	<i>Jueves</i>	<i>Viernes</i>
			Selección de casos por parte clínicos	Comité Molecular
Extracción DNA +RNA 2	Cuantificación 3	Extracción DNA+RNA 2	Comité Molecular	 Análisis 5
	Carga Genexus 4	Análisis  5	Cuantificación 3	Selección del material  1
	Selección del material 1 	Envío casos NGS semana	Carga Genexus 4	



Comités Moleculares inter-hospitalarios 2021

Septiembre 21

Noviembre 21





Comité Molecular Inter-hospitalario

Viernes 25 Noviembre 2022

Agenda

Caso 01 NHC: 11895142 VH21B8984 HUVH

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Origen de la mostra: citologia
Tipus de mostra: FFPE
Indicació: Càncer de pulmó
Referència DNA: 22-254, Mostra Valorable
Referència RNA: 22-255, Mostra Valorable
Contingut tumoral: 70%

RESULTATS

1. Variants ACCIONABLES

No s'han detectat alteracions accionables

2. ALTRES alteracions patogèniques

BRAF NM_004333.6: c.1799T>A, p.Val600Glu VAF 32,8%

3. Variants DE SIGNIFICAT INCERT

No s'han detectat alteracions de significat incert

En funció del coneixement actual no es pot determinar la rellevància clínica de les variants de significat incert, per tant, no es poden tenir en compte en el maneig clínic del pacient. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

COMENTARIS I RECOMANACIONS

Els resultats obtinguts es presentaran en el comitè molecular de tumors

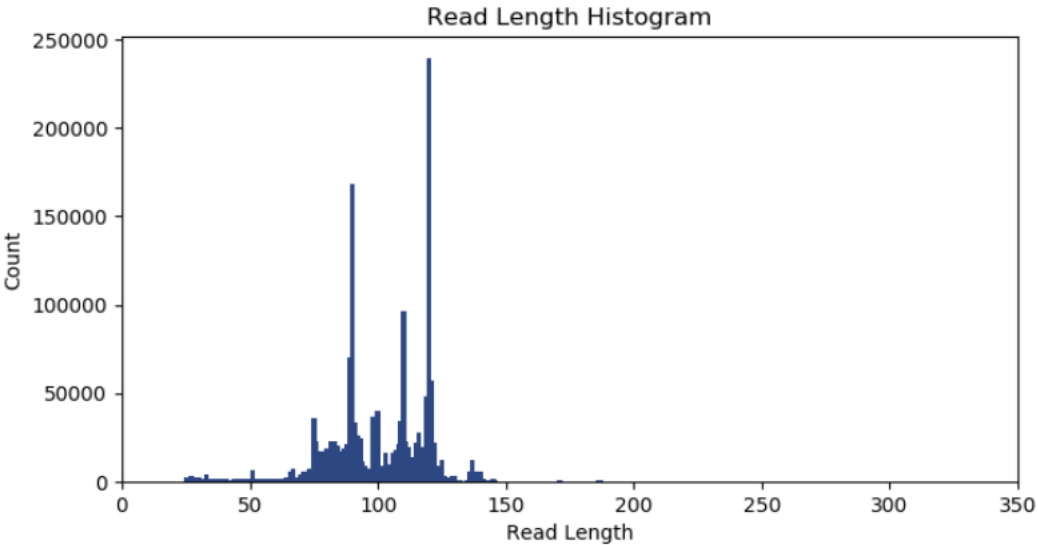
METODOLOGIA

Panell NGS: Oncomine Precision Assay (ThermoFisher Scientific)

Sample QC - DNA



MAPD	✓	0,19
[0-0.5]		
Mapped Reads	✓	1.679.025
>=350000		
Mean AQ20 Read Length (bp)	—	93
Not Set		
Mean Read Length (bp)	✓	100
>=80		
Uniformity Of Base Coverage	✓	99,39
>=94		



22_254D_22_255R

QC 

Key Findings

Variants

Plugins

Reports

Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
22_254D_22_255R	19 NOV 2022	Male	FFPE	Solid Tumor	Non-Small Cell Lung Cancer	Stage IV	70

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity of Amplicon Coverage
100.00%	100.00%	6128	99.39%	93.54%	99.60%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

BRAF

SNV/Indel

COSM476

AA Change: p.V600E

Allele Frequency: 0.328

ALK

EGFR

ERBB2

KRAS

MET

ROS1

22_254D_22_255R [QC](#) [Key Findings](#) **[Variants](#)** [Plugins](#) [Reports](#)

OncoPrint Extended (5.14) Filter Chain Applied 1 of 3,180 Variants												Edit Filters	Export	Column
User Classification	Variant ID	Key Variant	OncoPrint Gene Class	Gene	Locus	AA Change	Allele Frequency	Allele Frequency (%)	Nuc Change	Raw Alt Allele Read Counts				
Classification	COSM476	Yes	Gain-of-Function	BRAF	chr7:140453135	p.V600E	0.328	32.8	c.1799T>A	2627				

1 50 items per page

1 - 1 of 1

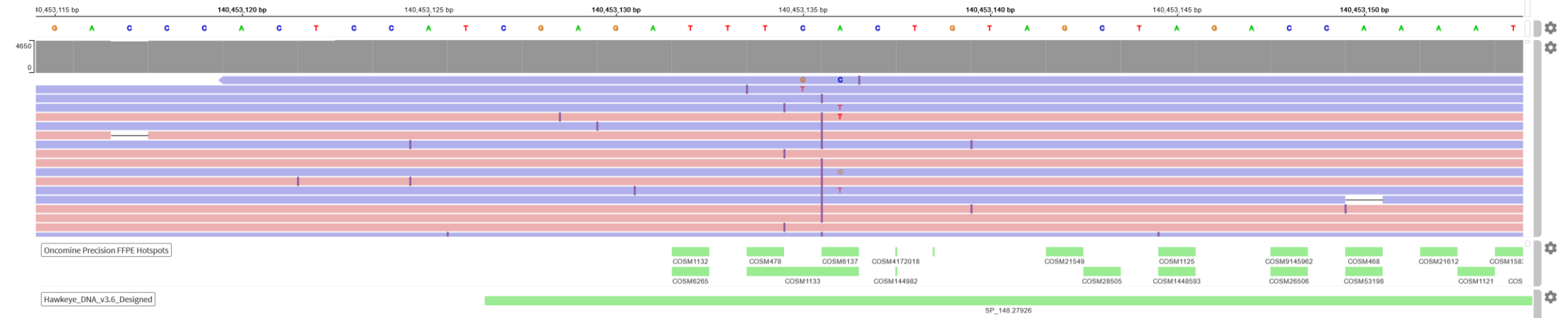
** More than 1 gene is detected. Click on the variant row to view the annotation details.

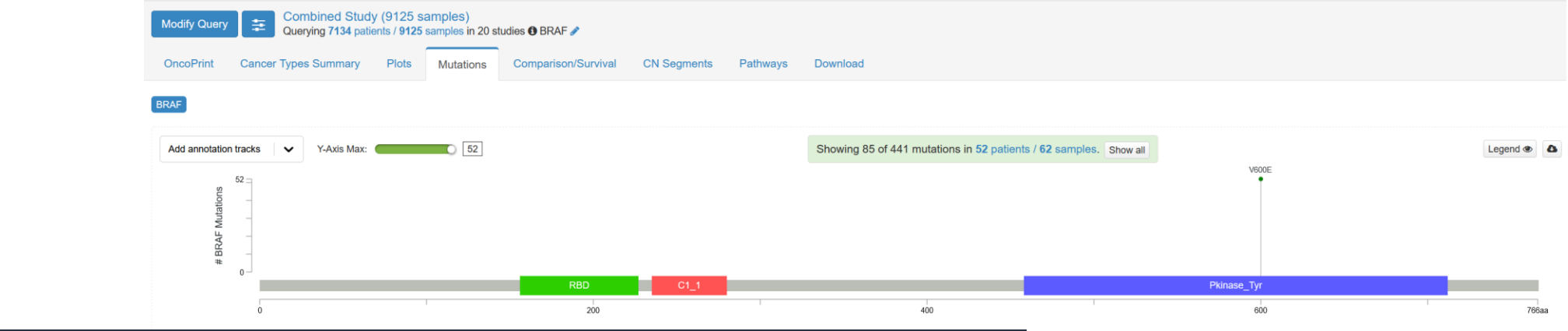
Pileup [Annotations](#)

Gene Browser 40 bp

[View in IGV](#)

[Cursor Guide](#) [Center Line](#) [Track Labels](#)   





V600E RS113488022, VAL600GLU, V640E, VAL640GLU

Summary Comments Revisions Flags Events

Description

BRAF V600E has been shown to be recurrent in many cancer types. It is one of the most widely studied variants in cancer. This variant is correlated with poor prognosis in certain cancer types, including colorectal cancer and papillary thyroid cancer. The targeted therapeutic dabrafenib has been shown to be effective in clinical trials with an array of BRAF mutations and cancer types. Dabrafenib has also shown to be effective when combined with the MEK inhibitor trametinib in colorectal cancer and melanoma. However, in patients with TP53, CDKN2A and KRAS mutations, dabrafenib resistance has been reported. Ipilimumab, regorafenib, vemurafenib, and a number of combination therapies have been successful in treating V600E mutations. However, cetuximab and panitumumab have been largely shown to be ineffective without supplementary treatment.

Sources

None specified

Aliases

RS113488022 VAL600GLU V640E VAL640GLU

Variant Type

Missense Variant

HGVs Descriptions

NM_004333.4:c.1799T>A NP_004324.2:p.Val600Glu NC_000007.13:g.140453136A>T ENST00000288602.6:c.1799T>A

V600E Evidence 174 total, showing 35

EID	Disease	Drugs
EID1409	Skin Melanoma	Vemurafenib
EID9851	Colorectal Cancer	Encorafenib Cetuximab
EID3017	Lung Non-small Cell...	Dabrafenib Trametinib
EID10785	Thyroid Gland Anaplastic...	Dabrafenib/Trametini...
EID2116	Colorectal Cancer	N/A
EID2120	Colorectal Cancer	Bevacizumab
EID2126	Melanoma	Dabrafenib

BRAF V600E in lung adenocarcinoma

Oncogenic**Gain-of-function**

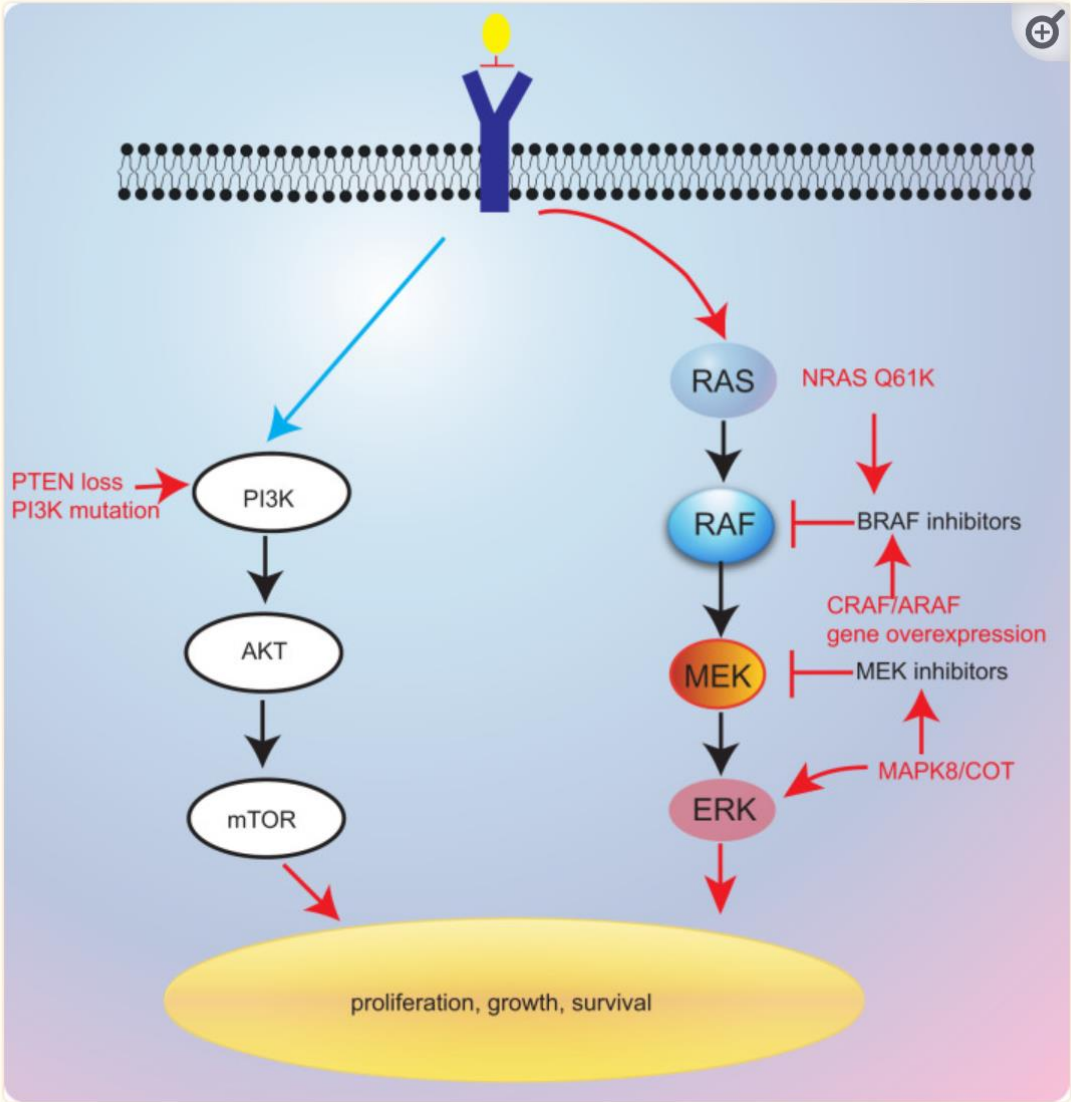
BRAF, an intracellular kinase, is frequently mutated in melanoma, thyroid and lung cancers among others.

The BRAF V600E mutation is known to be oncogenic.

The RAF-targeted inhibitor dabrafenib in combination with the MEK1/2-targeted inhibitor trametinib is FDA-approved for the treatment of patients with BRAF V600E mutant non-small cell lung cancer.

Biological Effect **1** Therapeutic Implications

Level	Alteration(s)	Drug(s)	Level-associated cancer type(s)	
1	V600E	Dabrafenib + Trametinib	All Solid Tumors (excluding Colorectal Cancer)	
3B	V600E	Dabrafenib	Melanoma	
3B	V600E	Encorafenib + Cetuximab	Colorectal Cancer	
3B	V600E	Selumetinib	Pilocytic Astrocytoma	
3B	V600	Vemurafenib + Atezolizumab + Cobimetinib	Melanoma	
3B	V600E	Encorafenib + Panitumumab	Colorectal Cancer	



Current Treatment Status and Future Perspective. Front Oncol. 2022 Mar 31;12:863043.

Table 2

Targeted therapy for BRAF-mutated NSCLC.

Trial	Treatment lines	Agents	ORR	PFS	OS
NCT01524978	≥2	Vemurafenib	42%	7.3	NA
EURAF Cohort	≥2	Vemurafenib, dabrafenib, or sorafenib	53%	5.0	10.8
AcSé	≥2	Vemurafenib	44.9%	5.2	10
NCT01336634	≥2	Dabrafenib	33%	5.5	NA
NCT02610361	≥2	BGB-283	20%	NA	NA
NCT01336634	≥2	Dabrafenib+Trametinib	63%	10.2	18.2
NCT01336634	1	Dabrafenib+Trametinib	64%	10.9	24.6
NCT02974725	≥2	LXH254+LTT462	66.7%	NA	NA

Table 3

Several ongoing trials of ICIs combined with targeted therapy.

Trial	Phase	Treatment lines	Experimental arm	Enrolled population	Status
NCT03600701	II	≥2	Atezolizumab+combimetinib	Metastatic, Recurrent, or Refractory non-small cell lung cancer	Recruiting
NCT03299088	Ib	≥2	Pembrolizumab+trametinib	Stage IV non-small cell lung cancer with <i>K-ras</i> gene mutations	Active
NCT03225664	Ib/II	≥2	Pembrolizumab+trametinib	Recurrent non-small cell lung cancer	Active

LA NGS LO VE TODO



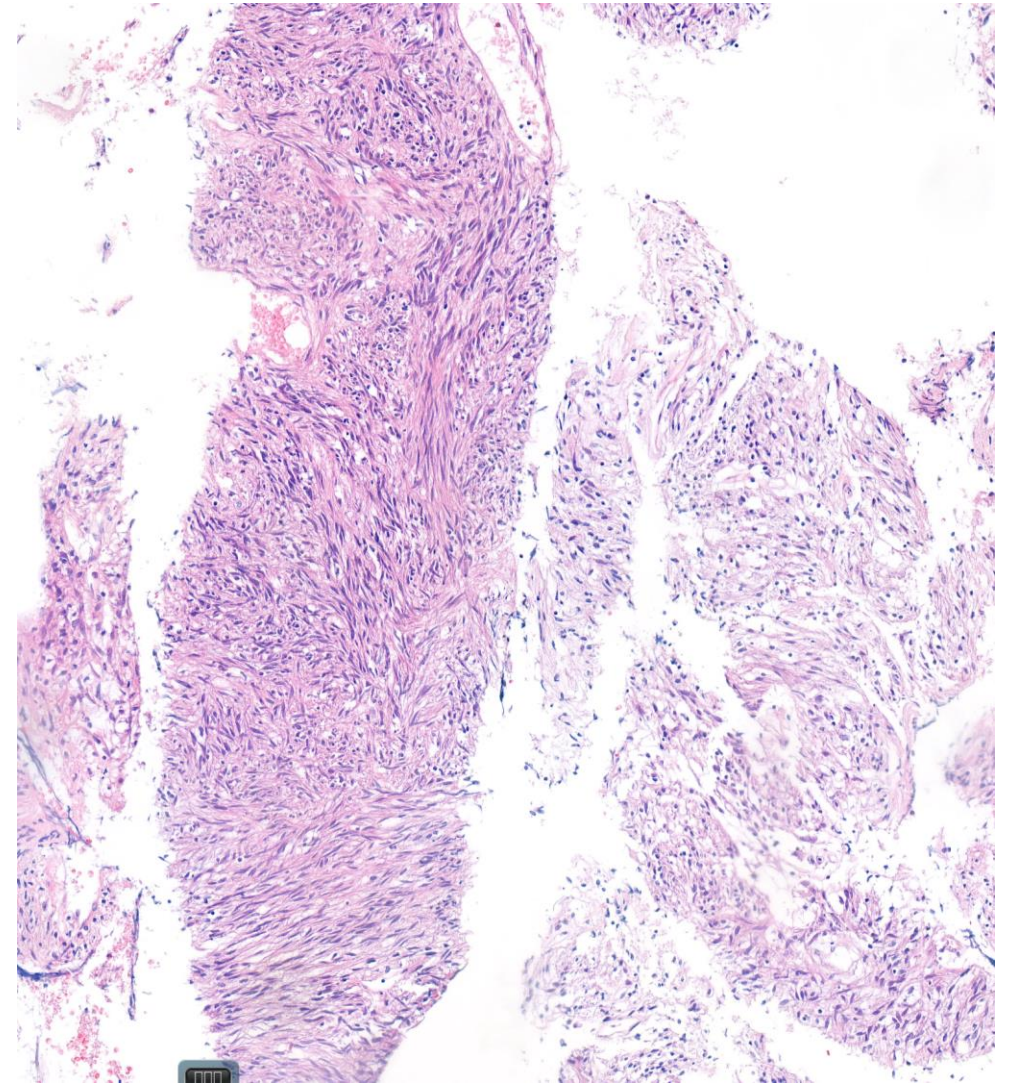
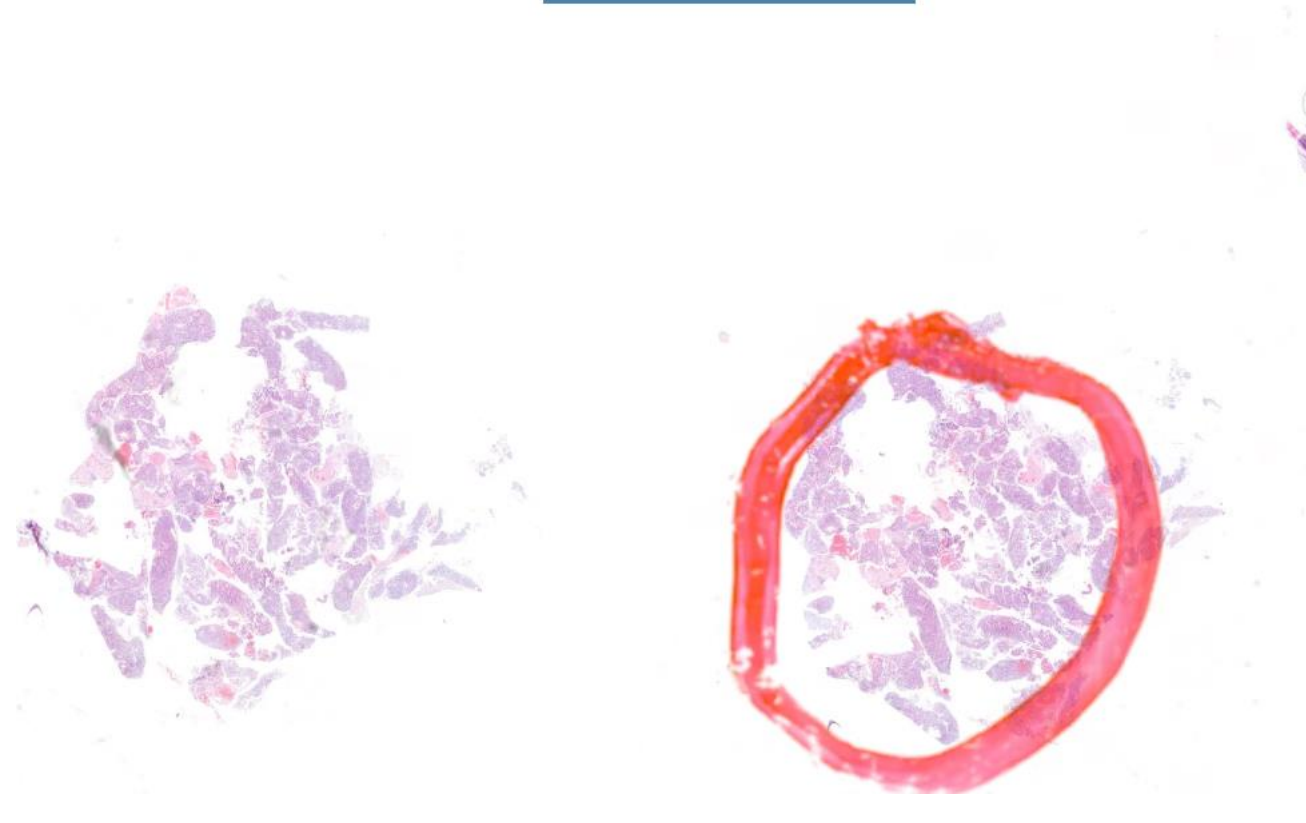
NHC: 17756078 VH22P24894

1 / Case: VH22P024894A001003 Patient: ANA MARIA PEREZ COLLADO 

Integration HE (HEMATOXILINA-EOSINA)

ANA MARIA PEREZ COLLADO 12/31/1945

VH22P024894A001003



VH-22-P-24894

INFORME ANATOMIA PATOLÒGICA

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT
SEQÜENCIACIÓ MASSIVA (NGS)

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Centre sol·licitant: Althaia, Xarxa Assistencial Manersa

Referència origen: 22C19156A2

Origen de la mostra:

Tipus de mostra: FFPE

Indicació: GIST

Referència DNA: VH22P024894A001, Mostra Valorable

Referència RNA: NO REALITZAT

Contingut tumoral: 80%

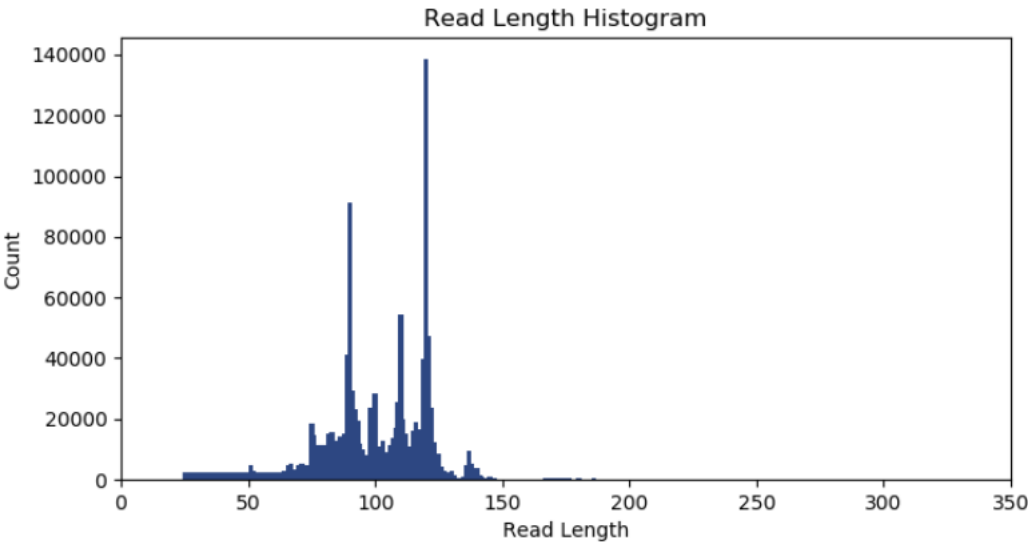
- RESULTATS
1. Variants ACCIONABLES
- No s'han detectat alteracions accionables
2. ALTRES alteracions patogèniques
- No s'han detectat
3. Variants DE SIGNIFICAT INCERT

Informe Versión 01

Sample QC - DNA



MAPD	✓	0,2
[0-0.5]		
Mapped Reads	✓	1.235.168
>=350000		
Mean AQ20 Read Length (bp)	—	88
Not Set		
Mean Read Length (bp)	✓	99
>=80		
Uniformity Of Base Coverage	✓	99,4
>=94		



VH22P024894A001002

QC

✖

Key Findings

Variants

Plugins

Reports

Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
VH22P024894A001002	08 NOV 2022	Female	FFPE	Solid Tumor	Gastrointestinal Stromal Tumor	Stage IV	80

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity of Amplicon Coverage
100.00%	100.00%	4426	99.40%	92.97%	99.60%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

✔

Not Assayed (1)

KIT

PDGFRA

NF1

Filtro Oncomine Extended

Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
VH22P024894A001002	08 NOV 2022	Female	FFPE	Solid Tumor	Gastrointestinal Stromal Tumor	Stage IV	80

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity of Am
100.00%	100.00%	4426	99.40%	92.97%	99.60%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

☒ Not Assayed (1)

KIT

SNV/Indel

.

AA Change: p.D579_H580insLDPTQLPYD

Allele Frequency: 0.312

PDGFRA

SNV/Indel

.

AA Change: p.P567=

Allele Frequency: 0.999

KIT

PDGFRA

NF1

Sin Filtro

VH22P024894A001002 QC ✕ Key Findings **Variants** Plugins Reports

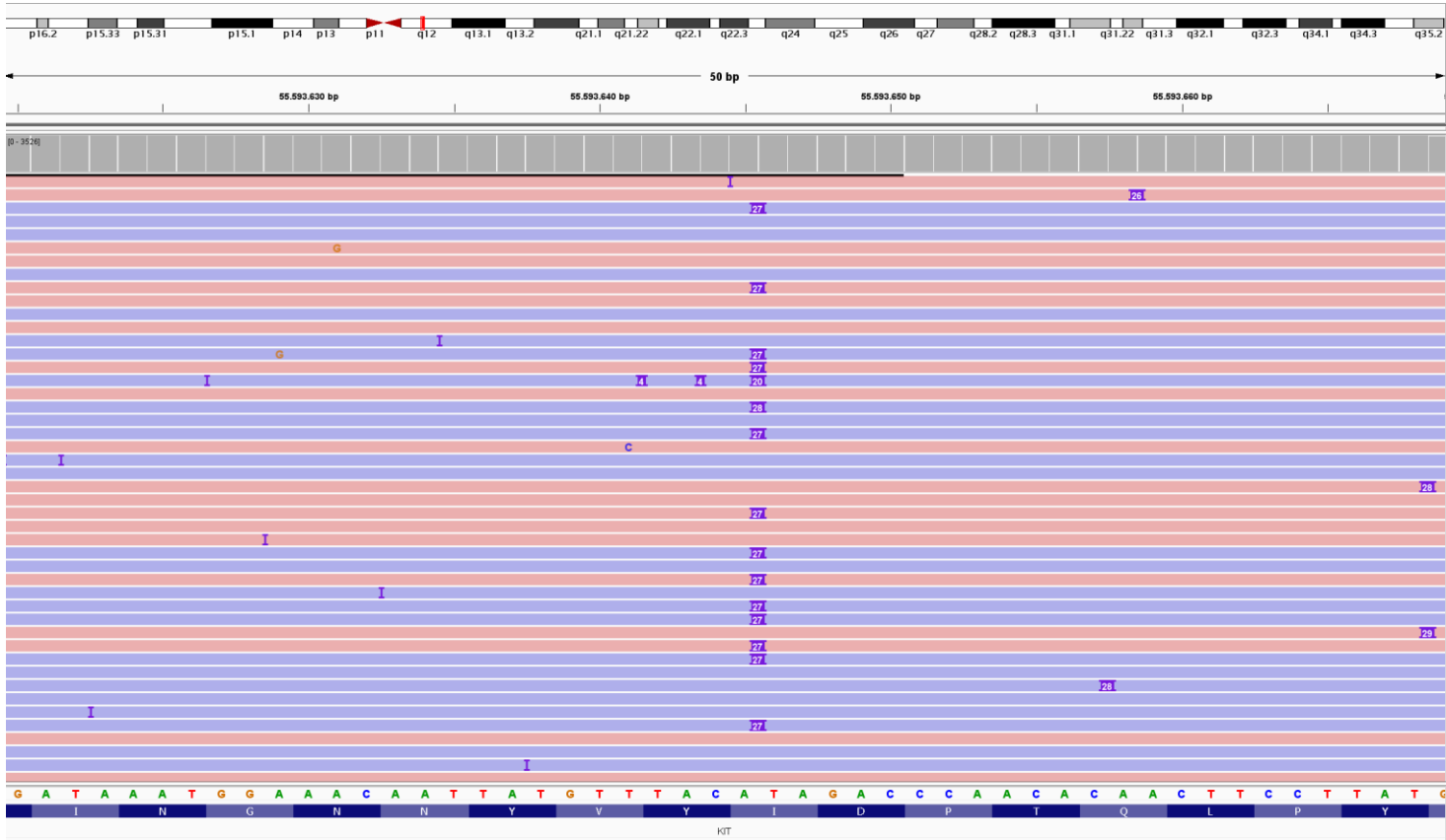
All SNVs/Indels Fusions CNVs

☐ Gene List Only

No Filter Chain Applied
3,175 of 3,175 Variants

Edit Filters

	Gene	Locus	AA Change	Allele Frequency (%)	Ref	Alt	Call	Nuc Change	Raw Read Depth	Genes (E
	KIT	chr4:55593645	p.D579_H580insLDPTQLPYD	31.2	A	ATAGACCCCAACAACTTCCTTATGATC	PRESENT (HETEROZYGOUS)	c.1738_1739insTAGACCCCAACAACTTCCTTATGATC	3169	
	NTRK2	chr9:87563562	p.?	50.1	G	A	PRESENT (HETEROZYGOUS)	c.1937+13G>A	4622	
	RET	chr10:43613843	p.L769=	51.8	G	T	PRESENT (HETEROZYGOUS)	c.2307G>T	3873	



ExAC LAF (1) • 0.0 (pos.55593645)

ExAC GAF (1) • 1.65E-5 (pos.55593645)

ExAC EAAF (1) • 2.316E-4 (pos.55593645)

ExAC SAAF (1) • 0.0 (pos.55593645)

ExAC AAF (1) • 0.0 (pos.55593645)

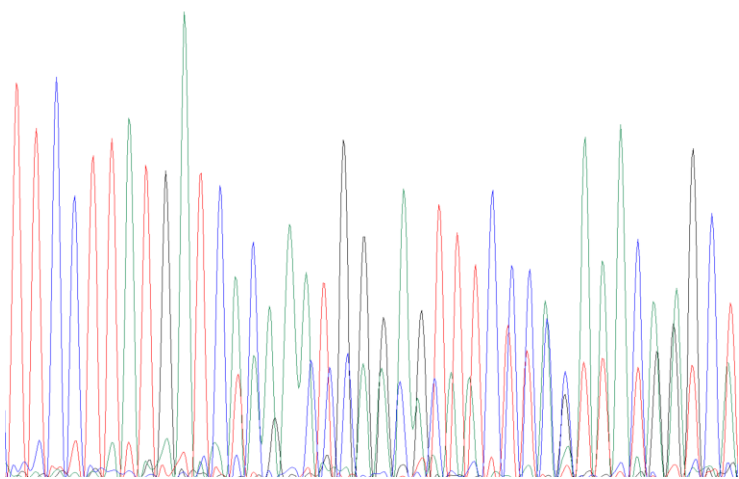
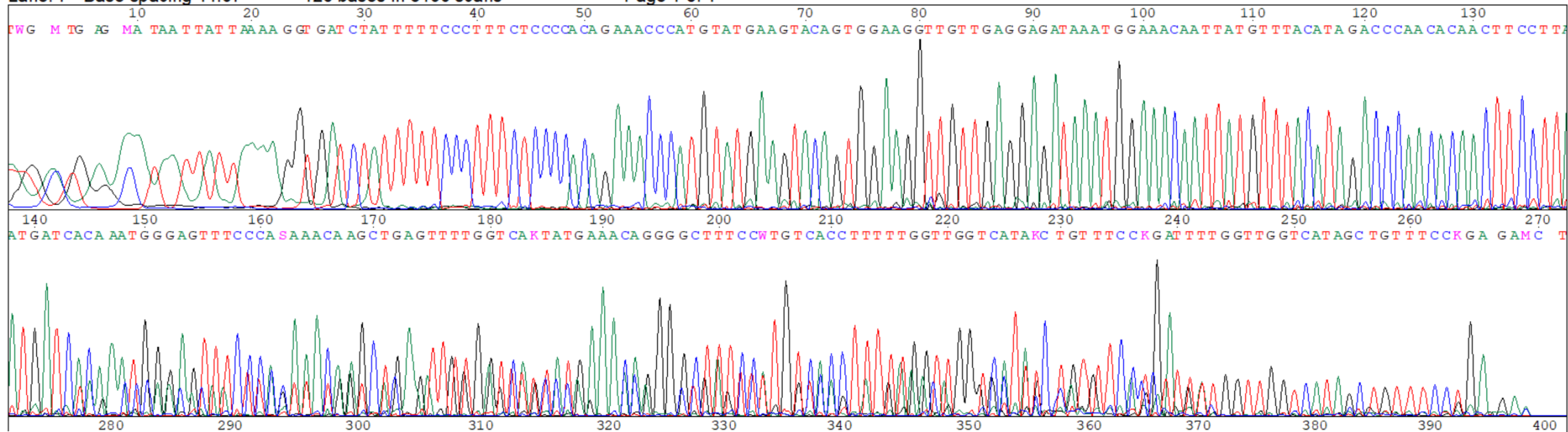
ExAC EFAF (1) • 0.0 (pos.55593645)

ExAC OAF (1) • 0.0 (pos.55593645)

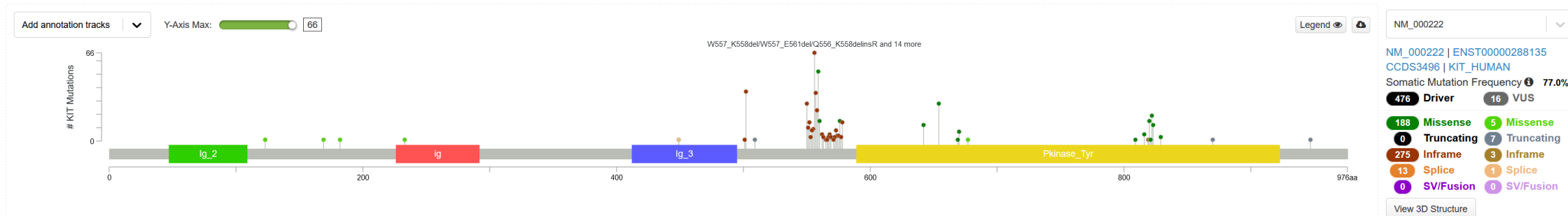
ExAC ENFAF (1) • 0.0 (pos.55593645)

Filtro aplicado Poblacional 10⁻⁶

File: C07_22P24894 CK 11F.ab1 Run Ended: Nov 16, 2022, 18:16:19 Signal G:1283 A:1568 T:1858 C:1603 Sequence: 51
Lane: 7 Base spacing 11.07 426 bases in 5190 scans Page 1 of 1



KIT p.Asp579_His580insLDPTQLPYD
c. 1738_1739insTAGACCCAACACAACCTTCCTTATCATC



KIT D579_H580insSQDPTQLPYD in gastrointestinal stromal tumors

Likely Oncogenic

Likely Gain-of-function

KIT, a receptor tyrosine kinase, is recurrently mutated in gastrointestinal stromal tumors.

The KIT D579_H580insSQDPTQLPYD alteration has been identified as a statistically significant hotspot and is likely to be oncogenic.

The multikinase KIT and PDGFRA inhibitors imatinib, sunitinib, regorafenib and ripretinib are FDA-approved for the treatment of patients with gastrointestinal stromal tumors (GISTs) harboring KIT exon 11 alterations, such as KIT D579_H580insSQDPTQLPYD.

Biological Effect

Therapeutic Implications

Level	Alteration(s)	Drug(s)	Level-associated cancer type(s)	
1	Y578C and 65 other alterations	Imatinib	Gastrointestinal Stromal Tumor	
1	Y578C and 65 other alterations	Sunitinib	Gastrointestinal Stromal Tumor	
1	Y578C and 65 other alterations	Regorafenib	Gastrointestinal Stromal Tumor	
1	Y578C and 65 other alterations	Ripretinib	Gastrointestinal Stromal Tumor	

The information above is intended for research purposes only and should not be used as a substitute for professional diagnosis and treatment.

Levels of Evidence



Onc[®]KB

Feedback

Overview

This section shows a general overview of the selected mutation. It describes the source of the mutation i.e gene n sequence level. You can see more information on our [help pages](#).

Genomic Mutation ID  COSV55405342

Legacy Identifier  COSM133712

Gene name [KIT](#)

AA mutation p.D579_H580insLNTQLPYD (Insertion - In frame)

CDS mutation c.1738_1739insTAAACACACAACCTTCCTTATGATC (Insertion)

SNP No

Nucleotides inserted TAAACACACAACCTTCCTTATGATC

Genomic coordinates GRCh38, [4:54727506..54727507](#), view [Ensembl contig](#) 

CDD [NP_001087241.1](#) 

HomoloGene n/a

Ever confirmed somatic? No

FATHMM prediction n/a

Remark n/a

Recurrent n/a

Drug resistance n/a

Alternative Ids  n/a

General Information Insertion KIT(ENST00000288135.6):c.1738_17... (p.Asp579_His580insLeuAsnThrGln...	PharmGKB No data available	ACMG Classification Pathogenic 11 points = 11 P - 0 B	Frequencies exomes: not found genomes: not found (cov:31.4)	Pathogenicity Scores	Expression Data Top: Esophagus - Muscularis Tissues: 51	Beacon Network
Genes KIT	Publications Variant: 0 Gene: 287	ClinVar No data available	MitoMap No data available	ClinGen No data available	GWAS No data available	Protein Viewer
Community Contributions	Transcripts ENST00000288135.6 - in frame MANE Select	Uniprot Variants No data available	Conservation Scores phyloP100: 9.137	Region Browser	Structural Variants	OMIM No data available

Variant

Chromosome	Position	REF Sequence	ALT Sequence	Variant type	Cytoband	HGVS	Gene symbol
chr4	54727507		TA...TC (24bp)	Insertion	4q12	KIT(ENST00000288135.6):c.1738_1739insTAAACACACAACTTCCTTATGATC (p.Asp579_His580insLeuAsnThrGlnLeuProTyrAsp)	KIT
<a>UCSC genome browser							

CIVIC

CLINICAL INTERPRETATION OF
VARIANTS IN CANCER

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Assertions

Evidence

Genes

Variants

Variant Groups

Clinical Trials

Diseases

Drugs

Phenotypes

Sources

Variant Types

Curation

Activity

Queues

Community

Contributors

Organizations

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Variants / KIT D579_H580insIDPTQLPYD / Summary

D579_H580insIDPTQLPYD Parent: KIT

D579_H580insIDPTQLPYD

SummaryCommentsRevisionsFlagsEvents

Description

No description provided

Sources

None specified

Aliases

None specified

Variant Type

Inframe Insertion

HGVS Description

None specified

Gene

KIT

CIVIC Variant Evidence Score

0

Allele Registry ID

None provided

ClinVar ID

None provided

Representative Variant Coordinates

Ref. BuildGRCH37Ensembl Version

Coordinates

Chr.4Start55593671Stop55593672Ref. Bases--

Var. Bases--Transcript

MyVariantInfo

Not available, provide Representative Variant Coordinates

D579_H580insIDPTQLPYD Evidence 1 total, showing 1

EID

Disease

Drugs

DIT

DESC

EL

ET

ED

CS

VO

R

EID4585

Gastrointestinal Stromal...

Sunitinib

N/A

D

Flumatinib, a selective inhibitor of BCR-ABL/PDGFR/KIT, effectively overcomes drug resistance of certain KIT mutants

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Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Key words

Drug resistance, flumatinib, gastrointestinal stromal tumors, imatinib mesylate, sunitinib malate

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Funding information

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Cancer Sci 105 (2014) 117–125

doi: 10.1111/cas.12320

Activating mutations in KIT have been associated with gastrointestinal stromal tumors (GISTs). The tyrosine kinase inhibitor imatinib mesylate has revolutionized the treatment of GISTs. Unfortunately, primary or acquired resistance to imatinib does occur in GISTs and forms a major problem. Although sunitinib malate, a multi-kinase inhibitor, has shown effectiveness against imatinib-resistant GISTs, recent studies have indicated that some imatinib-resistant GISTs harboring secondary mutations in the KIT activation loop were also resistant to sunitinib. Therefore, new drugs capable of overcoming the dual drug resistance of GISTs probably have potential clinical utility. In this study, we investigated the efficacy of flumatinib, an inhibitor of BCR-ABL/PDGFR/KIT, against 32D cells transformed by various KIT mutants and evaluated its potency to overcome the drug resistance of certain mutants. Interestingly, our *in vitro* study revealed that flumatinib effectively overcame the drug resistance of certain KIT mutants with activation loop mutations (i.e., D820G, N822K, Y823D, and A829P). Our *in vivo* study consistently suggested that flumatinib had superior efficacy compared with imatinib or sunitinib against 32D cells with the secondary mutation Y823D. Molecular modeling of flumatinib docked to the KIT kinase domain suggested a special mechanism underlying the capability of flumatinib to overcome the drug-resistance conferred by activation loop mutations. These findings suggest that flumatinib could be a promising therapeutic agent against GISTs resistant to both imatinib and sunitinib because of secondary mutations in the activation loop.

Table 1. Comparative effects of imatinib, flumatinib, and sunitinib on the proliferation of 32D cell lines expressing transforming KIT mutants

Cell line	Mean $\pm$ SD (nM)		
	Imatinib	Flumatinib	Sunitinib
WT + mL3	>10000	>5000	>10000
WT + rmSCF	351.8 $\pm$ 30.6	517.6 $\pm$ 110.0	16.3 $\pm$ 6.1
Del(T417Y418D419) ins Ile	32.9 $\pm$ 11.9	6.3 $\pm$ 1.1	7.4 $\pm$ 3.1
Y503-F504 ins AY	192.0 $\pm$ 9.2	275.0 $\pm$ 36.9	10.9 $\pm$ 1.4
V559D	3.0 $\pm$ 0.5	4.3 $\pm$ 0.9	2.0 $\pm$ 0.3
Del(V559V560)	2.9 $\pm$ 0.6	4.2 $\pm$ 1.2	2.8 $\pm$ 0.7
D579-H580 ins IDPTQLPYD	59.0 $\pm$ 6.3	76.4 $\pm$ 4.5	47.4 $\pm$ 7.3
V559D+V654A	108.5 $\pm$ 14.8	99.0 $\pm$ 28.8	3.0 $\pm$ 0.5
V559D+T670I	6552 $\pm$ 354.5	419.2 $\pm$ 48.0	2.0 $\pm$ 0.3
D816H	208.8 $\pm$ 48.7	34.4 $\pm$ 11.8	17.5 $\pm$ 3.9
D816V	8585 $\pm$ 600.4	1792 $\pm$ 451.2	294.7 $\pm$ 121.9
D816Y	1046 $\pm$ 229.9	302.7 $\pm$ 28.6	73.1 $\pm$ 21.4
V559D + D816H	963.4 $\pm$ 340.9	109.0 $\pm$ 43.5	704.4 $\pm$ 255.9
V559D+D820G	50.0 $\pm$ 9.1	11.2 $\pm$ 5.1	80.7 $\pm$ 16.8
N822K	252.5 $\pm$ 33.1	16.5 $\pm$ 5.1	37.0 $\pm$ 6.1
V559D + N822K	67.4 $\pm$ 30.4	10.4 $\pm$ 3.9	112.9 $\pm$ 60.9
V559D + Y823D	219.8 $\pm$ 48.5	6.3 $\pm$ 2.3	579.0 $\pm$ 160.3
V559D + A829P	92.4 $\pm$ 15.0	11.2 $\pm$ 4.1	192.6 $\pm$ 36.1

Cells were plated in 96-well plates and incubated with different concentrations of each drug for 72 h in triplicate. Cell proliferation was determined using the MTT assay. Values represent the means $\pm$ SDs of at least three independent experiments. mL-3, mouse interleukin 3; rmSCF, recombinant mouse stem cell factor; WT, wild-type.



LA NGS LO RESUELVE TODO

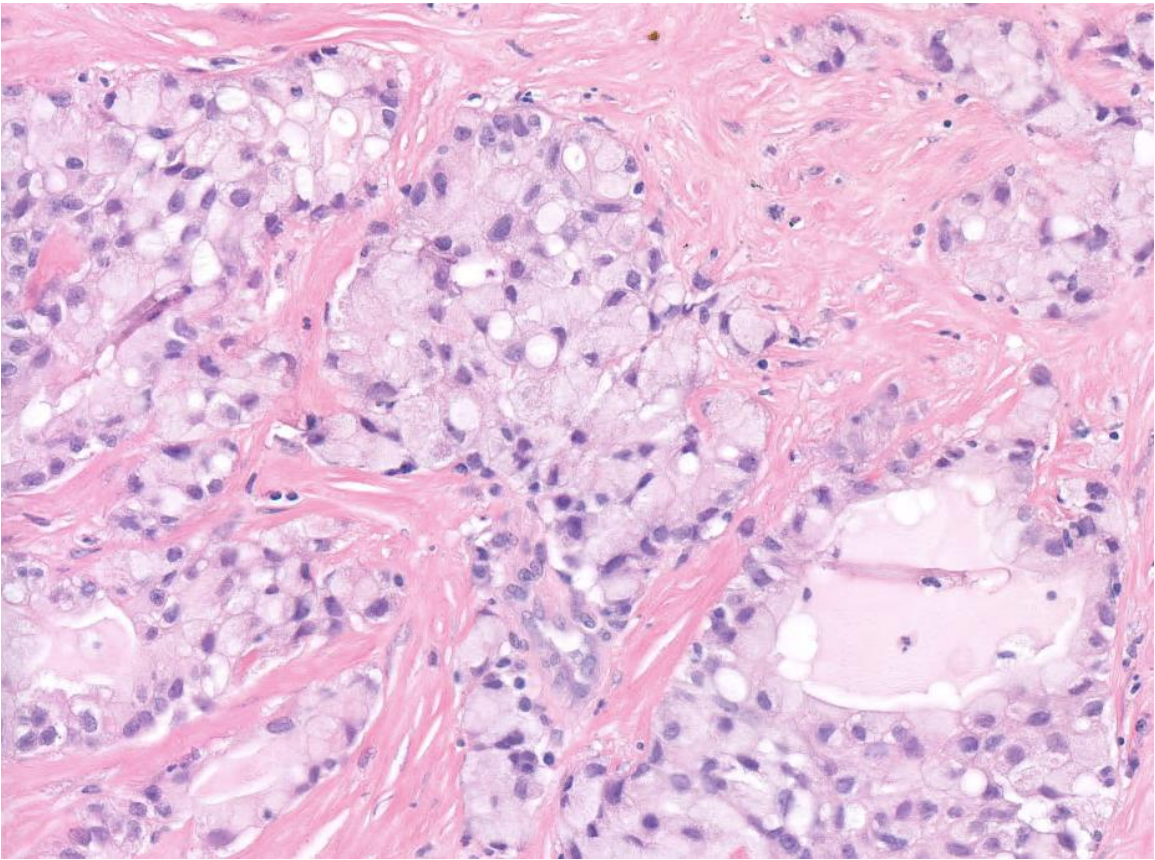


Historia Clinica

- Mujer 76 años Nunca Fumadora
- A raíz de una disfonia se realizan estudios de imagen detectando una masa pulmonar LSI de 5cm que afectando Hilio
- Adenocarcinoma Mucinoso T3N2 con PDL1-Negativo
- Se solicita estudio de NGS

NHC: 17790999

Presencia de Células en Anillo de sello
Adenocarcinoma Mucinoso



qPCR de EGFR
IHQ ALK
IHQ ROS1

			G	H	I	J	K	L	M	N	
NHC	CIP	Num DNA	Num RNA	RESULTAT	[DNA] Qubi	[RNA] Qubi	% Tumora	Fecha solicit	DATA SEQ	INFORME	
17790999	COUR1470312003	VH23P031858A001002	VH23P031858A002001	single gene?	0,5	too low	40%	13/12/2023	no realitzat	7/1/2024	

NHC: 17790999

ANÀLISI MUTACIONAL DEL GEN EGFR

Informació de la mostra:

Origen de la mostra: pulmó

Tipus de mostra: FFPE

Contingut tumoral: 40%

Resultat: NO MUTAT

Metodologia:

PCR a temps real emprant el test cobas® EGFR Mutation Test v2 (Roche) que identifica 42 mutacions en els exons 18, 19, 20 i 21 del gen epidermal growth factor receptor (EGFR). En mostres d'ADN genòmic, aquesta metodologia permet una sensibilitat de detecció de fins un 5% d'al·lels mutats a la mostra analitzada.



H/E

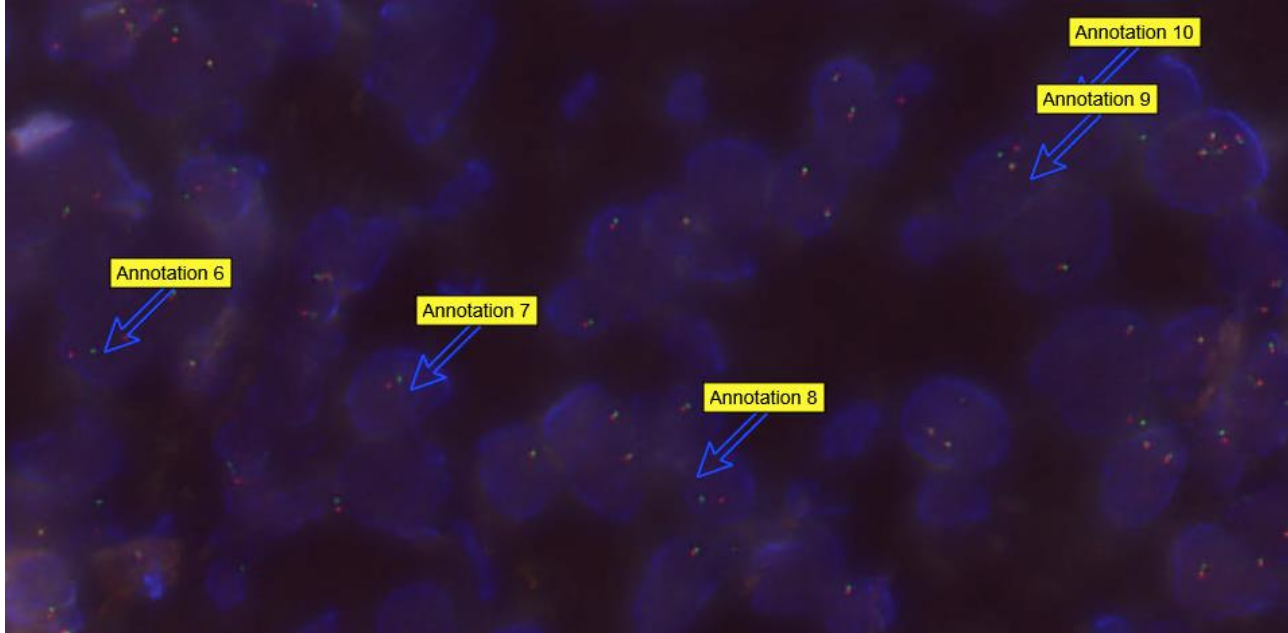


ALK



ROS1

NHC: 17790999



Molecular VH23P031858A003005- ALK FISH BA

Surt al informe

Normal	t	B	I	U						µm	²	³	α	β	A	K
FISH para el gen ALK (2p23) realizado con la sonda dual color break apart rearrangement probe (ref. 53206N38-020 de Vysis) sobre tejido parafinado. Se considera la muestra adecuada tras identificarse más de 100 células tumorales.																
RESULTADO: Se observan señales separadas/ rojas aisladas en 23 de 50 células contabilizadas. PRESENCIA DE TRASLOCACIÓN DE ALK (46%)																
Nota: para la determinación e interpretación de este biomarcador se han utilizado los criterios y recomendaciones de la guía de la IASCI vigente.																

NHC: 17790999



Tipo de Test GeneFusion_RU0/1.2		Cartucho
Operador Sandra Valera		ID del cartuch
Solicitud completada 09 Jan 2024 (11:22)		Cartuch
Instrumento SER1525		Fecha expi
Test completado 09 Jan 2024 (14:18)		Número de lot
Muestra		
ID de muestra 23p31858		
Tipo de muestra Tejido FFPE		
Idylla™ GeneFusion Assay		
Solo para uso en investigación. No para uso en procedimientos de diagnóstico.		
OVERALL GENEFUSION RESULT		DETECTED
ALK FUSION		
ALK Specific Fusion		Detected
Cq ALK Specific Fusion		25.5
ALK Expression Imbalance		Detected
Cq of the 5' ALK		34.7
ΔCq ALK Expression Imbalance (3' - 5')		-8.1
ROS1 FUSION		
ROS1 Specific Fusion		Not Detected
ROS1 Expression Imbalance		Not Detected
Cq of the 5' ROS1		23.3
ΔCq ROS1 Expression Imbalance (3' - 5')		0.5
RET FUSION		
RET Specific Fusion		Not Detected
RET Expression Imbalance		Not Detected
Cq of the 5' RET		30.4
ΔCq RET Expression Imbalance (3' - 5')		2.5
MET EXON 14 SKIPPING		
MET Exon 14 Skipping		Not Detected
Cq MET Wild Type		23.4
NTDK1		

IDYLLA™ GENEFUSION PANEL DETECTION OF KNOWN AND NOVEL FUSIONS

A unique combination of 2 detection technologies:



Highly sensitive detection of the most relevant gene fusions directly from RNA transcripts by real-time PCR (RT-qPCR).



Expression imbalance detects gene fusions, irrespective of the fusion partner, based on the 3' kinase overexpression caused by that partner gene. Expression imbalance results are indicative for the presence of a fusion and should be confirmed with another technology.

Detection Method	ALK	ROS1	RET	MET ex14
Specific fusion detection	•	•	•	•
Expression imbalance	•	•	•	-

VH-23-P-191

INFORME ANATOMIA PATOLÒGICA



**ESTUDI D'ALTERACIONS GENÈTIQUES EN TUMORS SÒLIDS
MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)**

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Origen de la mostra: gangli limfàtic

Tipus de mostra: PAAF

Indicació: Càncer de pulmó

Referència DNA: VH23P000191A001, Mostra Valorable

Referència RNA: VH23P000191A002, Mostra NO Valorable

Contingut tumoral: 70%

RESULTATS

1. Variants ACCIONABLES

No s'han detectat alteracions accionables

2. ALTRES alteracions patogèniques

TP53 NM_000546.5: c.637C>T p.Arg213Ter VAF 6,2%

3. Variants DE SIGNIFICAT INCERT

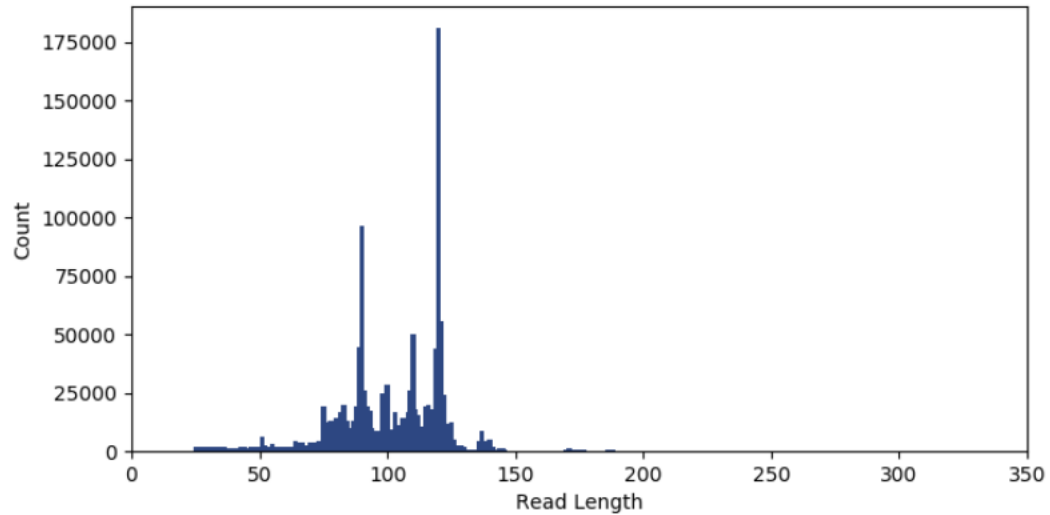
No s'han detectat alteracions de significat incert

Sample QC - DNA



MAPD	✓	0,26
[0-0.5]		
Mapped Reads	✓	1.277.479
>=350000		
Mean AQ20 Read Length (bp)	—	92
Not Set		
Mean Read Length (bp)	✓	101
>=80		
Uniformity Of Base Coverage	✓	100
>=94		

Read Length Histogram

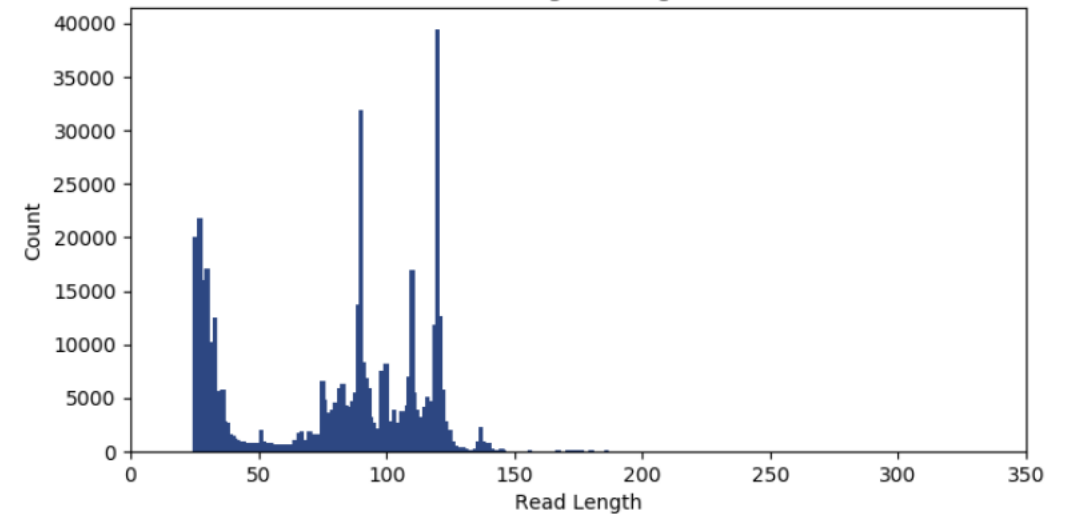


Sample QC - RNA



Mapped Reads	✓	8796
>=0		
Mean Read Length (bp)	✓	77
>=0		
RNA Expression Ctrls Detected	✗	4
>=5		

Read Length Histogram



Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
VH23P000191A001D_VH23P000191A002R	31 JAN 2023	Female	FFPE	Solid Tumor	Non-Small Cell Lung Cancer	Stage IV	45

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity of Amplicon Coverage
100.00%	100.00%	4925	100.00%	93.56%	100.00%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

Oncomine Extended (5.16) filter chain and Non-Small Cell Lung Cancer gene list applied

Edit Filters

ALK

BRAF

EGFR

ERBB2

KRAS

MET

NTRK1

NTRK2

NTRK3

RET

ROS1

All

SNVs/Indels

Fusions

CNVs

☐ Gene List Only

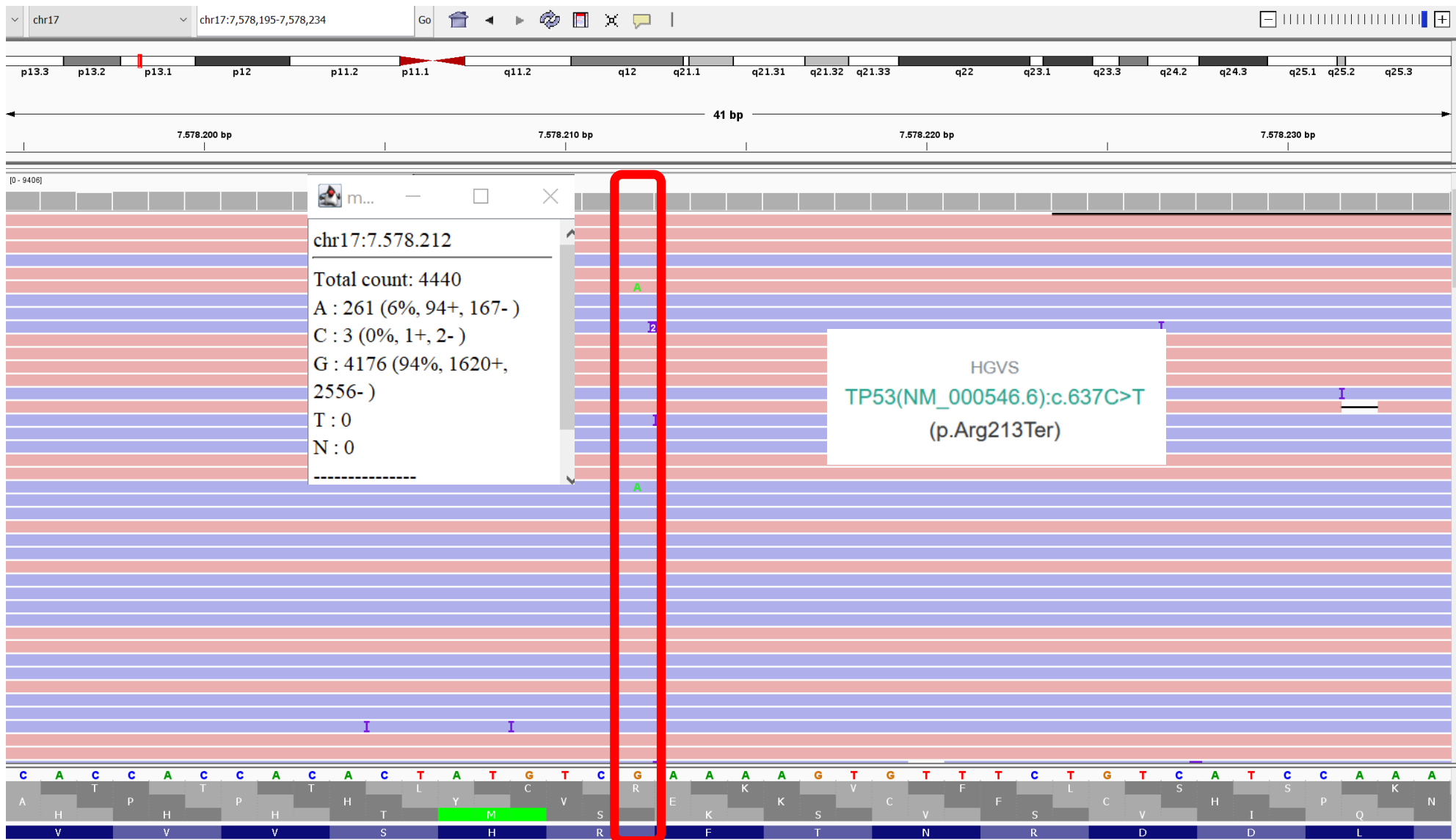
Oncomine Extended (5.16) Filter Chain Applied

1 of 2,154 SNVs/Indels

Edit Filters

Exp

User Classification	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	AA Change	Allele Frequency (%)	Call	Coverage	Nuc Change	Effective Read De
Classification ▼	chr17:7578212	Truncating	Loss-of-Function	TP53	p.R213*	6.2	PRESENT (HETEROZYGOUS)	4352	c.637C>T	4352



General Information SNV TP53(NM_000546.6):c.637C>T (p.Arg213Ter)	PharmGKB No data available PREMIUM	ACMG Classification Pathogenic 17 points = 17 P - 0 B	Frequencies exomes: $f = 0$ genomes: $f = 0$ (cov:32.4)	Pathogenicity Scores 52	Beacon Network
Genes TP53	Transcripts NM_000546.6 - nonsense MANE Select	ClinVar Pathogenic ☆☆☆ Submissions: 14	MitoMap No data available	ClinGen No data available New	Protein Viewer
Community Contributions	Region Browser New	LOVD No data available PREMIUM New	OMIM ® No data available PREMIUM New	GWAS No data available	
Publications Variant: 41 Gene: 2088	Expression Data Top: Cells - EBV-transformed lymphocytes Tissues: 54	Uniprot Variants No data available	Conservation Scores phyloP100: 0.754	Structural Variants New	

Variant

Explain

Chromosome	Position	REF Sequence	ALT Sequence	Variant type	Cytoband	HGVS	RS ID	Gene symbol
chr17	7674894	G	A	SNV	17p13.1-17p13.1	TP53(NM_000546.6):c.637C>T (p.Arg213Ter)	rs397516436 dbSNP	TP53
UCSC genome browser Mastermind TraP Score								

This variant has been viewed 344 times on VarSome.



Genes (Exons)	Read Counts	Type	Call	Gene Isoform
G6PD	463	ProcControl	PRESENT	G6PD.E7E8.NM_000402.3
EIF2B1	115	ProcControl	PRESENT	EIF2B1.E8E9.NM_001414.3
HMBS	61	ProcControl	PRESENT	HMBS.E3E4.NM_000190.3
ANKRD17	53	ProcControl	PRESENT	ANKRD17.E25E26.NM_032217.3
ALAS1	3	ProcControl	ABSENT	ALAS1.E3E4.NM_000688.4
TRIM27	3	ProcControl	ABSENT	TRIM27.E7E8.NM_006510.4
TBP	0	ProcControl	ABSENT	TBP.E3E4.NM_001172085.1



Idylla™ GeneFusion Panel*



Detection of

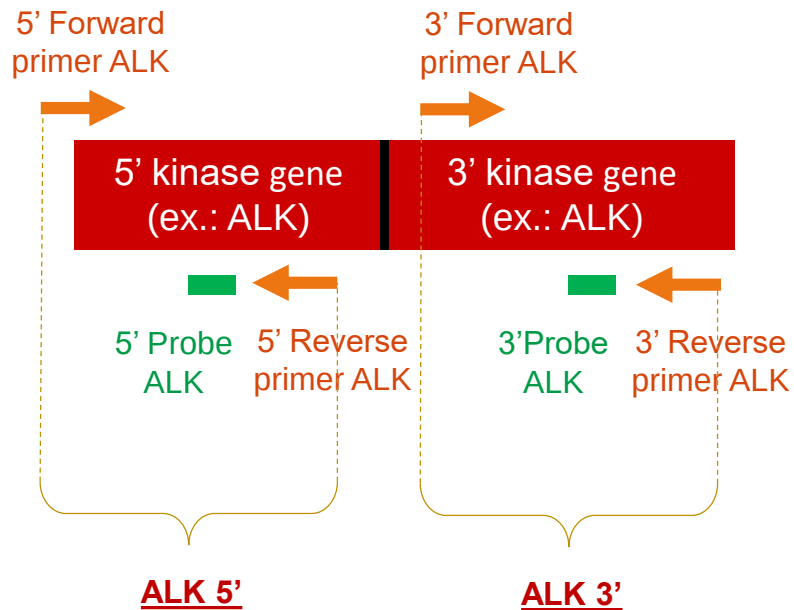
- ALK - ROS1 - RET fusion transcripts
- MET exon 14 skipping transcripts
- Indication of the presence of ALK, RET, ROS1 based on expression imbalance assay

in FFPE sections of tumor tissue from NSCLC patients, incl. resected samples and core needle biopsies



GENERIC DETECTION BASED ON EXPRESSION IMBALANCE

No expression imbalance

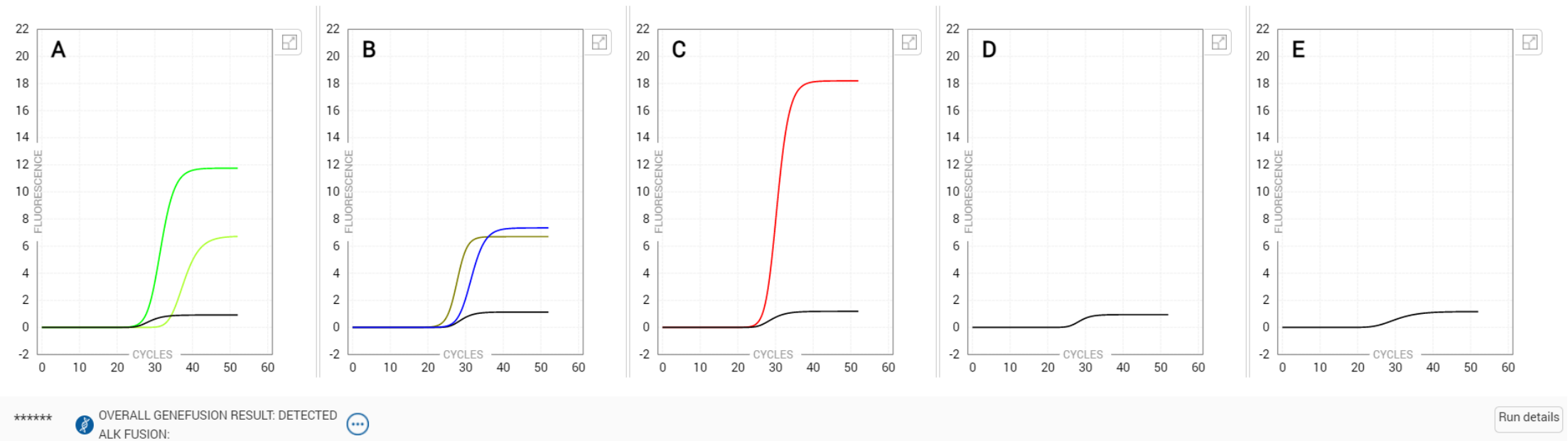


$$Cq_{ALK\ 5'} \approx Cq_{ALK\ 3'}$$

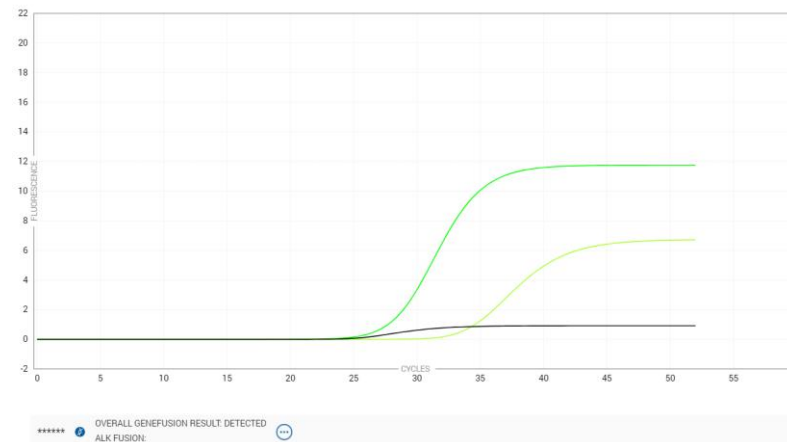
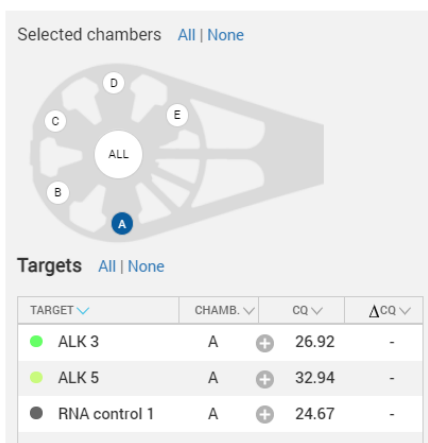
Expression imbalance



$$Cq_{ALK\ 5'} > Cq_{ALK\ 3'}$$



TARGET	CQ	Δ CQ	TARGET	CQ	Δ CQ	TARGET	CQ	Δ CQ	TARGET	CQ	Δ CQ	TARGET	CQ	Δ CQ
ALK 3	26.92	-	MET WT	23.79	-	DNA control	26.43	-	RNA control 1	24.85	-	RNA control 1	23.53	-
ALK 5	32.94	-	RNA control 1	25.19	-	RNA control 1	24.77	-						
RNA control 1	24.67	-	RNA control 2	26.86	-									



+ OVERALL GENE FUSION RESULT: DETECTED

ALK FUSION:

- ALK Specific Fusion: Not Detected

+ ALK Expression Imbalance: Detected

Cq of the 5' ALK: 32.9

Δ Cq ALK Expression Imbalance (3' - 5'): -6.0

INFORME DE RESULTADO DEL TEST

Prueba realizada: ANATOMIA PATOLOGICA, Passeig Vall D'Hebron 119-129 BARCELONA, Spain, Javier Herandez Losa, 0034 934894582



muestra ID	VH23P000191A003004		
Tipo de muestra	Tejido FFPE		
ID de Cartucho	61040750		
Tipo Test	GeneFusion_RUO/1.0	Versión de TTP	1.6
ID del lote	00006104	Fecha expir.	13 dic. 2023

N.º de serie del Instrumento	SER1524
Versión software Instrumento	27.0
N.º serie de la Consola	C00001077
Versión de software de la Consola	4.4.0.630
Solicitud de Test completada	08 feb. 2023 (19:04)
Test iniciado	08 feb. 2023 (19:05)
Prueba finalizada	08 feb. 2023 (22:02)
Estado Test	Resultado publicado: Automático, 08 feb. 2023 (22:02)
Operador	Ariadna Mendez

Resultado del Test Solo para uso en investigación. No para uso en procedimientos de diagnóstico.

Idylla™ GeneFusion Assay

OVERALL GENEFUSION RESULT	DETECTED
ALK FUSION	
ALK Specific Fusion	Not Detected
ALK Expression Imbalance	Detected
Cq of the 5' ALK	32.9
ΔCq ALK Expression Imbalance (3' - 5')	-6.0

Aplicación reordenamientos Sin filtro.

No Filter Chain Applied
1,007 of 1,007 Fusions

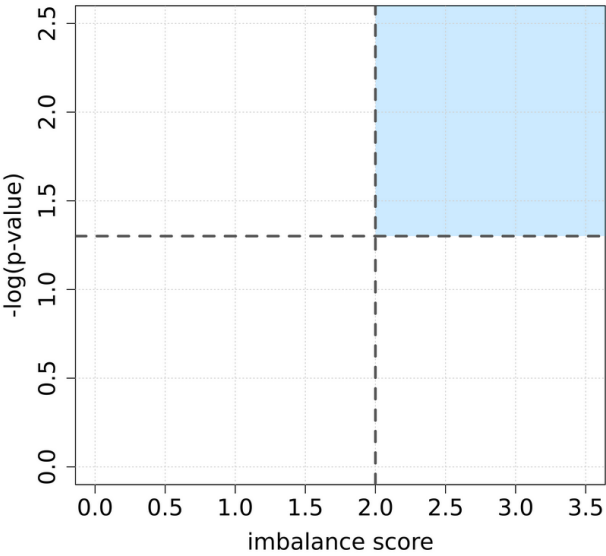
Edit Filters

Export

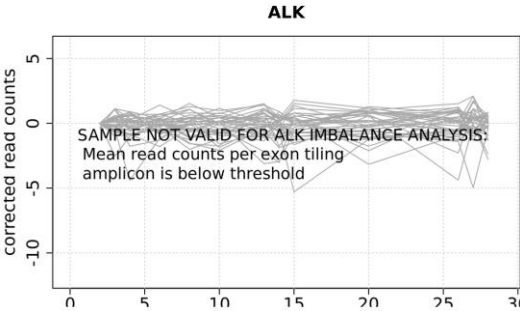
Columns ▾

Genes (Exons)	Read Counts	Type	Call	Gene Isoform	Oncomin
ALK	0	RNAExonTiles	NO CALL	ALK	ALK
A2M(22) - ALK(19)	0	Fusion	ABSENT	A2M-ALK.A22A19	ALK
ACTG2(1) - ALK(17)	0	Fusion	ABSENT	ACTG2-ALK.A1A17	ALK
ACTG2(1) - ALK(19)	0	Fusion	ABSENT	ACTG2-ALK.A1A19	ALK
ACTG2(2) - ALK(18)	0	Fusion	ABSENT	ACTG2-ALK.A2A18	ALK
AGAP1(6) - ALK(18)	0	Fusion	ABSENT	AGAP1-ALK.A6A18	ALK
ATG16L1(2) - ALK(20)	0	Fusion	ABSENT	ATG16L1-ALK.A2A20	ALK
ATG16L1(2) - ALK(20)	0	Fusion	ABSENT	ATG16L1-ALK.A2ins25ins97A20	ALK
ATIC(7) - ALK(20)	0	Fusion	ABSENT	ATIC-ALK.A7A20.COSF444	ALK
ATRNL1(19) - ALK(20)	0	Fusion	ABSENT	ATRNL1-ALK.A19A20	ALK
BCL11A(4) - ALK(20)	0	Fusion	ABSENT	BCL11A-ALK.B4A20	ALK
BEND5(3) - ALK(20)	0	Fusion	ABSENT	BEND5-ALK.B3A20	ALK
BIRC6(10) - ALK(20)	0	Fusion	ABSENT	BIRC6-ALK.B10A20	ALK

Imbalance Calls: VH23P000191A001D_VH23P000191A002R_LibPre...



+ imbalance positive o imbalance negative ♦ control gene



LA NGS NOS DA ALEGRIAS



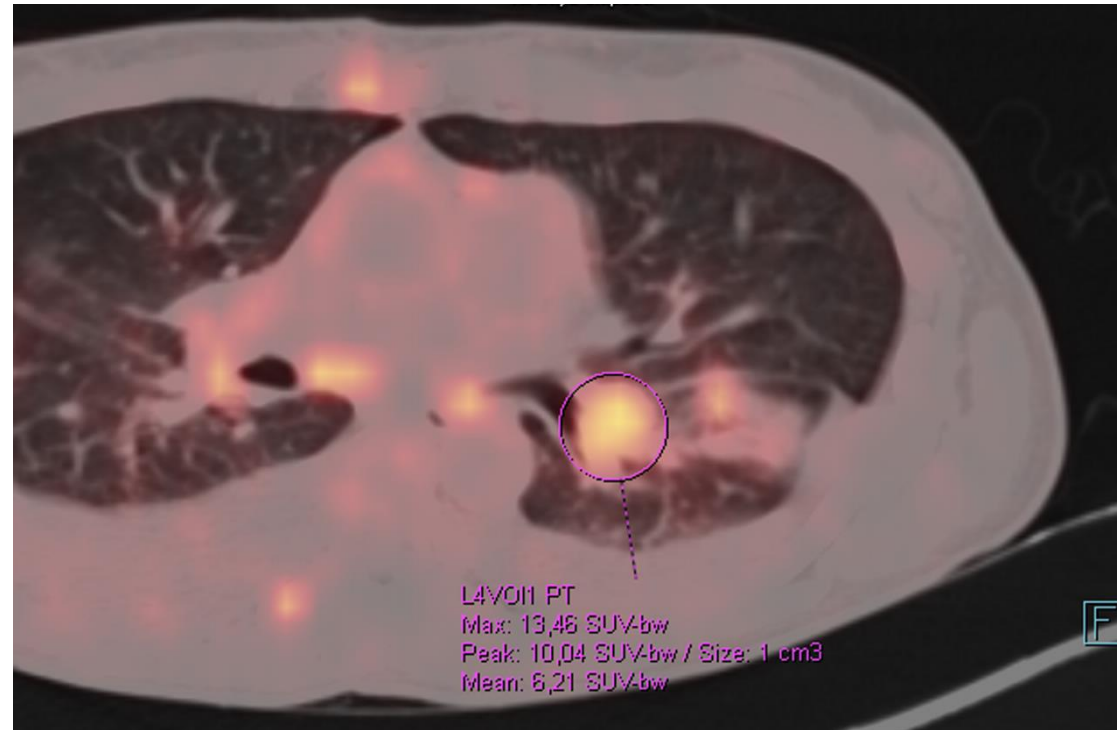
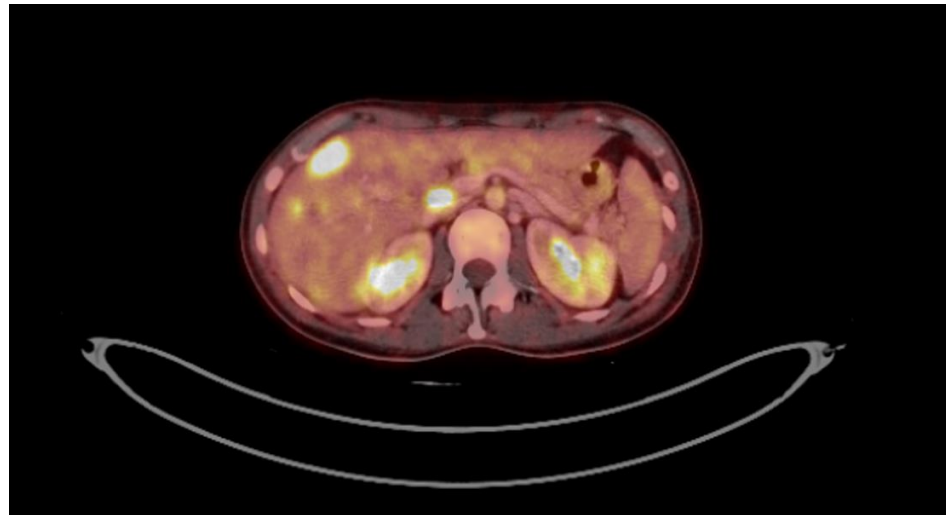
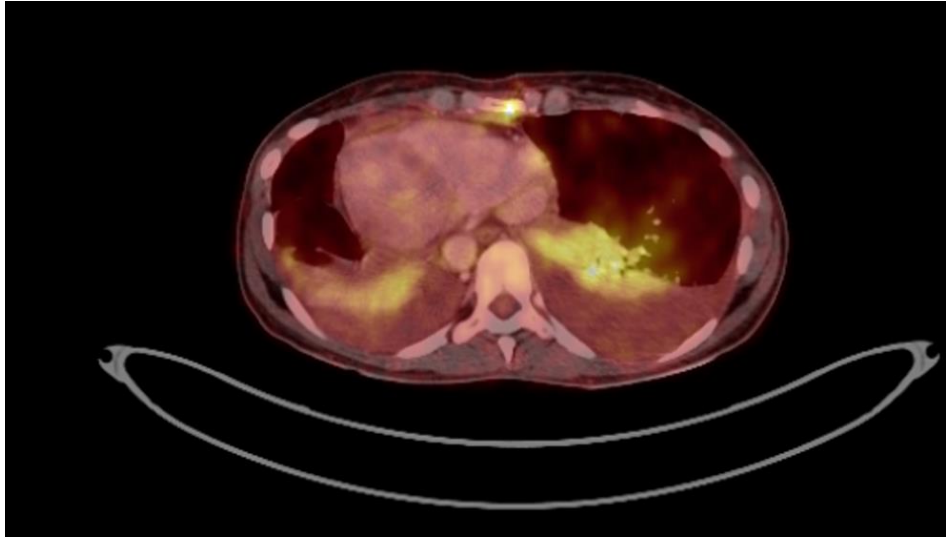
PRESENTACIÓN DEL CASO

- Mujer de 48 años
- No fumadora, profesora sin exposición a tóxicos
- No antecedentes familiares
- Sin Antecedentes Médicos Conocidos ni comorbilidades

PRESENTACIÓN DEL CASO

- Primer síntoma: disnea -> ingreso por taponamiento cardíaco
- Proceso diagnóstico:
 - AngioTC Tx Urgencias 7/10/22: Derrame pericárdico. Derrame pleural bilateral
 - PET-TC 19/10/22: **nódulo** hipermetabólico en **LII (8mm; SUVmax: 9.1)**. **Adenopatías** hipermetabólicas en **mediastino**, hilios pulmonares bilaterales (16mm; SUVmax: 13.4). Múltiples **lesiones hepáticas** hipermetabólicas. Adenopatías hipermetabólicas en hilio hepático (SUVmax: 17.9). Lesiones **osteolíticas** hipermetabólicas en columna vertebral, parrilla costal y pelvis
 - TC SNC: no M1
 - Citología pericárdica 24/10/22: **adenocarcinoma de pulmón**. Oncomine: **EML4 - ALK**. Otras alteraciones patogénicas: No se han detectado
 - Biopsia hepática 28/10/22: M1 adenocarcinoma pulmonar. IHQ ALK positivo.

PET-TC 19/10/22



PRESENTACION DEL CASO



Angio TC + PET-TAC

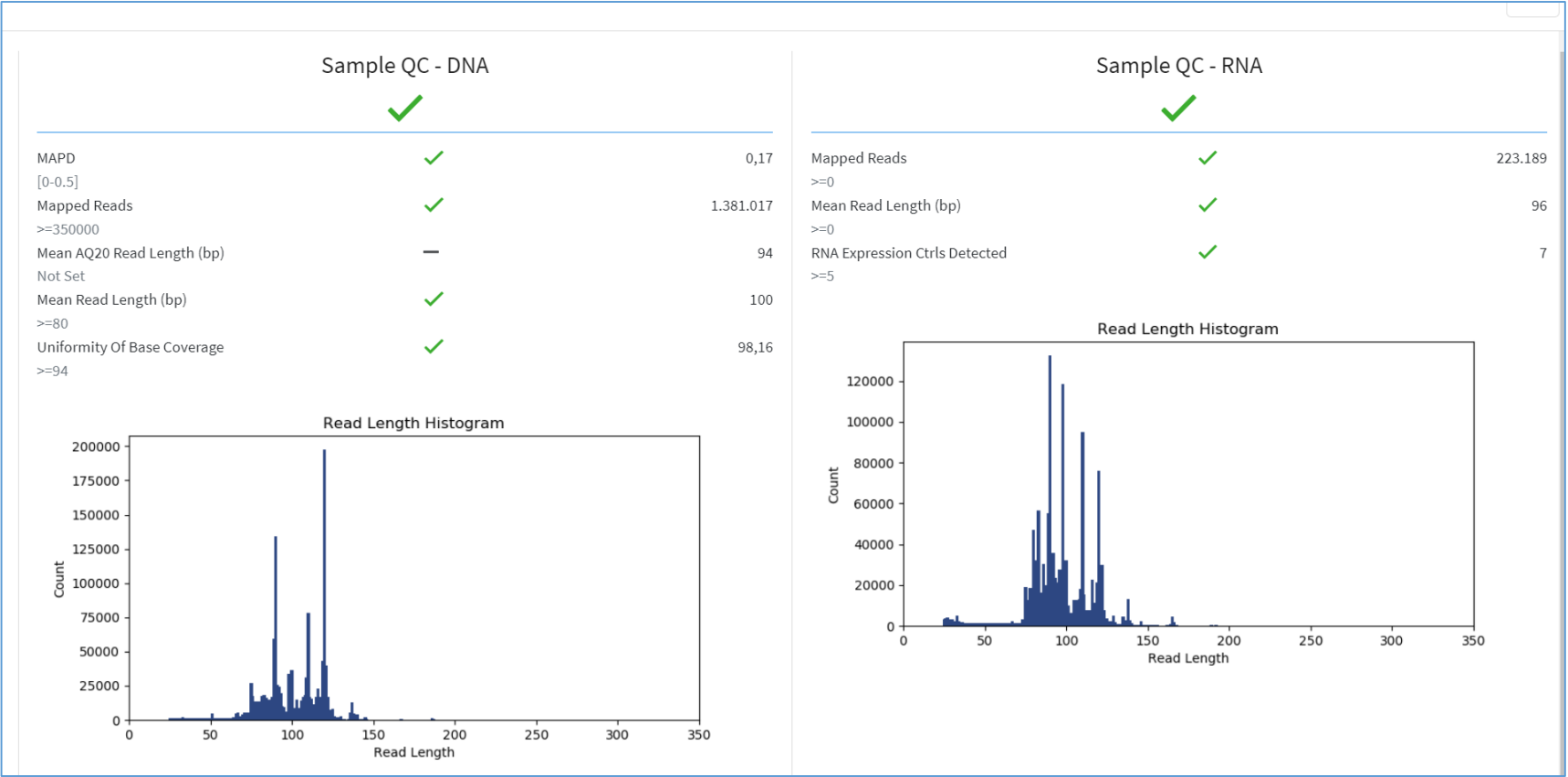
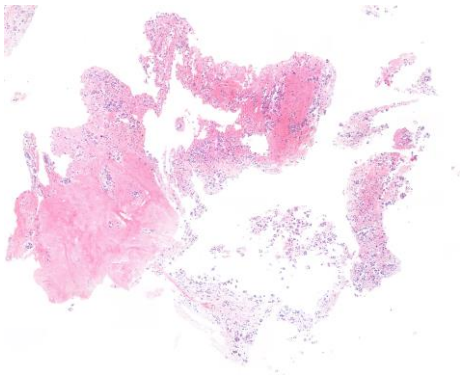
Derrame Pleura
Nodulo Hipermetabolico
pulmonar
Adenopatias
mediastinicas.
Lesionas hepáticas
• Lesiones osteolíticas

Citologia Liquido pericardico

Biopsia Hepática

ANALISIS NGS DE LA MUESTRA LIQUIDO PLEURAL

Liquido Pleural



2A003R

QC

Key Findings

Variants

Plugins

Reports

CNVs

☐ Gene List Only

Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Genes (Exons)	Read Counts
ALK	Yes	chr2:29455169	ExpressionImbalance	Gain-of-Function	ALK	0
EML4-ALK.E13A20.COSF408.2	Yes	chr2:42522656 - chr2:29446394	Fusion	Gain-of-Function	EML4(13) - ALK(20)	500

PRESENTACION DEL CASO



Angio TC + PET-TAC

- Derrame Pleura
- Nodulo Hipermetabolico pulmonar
- Adenopatias mediastinicas.
- Lesionas hepáticas
- Lesiones osteolíticas



Alectinib



- Disminuye M1 Hepáticas
- Mantiene derrame pleural

Biopsia Pleural

- IHQ ALK Positivo
- FISH ALK Positivo (62%)

TC 23/11/22



TC 30/01/23



PRESENTACION DEL CASO



Angio TC + PET-TAC

Derrame Pleura
Nodulo Hipermetabolico
pulmonar
Adenopatias
mediastinicas.
Lesionas hepáticas
Lesiones osteolíticas



Alectinib



Disminuye M1 Hepáticas
Mantiene derrame pleural



Progresión

No mejora de derrame
pleural.

Citologia Pleural 03/02/2023

Panel 300 genes VHIO: EML4- ALK; FANCA V372A VAF 49,6%
Guardant Suero: EML4- ALK

Lorlatinib



CASO CLINICO

- Dos visitas a Urgencias con toracocentesis -> Ingresa por insuficiencia respiratoria por DP bilateral -> drenaje 15/2/23 -> 20/2/23 pleurX bilateral
 - Bx pleural 20/2/23: reacción inflamatoria a talcaje
 - Citología pleural 20/2/23: Oncomine: EML4 - ALK. Otras alteraciones patogénicas: MYC. Amplificación del número de copias (CNV): 8,18; FANCA V372A VAF 47,4%
 - Alta a domicilio 23/2/23 con pleurex bilateral + ODC
- Por mala evolución se adelanta TC TA 25/02 con pericárdico severo (grosor máximo 33 mm, previo 07/02 de 15 mm), así como TEP arterias inferiores derechas -> pericardiocentesis + enoxa

IMAGEN TRAS TRATAMIENTO 2 LINEA

TC 25/02/23



POSIBLES MECANISMOS DE RESISTENCIA A TRTAMIENTOS ALK

nature cancer

Review Article

<https://doi.org/10.1038/s43018-023-00515-0>

ALK-positive lung cancer: a moving target

Received: 3 March 2022

Jaime L. Schneider^{1,2}, Jessica J. Lin^{1,2} & Alice T. Shaw^{1,2}✉

Accepted: 10 January 2023

Published online: 16 February 2023

[Check for updates](#)

Anaplastic lymphoma kinase (ALK) is a potent oncogenic driver in lung cancer. ALK tyrosine kinase inhibitors yield significant benefit in patients with ALK fusion-positive (ALK⁺) lung cancers; yet the durability of response is limited by drug resistance. Elucidation of on-target resistance mechanisms has facilitated the development of next-generation ALK inhibitors, but overcoming ALK-independent resistance mechanisms remains a challenge. In this Review, we discuss the molecular underpinnings of acquired resistance to ALK-directed therapy and highlight new treatment approaches aimed at inducing long-term remission in ALK⁺ disease.

[Cancer Discov.](#) Author manuscript; available in PMC 2018 Feb 1.

PMCID: PMC5296241

Published in final edited form as:

NIHMSID: NIHMS838154

[Cancer Discov.](#) 2017 Feb; 7(2): 137–155.

PMID: [28122866](#)

Published online 2017 Jan 25. doi: [10.1158/2159-8290.CD-16-1123](#)

Targeting ALK: Precision Medicine Takes On Drug Resistance

Jessica J. Lin,¹ Gregory J. Riely,² and Alice T. Shaw¹

► Author information ► Copyright and License information ► [Disclaimer](#)

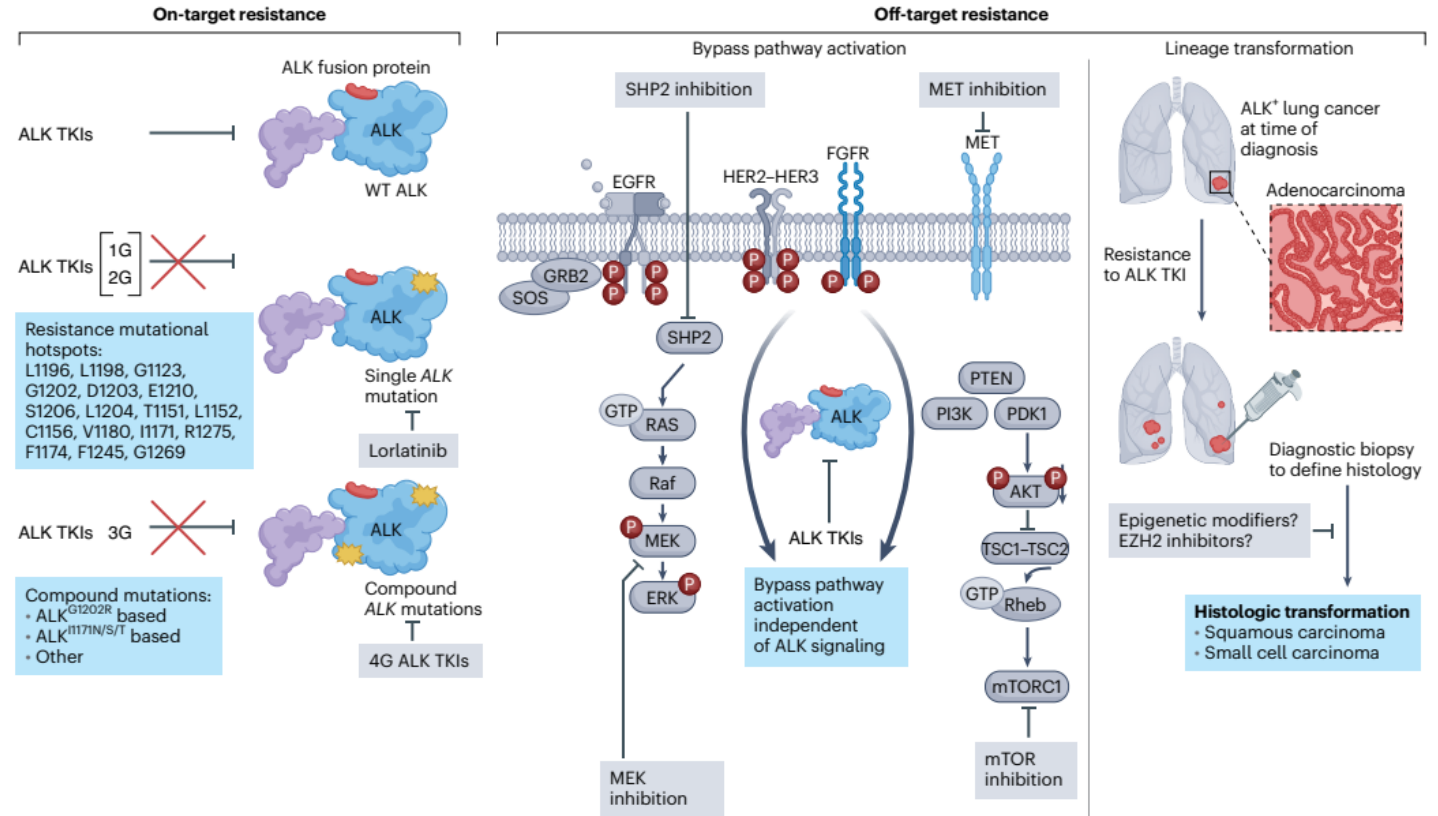
The publisher's final edited version of this article is available free at [Cancer Discov](#)

Abstract

Go to: ►

Anaplastic lymphoma kinase (ALK) is a validated molecular target in several ALK-rearranged malignancies, including non-small-cell lung cancer (NSCLC). However, the clinical benefit of targeting ALK using tyrosine kinase inhibitors (TKIs) is almost universally limited by the emergence of drug resistance. Diverse mechanisms of resistance to ALK TKIs have now been discovered, and these basic mechanisms are informing the development of novel therapeutic strategies to overcome resistance in the clinic. In this Review, we summarize the current successes and challenges of targeting ALK.

Keywords: ALK, anaplastic lymphoma kinase, TKI, resistance, NSCLC



MYC COMO MECANISMO DE RESISTENCIA A TRATAMIENTO ALK



Front Oncol. 2021; 11: 713530.

Published online 2021 Oct 1. doi: [10.3389/fonc.2021.713530](https://doi.org/10.3389/fonc.2021.713530)

PMCID: PMC8517331

PMID: [34660278](https://pubmed.ncbi.nlm.nih.gov/34660278/)

The Resistance Mechanisms and Treatment Strategies for ALK-Rearranged Non-Small Cell Lung Cancer

Yue Pan,¹ Chao Deng,¹ Zhenhua Qiu,¹ Chenghui Cao,² and Fang Wu^{1, 3, 4, 5, *}

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Abstract

Go to: ►

Anaplastic lymphoma kinase (ALK) is a validated molecular target for non-small-cell lung cancer (NSCLC). The use of tyrosine kinase inhibitors (TKIs) has led to significantly improved survival benefits. However, the clinical benefits of targeting ALK using TKIs are limited due to the emergence of drug resistance. The landscape of resistance mechanisms and treatment decisions has become increasingly complex. Therefore, continued research into new drugs and combinatorial therapies is required to improve outcomes in NSCLC. In this review, we explore the resistance mechanisms of ALK TKIs in advanced NSCLC in order to provide a theoretical basis and research ideas for solving the problem of ALK drug resistance.

Keywords: anaplastic lymphoma kinase (ALK), TKI - tyrosine kinase inhibitor, resistance, NSCLC, therapy

2.3 Primary ALK TKI Resistance

Progression of ALK-TKI within 3 months is considered primary resistance. In theory, any of these mechanisms of acquired resistance that existed before the use of TKI could also lead to primary resistance ([23](#)).

BIM is a Bcl-2 (B lymphocytoma-2) -like protein 11 that activates programmed cell death in cells. The study found that patients with BIM with missing polymorphisms had shortened PFS and reduced objective response rate, which was an independent predictor of patients treated with crizotinib and was related to primary drug resistance ([97](#)). In addition, the low minimum allele frequency (MAF) of the *EML4-ALK* rearrangement may also be a mechanism of primary resistance to crizotinib ([98](#)).

Rihawi K reported a patient with primary resistance to crizotinib. **MYC** amplification was a potentially new mechanism of primary ALK-TKI, resistance and proposed as a potential **MYC**-oriented inhibition strategy to overcome primary resistance of advanced *ALK*-rearrangement NSCLC ([99](#)). Similarly, the results of Pilling AB et al. showed a dual oncogene mechanism, in which ALK positively regulates the **MYC** signaling axis, providing an additional oncogene target ([100](#)).



NECESITO VER MAS COSAS POR NGS

OPA_CNVs

Gene	Copy Number	Call
ALK	2.33	ABSENT
AR	1.91	ABSENT
CD274	1.84	ABSENT
CDKN2A	2	ABSENT
EGFR	2.29	ABSENT
ERBB2	1.51	ABSENT
ERBB3	2	ABSENT
FGFR1	2.18	ABSENT
FGFR2	2.24	ABSENT
FGFR3	1.56	ABSENT
KRAS	1.96	ABSENT
MET	1.64	ABSENT
PIK3CA	2.11	ABSENT
PTEN	1.96	ABSENT



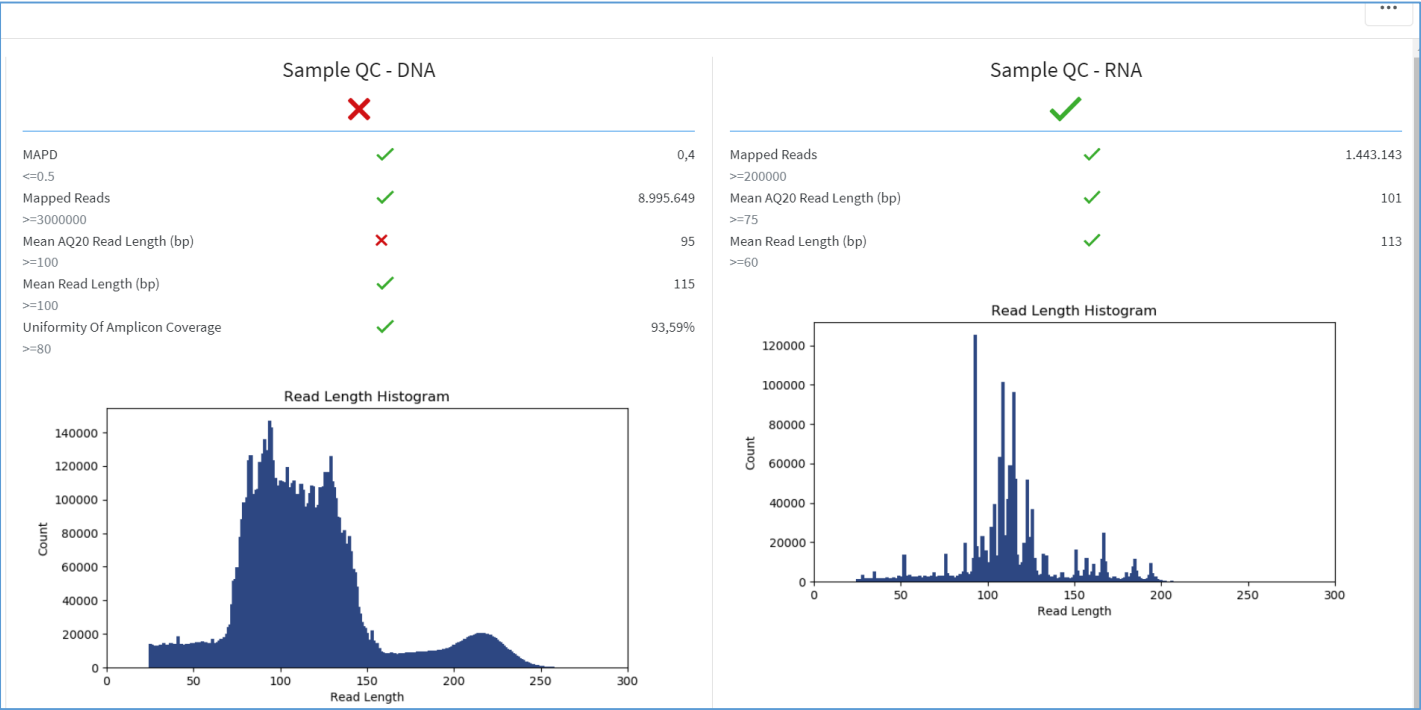
ion torrent

Hotspot genes				Full-length genes			Copy number genes		Gene fusions (inter- and intragenic)		
AKT1	ESR1	KIT	PDGFRB	ARID1A	FBXW7	PTEN	AKT1	FGFR4	AKT2	FGFR2	NUTM1
AKT2	EZH2	KNSTRN	PIK3CB	ATM	MLH1	RAD50	AKT2	FLT3	ALK	FGFR3	PDGFRA
AKT3	FGFR1	KRAS	PIK3CA	ATR	MRE11	RAD51	AKT3	IGF1R	AR	FGR	PDGFRB
ALK	FGFR2	MAGOH	PPP2R1A	ATRX	MSH6	RAD51B	ALK	KIT	AXL	FLT3	PIK3CA
AR	FGFR3	MAP2K1	PTPN11	BAP1	MSH2	RAD51C	AXL	KRAS	BRCA1	JAK2	PRKACA
ARAF	FGFR4	MAP2K2	RAC1	BRCA1	NBN	RAD51D	AR	MDM2	BRCA2	KRAS	PRKACB
AXL	FLT3	MAP2K4	RAF1	BRCA2	NF1	RNF43	BRAF	MDM4	BRAF	MDM4	PTEN
BRAF	FOXL2	MAPK1	RET	CDK12	NF2	RB1	CCND1	MET	CDKN2A	MET	PPARG
BTX	GATA2	MAX	RHEB	CDKN1B	NOTCH1	SETD2	CCND2	MYC	EGFR	MYB	RAD51B
CBL	GNA11	MDM4	RHOA	CDKN2A	NOTCH2	SLX4	CCND3	MYCL	ERBB2	MYBL1	RAF1
CCND1	GNAQ	MED12	ROS1	CDKN2B	NOTCH3	SMARCA4	CCNE1	MYCN	ERBB4	NF1	RB1
CDK4	GNAS	MET	SF3B1	CHEK1	PALB2	SMARCB1	CDK2	NTRK1	ERG	NOTCH1	RELA
CDK6	H3F3A	MTOR	SMAD4	CREBBP	PIK3R1	STK11	CDK4	NTRK2	ESR1	NOTCH4	RET
CHEK2	HIST1H3B	MYC	SMO	FANCA	PMS2	TP53	CDK6	NTRK3	ETV1	NRG1	ROS1
CSF1R	HNF1A	MYCN	SPOP	FANCD2	POLE	TSC1	EGFR	PDGFRA	ETV4	NTRK1	RSPO2
CTNNB1	HRAS	MYD88	SRC	FANCI	PTCH1	TSC2	ERBB2	PDGFRB	ETV5	NTRK2	RSPO3
DDR2	IDH1	NFE2L2	STAT3				ESR1	PIK3CB	FGFR1	NTRK3	TERT
EGFR	IDH2	NRAS	TERT				FGF19	PIK3CA			
ERBB2	JAK1	NTRK1	TOP1				FGF3	PPARG			
ERBB3	JAK2	NTRK2	U2AF1				FGFR1	RICTOR			
ERBB4	JAK3	NTRK3	XPO1				FGFR2	TERT			
ERCC2	KDR	PDGFRA					FGFR3				

List of gene targets in the Oncomine Comprehensive Assay v3.

Liquido Pleural

04/04/2023



Fus_v01 / VH23P004331A001001D_VH23P004331A002001R

02001R QC X Key Findings Variants Plugins Reports

Gene List Only

Oncomine Extended (5.16) Filter Chain Applied 4 of 4,171 Variants

Oncomine Gene Class	Oncomine Variant Class	Gene	Locus	AA Change	Ref	Alt	Call	Nuc Change	Allele Frequency (%)
Loss-of-Function	Truncating	FANCA	chr16:89858441	p.Val372AlafsTer42	GCCAA	G	PRESENT (HETEROZYGOUS)	c.1115_1118delTTGG	47.4
		FGFR4	chr5:176520243	p.Gly388Arg	G	A	PRESENT (HETEROZYGOUS)	c.1162G>A	36
Gain-of-Function	Fusion	ALK	chr2:42522656 - chr2:29446394				PRESENT		
Gain-of-Function	Amplification	MYC	chr8:128748724				PRESENT (GAIN)		

PRESENTACION DEL CASO



Angio TC + PET-TAC

Derrame Pleura
Nodulo Hipermetabolico
pulmonar
Adenopatias
mediastinicas.
Lesionas hepáticas
Lesiones osteolíticas



Alectinib



Disminuye M1 Hepáticas
Mantiene derrame pleural



Lorlatinib



No mejora de derrame
pleural.

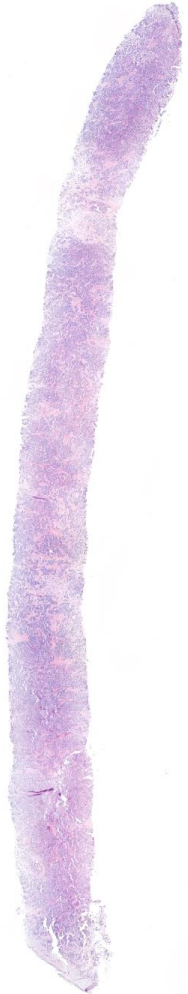


Progresión

¿Resistencia Primaria?

Biopsia Hepatica

H&E



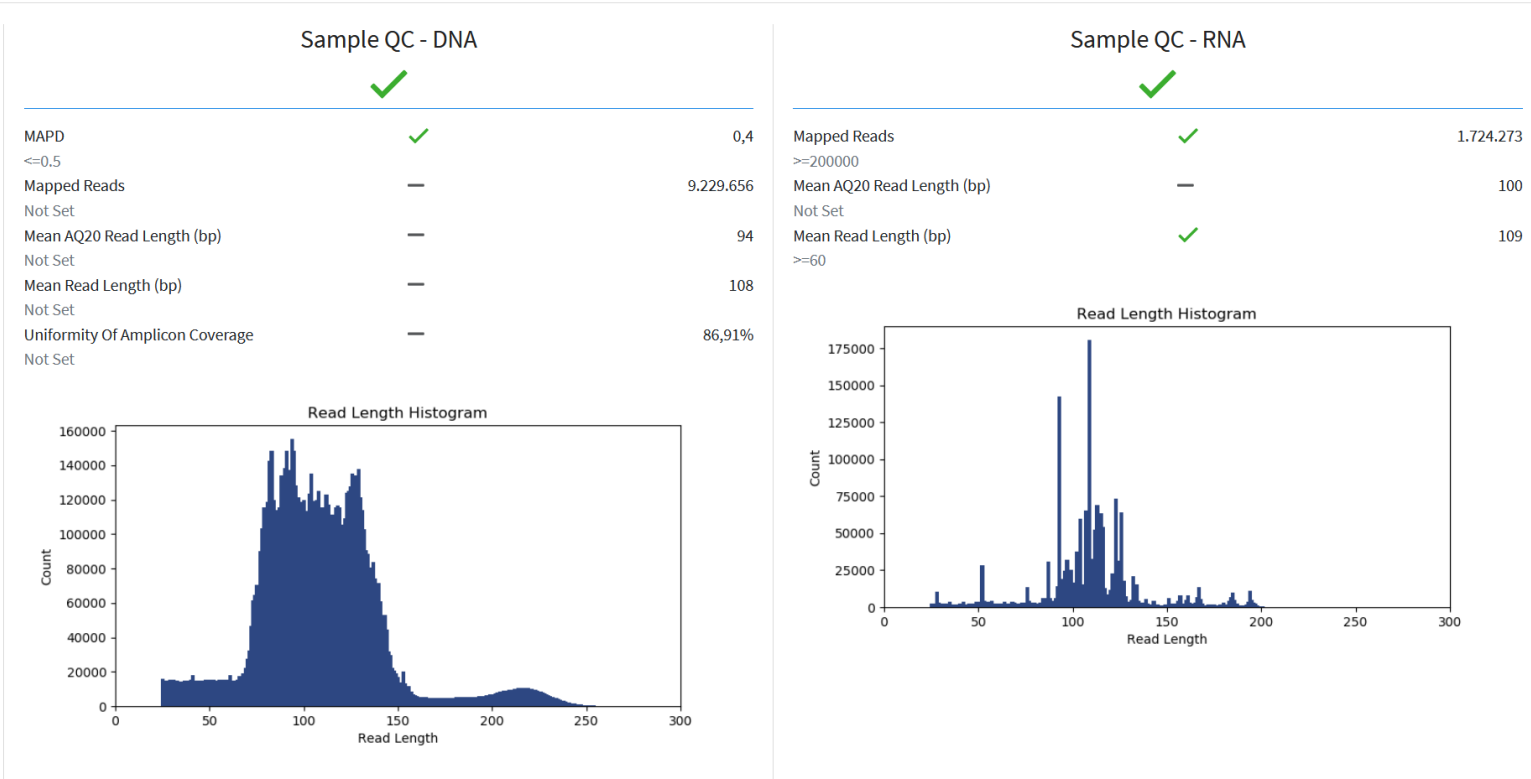
CK7



TTF1



ANALISIS NGS BIOPSIA HEPATICA



VH23P011236A001001D_VH23P011236A001001R QC ✓ [Key Findings](#) **[Variants](#)** [Plugins](#) [Reports](#)

[All](#) **[SNVs/Indels](#)** [Fusions](#) [CNVs](#)

☐ Gene List Only

Locus	Gene	AA Change	Type	Call	Effective Read Depth	Nuc Change	Allele Frequency (%)
chr16:89858441	FANCA	p.Val372AlafsTer42	del	PRESENT (HETEROZYGOUS)	1945	c.1115_1118delTTGG	49.6
chr19:4117608	MAP2K2	p.Gln38Ter	snp	PRESENT (HETEROZYGOUS)	75	c.112C>T	42.7
chr5:176520243	FGFR4	p.Gly388Arg	snp	PRESENT (HETEROZYGOUS)	1962	c.1162G>A	42

ANALISIS NGS BIOPSIA HEPATICA

VH23P011236A001001D_VH23P011236A001001R											
QC											
Key Findings											
Variants											
Plugins											
Reports											
All SNVs/Indels Fusions CNVs ☐ Gene List Only											
User Classification	Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Genes (Exons)	Read Counts	Type			
Classification	EML4-ALK.E13A20.COSF408.1	Yes	chr2:42522656 - chr2:29446394	Fusion	Gain-of-Function	EML4(13) - ALK(20)	103905	Fusion			

VH23P011236A001001D_VH23P011236A001001R											
QC											
Key Findings											
Variants											
Plugins											
Reports											
All SNVs/Indels Fusions CNVs ☐ Gene List Only											
User Classification	Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	Copy Number	Call	CNV Confidence	Oncomine Extended	
Classification	MYC	Yes	chr8:128748724	Amplification	Gain-of-Function	MYC	5.89	PRESENT (GAIN)	5%:4.57, 95%:7.55		

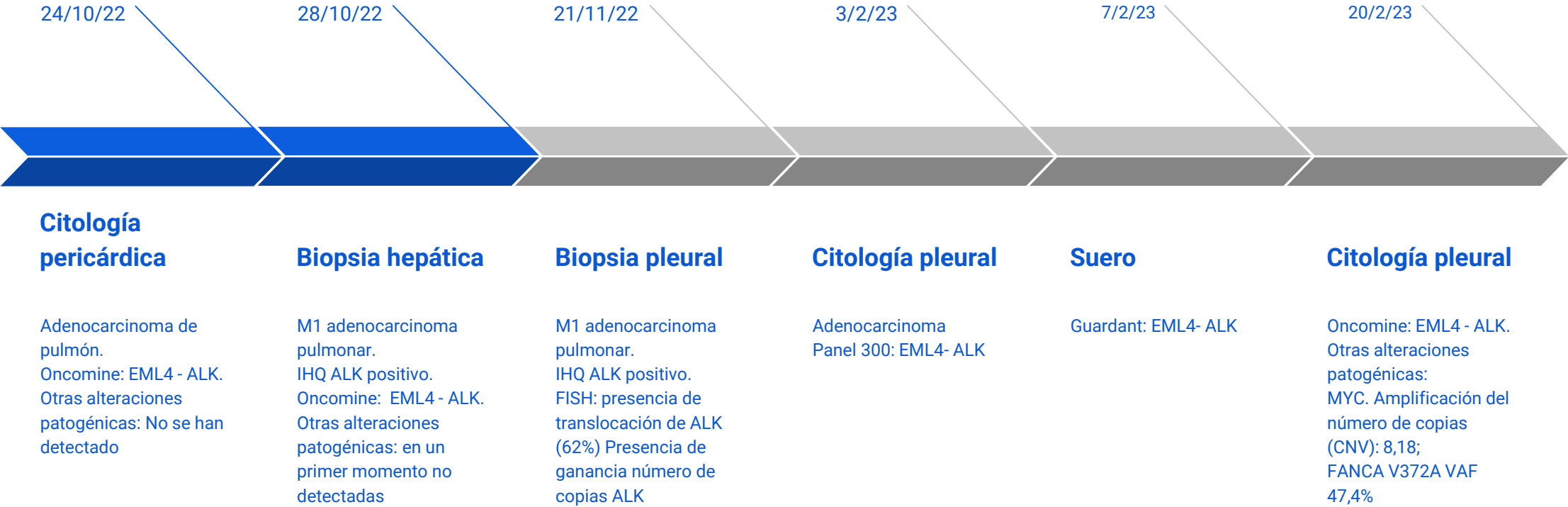
		Pre-Tratamiento (80% Tumor)		Post-Tratamiento (50%T)	
Gen	Variante	VAF	Reads	VAF	Reads
FANCA	p.Val372AlafsTer42	49,60%	1945	47.4%	1948
FGFR4	p.Gly388Arg	36%	1962	36%	1968
ALK	EML4(13)-ALK(20)	Fusion	103905		172376
MYC	CNV	5,89	4.77-7.55	8,18	6.05-10.87



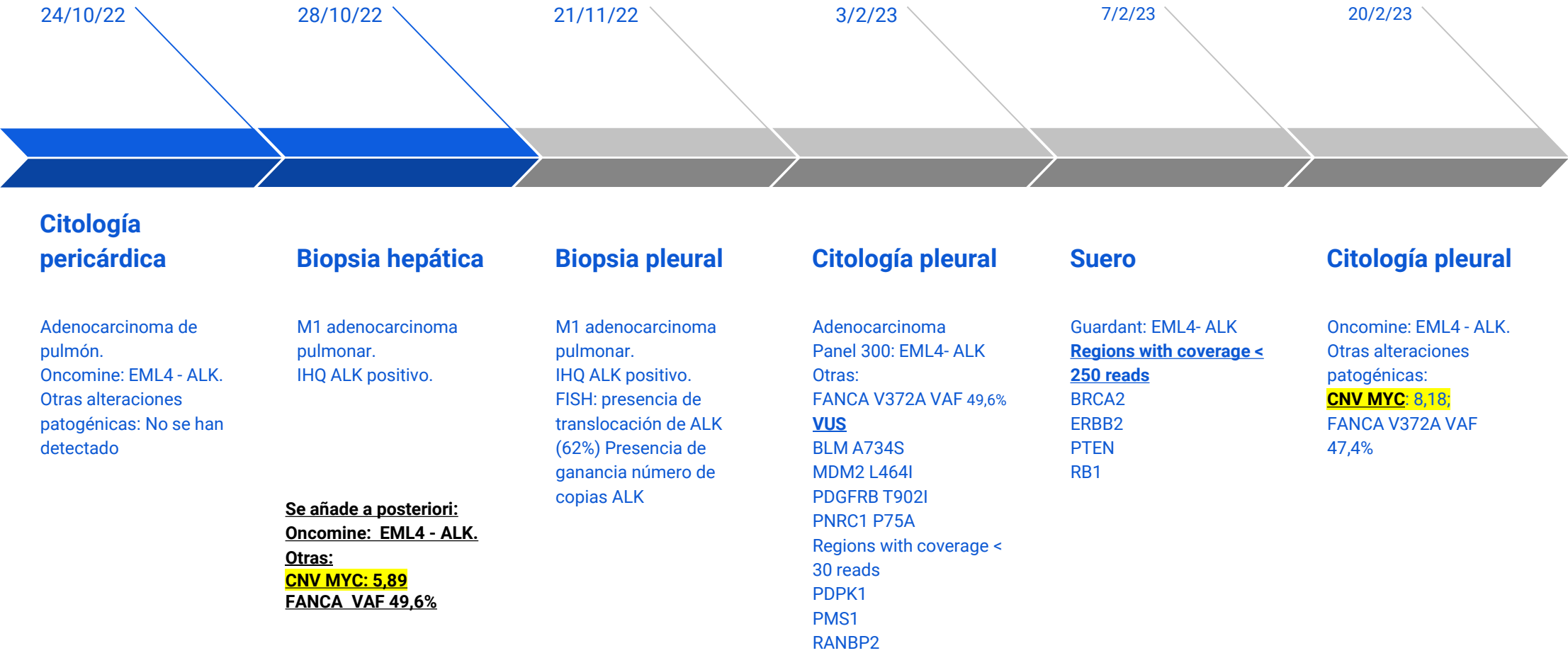
RESUMEN CLINICO



RESUMEN DE MUESTRAS DIAGNOSTICAS



RESUMEN DEL CASO



Comité Molecular Inter-hospitalario

Viernes 12 Enero 2024

Agenda

Caso 01 NHC 17790999 VH23P031858 Althaia – Cáncer de pulmón

Caso 02 NHC 20650398 HUVH – Cáncer de pulmón

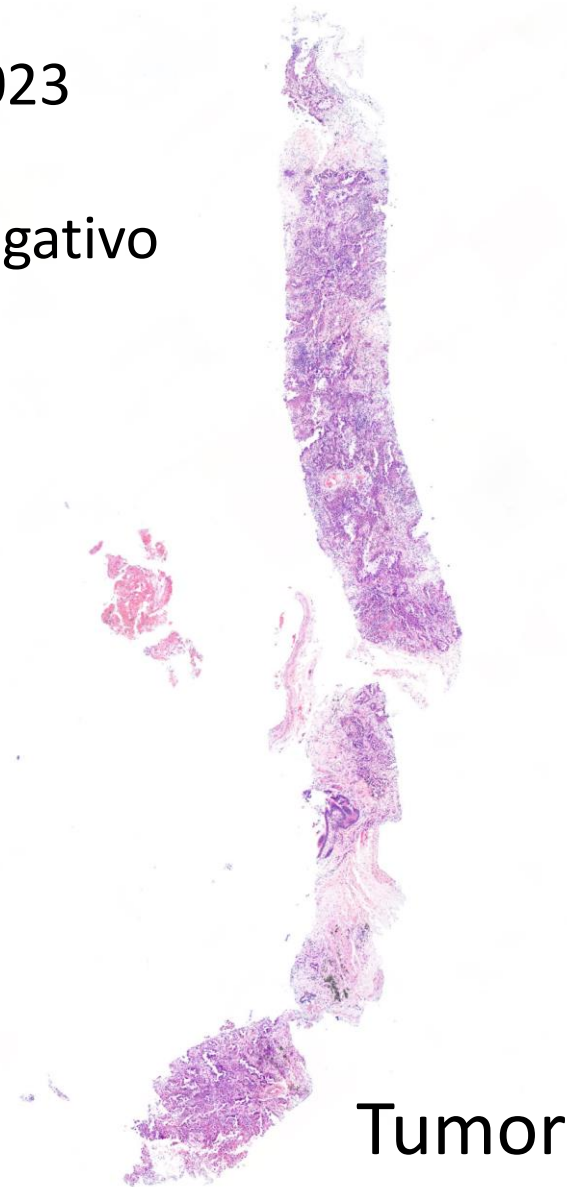
NHC: 20650398

Análisis VH23B036039 – VH23P026420

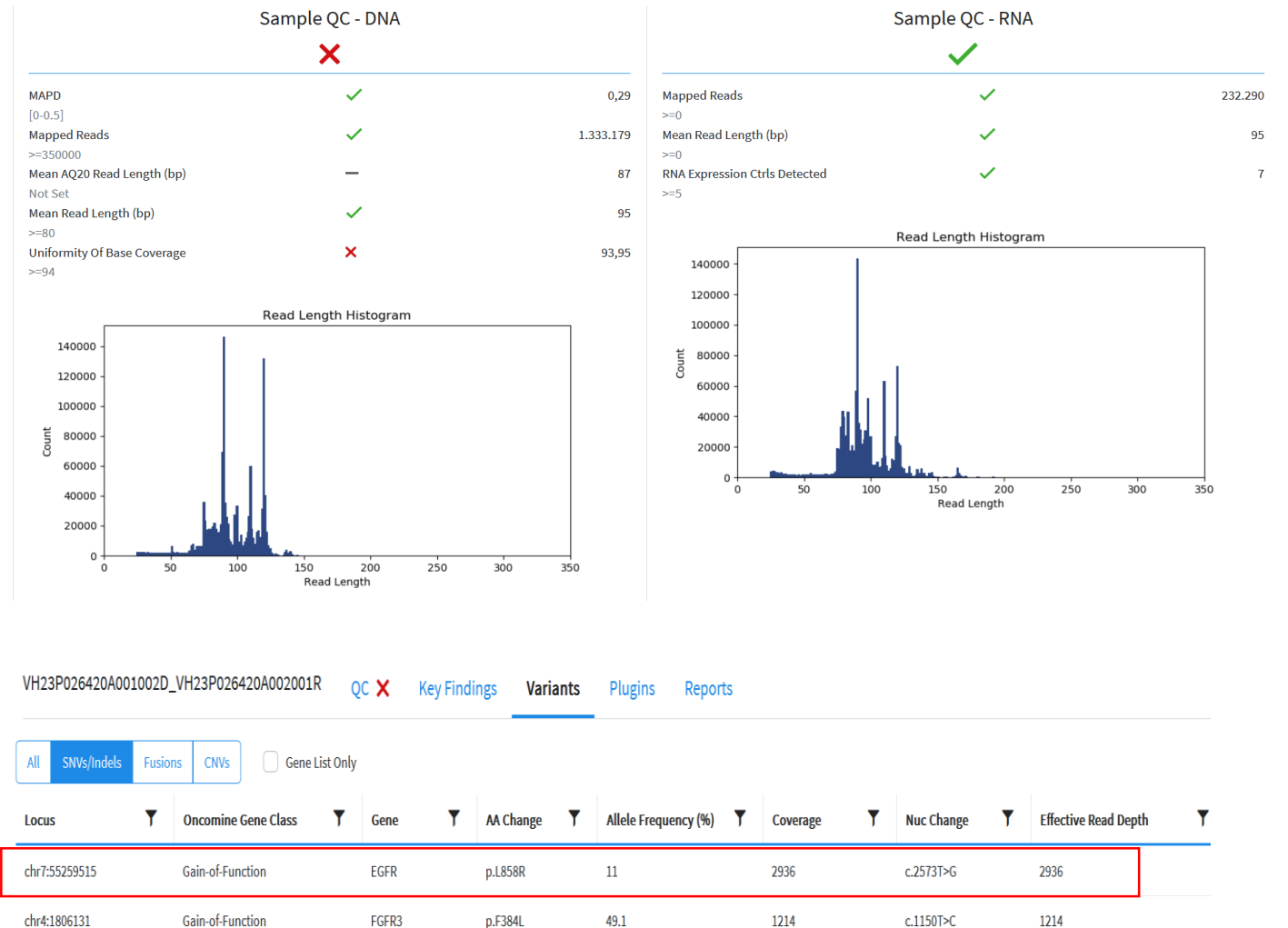
30/10/2023

BAG LID

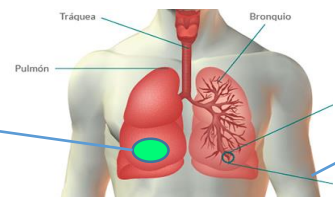
PDL-1 Negativo



Tumor 60%



30/10/2023



Muestra
Sangre

Molecular VH23P026420A001002- NGSpulmó DNA/RN

Surt al informe

Normal B I U μm ² ³ α β λ κ

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Origen de la mostra: Pulmó (localització no indicada), biòpsia per punció (VH23B036039A001)

Tipus de mostra: FFPE

Indicació: Adenocarcinoma compatible amb primari pulmonar

Referència DNA: VH23P026420A001, Mostra Valorable

Referència RNA: VH23P026420A002, Mostra Valorable

Contingut tumoral: 60%

RESULTATS

1. Variants ACCIONABLES

EGFR NM_005228.5:c.2573T>G, p.Leu858Arg VAF 11%

2. ALTRES alteracions patogèniques

No s'han detectat alteracions patogèniques

3. Variants DE SIGNIFICAT INCERT

No s'han detectat alteracions de significat incert

En funció del coneixement actual no es pot determinar la rellevància clínica de les variants de significat incert, per tant, no es poden tenir en compte en el maneig clínic del pacient. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

COMENTARIS i RECOMANACIONS

L'alteració L858R detectada a l'exó 21 del gen EGFR s'associa a sensibilitat a tractament amb inhibidors TKIs d' EGFR.

Els resultats obtinguts es presentaran en el comitè molecular de tumors.

VHIO VALL D'HEBRON Institute of Oncology

MUTATION TEST RESULTS

Patient initials

Extraction date

Lab ID

Results sent to

Sample tissue origin

Primary origin

Date

Time

E.L.

10-10-2023

C23/2485

Dr. Pardo

plasma

lung

10-10-2023

15H53

Results

Variants

Pathogenic/Likely pathogenic variants

Variant	MAF
BRAF:NM_004333.4:exon15:c.1799T>A.p.V600E	0.64%
PIK3CA:NM_006218.2:exon10:c.1633G>A.p.E545K	0.63%

VUS

No VUS were identified.

MAF: mutant allele fraction

VUS: variant of unknown significance

MSI Status

LOW

EGFR p.L858R

BRAF p.V600E
PIK3CA p.E545K



30/10/2023

PCR Digital en VHIO EGFR p.L858R

Idylla EGFR cortes sobrantes: EGFR p.L858R

Nueva extracción ADN. Panel NGS VHIO EGFR p.L858R



30/10/2023

Guardant BRAF V600E + PIK3CA E542K



04/12/2023

ddPCR BRAF V600E

11/12/2023

Muestra de Liquido Ascítico:
VH23C-41252
VH23P031588A001002

Muestra de Liquido Pleural:
VH23C-41029
VH23P031588A002001

ANÀlisi MUTACIONAL DEL GEN EGFR

Informació de la mostra:

Origen de la mostra: Líquid ascític (Bloc: VH-23-C-041252A002)

Tipus de mostra: Citologia

Resultat: NO MUTAT

Metodologia:

PCR a temps real emprant el test cobas® EGFR Mutation Test v2 (Roche) que identifica 42 mutacions en els exons 18, 19, 20 i 21 del gen epidermal growth factor receptor (EGFR). En mostres d'ADN genòmic, aquesta metodologia permet una sensibilitat de detecció de fins un 5% d'al·lels mutats a la mostra analitzada.

ANÀlisi MUTACIONAL DEL GEN EGFR

Informació de la mostra:

Origen de la mostra: Líquid pleural (Bloc: VH-23-C-041029A002)

Tipus de mostra: Citologia

Resultat: NO MUTAT

Metodologia:

PCR a temps real emprant el test cobas® EGFR Mutation Test v2 (Roche) que identifica 42 mutacions en els exons 18, 19, 20 i 21 del gen epidermal growth factor receptor (EGFR). En mostres d'ADN genòmic, aquesta metodologia permet una sensibilitat de detecció de fins un 5% d'al·lels mutats a la mostra analitzada.

Run name

22-DEC-2023 14:33 EGFR Tissue P1

Test status:

VALID

Controls

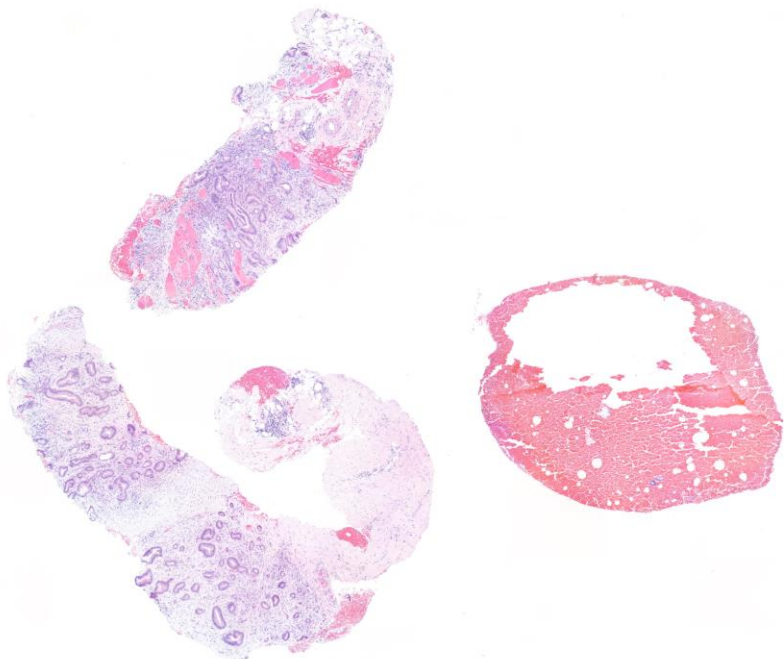
Position	Sample ID	Kit	Control Type	Result	Flags	Accepted by
A01:A02:A03	IA1J103552O00L0	1	Mutant Control	Valid		
B01:B02:B03	IA1J103552O00L0	1	Negative Control	Valid		

Specimens

Position	Sample ID	Kit	Result 1	Result 2	Flags	Accepted by
C01:C02:C03	VH23P031760A001003	1	Mutation Detected	L858R		
D01:D02:D03	VH23P031588A002001	1	No Mutation Detected	N/A		
E01:E02:E03	VH23P032010A001001	1	Mutation Detected	Ex20Ins		
F01:F02:F03	VH23P031883A003001	1	No Mutation Detected	N/A		

19/12/2023

Biopsia Toraco-Abdominal
20% Tumor



CK20+ STAB2+ CDX2+
CK7- TTF1 -



EGFR WT

ANÁLISIS MUTACIONAL DEL GEN EGFR

Información de la muestra:

Origen de la muestra: Pared toracoabdominal (Bloc: VH-23-B-043277A001)

Tipus de mostra: FFPE

Indicació: Adenocarcinoma compatible amb metàstasi de primari intestinal

Contingut tumoral: 20%

Resultat: NO MUTADO

Metodologia:

PCR a temps real emprant el test cobas® EGFR Mutation Test v2 (Roche) que identifica 42 mutacions en els exons 18, 19, 20 i 21 del gen epidermal growth factor receptor (EGFR). En mostres d'ADN genòmic, aquesta metodologia permet una sensibilitat de detecció de fins un 5% d'al·lels mutats a la mostra analitzada.

VH23B043277A001

VH23P031588A003001

22/12/2023

INFORME DE RESULTADO DEL TEST

Prueba realizada: ANATOMIA PATOLOGICA, Passeig Vall D'Hebron 119-129 BARCELONA, Spain, Javier Herandez Losa, 0034 934894582



muestra ID	23p 31588a3		
Tipo de muestra	Tejido FFPE		
ID de Cartucho	62621113		
Tipo Test	NRAS-BRAF_IVD/1.1	Versión de TTP	1.3
ID del lote	00006262	Fecha expir.	27 jun. 2024

N.º de serie del Instrumento	SER1525
Versión software Instrumento	27.0
N.º serie de la Consola	C00001077
Versión de software de la Consola	4.5.0.764
Solicitud de Test completada	22 dic. 2023 (17:36)
Test iniciado	22 dic. 2023 (17:36)
Prueba finalizada	22 dic. 2023 (19:26)
Estado Test	Resultado publicado: Automático, 22 dic. 2023 (19:26)
Operador	Rosa Somoza

Resultado del Test (1) Dispositivo médico de diagnóstico in vitro. Para uso en procedimientos de diagnóstico.

Idylla™ NRAS-BRAF Mutation Test

GENOTIPO NRAS	NO SE DETECTÓ MUTACIÓN EN LOS CODONES 12, 13, 59, 61, 117, 146 DE NRAS
GENOTIPO BRAF	SE DETECTÓ MUTACIÓN EN EL CODÓN 600 DE BRAF
Mutación	V600E/D
Proteína	p.Val600Glu / p.Val600Asp
Cambio nucleotídico	c.1799T>A ; c.1799_1800delinsAA / c.1799_1800delinsAC
Cq DEL CONTROL DE NRAS	37.3

(1) La información relativa al propósito planteado, al rendimiento clínico y a la interpretación clínica del ensayo aplicado está disponible en las instrucciones de uso.

ANÀLISI MUTACIONAL DEL GEN BRAF

Informació de la mostra:

Origen de la mostra: Pared toracoabdominal (Bloc: VH-23-B-043277A001)
Tipus de mostra: FFPE
Indicació: Adenocarcinoma compatible amb metàstasi de primari intestinal
Contingut tumoral: 20%

Resultat:

BRAF: PRESÈNCIA de la mutació p.V600E/D
NRAS: NO MUTAT

Metodologia:

PCR a temps real emprant el test "Idylla NRAS-BRAF Mutation Test" (Biocartis) que identifica 5 mutacions al codó 600 de l'exó 15 del proto-oncogen B-Raf (BRAF), i 18 mutacions als exons 2, 3 i 4 del gen neuroblastoma RAS viral oncogene homolog (NRAS). En mostres d'ADN genòmic, aquesta metodologia permet una sensibilitat de detecció de fins un 5% d'al·lels mutats a la mostra analitzada.

BRAF p.V600E/D

Conclusión:

Paciente con dos tumores sincrónicos

- Tumor de pulmón EGFR Mutado p.L858R tratado con Osimertinib.
- Tumor digestivo BRAF Mutado p.V600E

Comité Molecular Inter- hospitalario

Viernes 08 marzo 2024

Agenda **Caso 02 NHC xxxxxx VH24P00x4x5 HUVH** – Cáncer de pulmón

NHC: 1xxxxx706

Varón 67 años

Información remitida:

- Biopsia de nódulo de pulmón derecho
- Diagnóstico: Adenocarcinoma
- Paciente con mutación en EGFR en progresión
- Se solicita estudio molecular para detección de mutaciones de resistencia ...

INFORME ANATOMIA PATOLÒGICA

MACROSCÒPIA

A) Procedencia: Pulmón

Se recibe/n 5 cilindros, remitido/s en formol, de color blanquecino, de superficie lisa y consistencia friable, que mide/n 0,3-0,6 cm. Se procede a la inclusión total del tejido recibido en 1 bloque/s de parafina.

DIAGNÒSTIC

A) NÓDULO EN PULMÓN DERECHO, BIOPSIA BAG:

- ADENOCARCINOMA.

Estudi molecular sol·licitat

INFORME NGS OCA

DNA:

EGFR:NM_005228.5:exon19:c.2240_2264delinsCGAAAGA:p.(Leu747_Ala755delinsSerLysAsp) VAF 66.3% **[EGFRExon19Deletion]**

EGFR:NM_005228.5:exon20:c.2369C>T:p.(Thr790Met) VAF 17.9% **[T790M]**

EGFR:NM_005228.5:exon20:c.2390_2391delinsCT:p.(Cys797Ser) VAF 32.4% **[C797S]**

EGFR:NM_005228.5:exon19:c.2264C>A:p.(Ala755Asp) VAF 60.9% **[A755D]** - VUS

RNA: SIN ALTERACIONES DETECTABLES

NHC: 1xxxx06

CONTEXTO MOLECULAR DE ESTE CASO...

[Año 2009]

Secuenciación Sanger

- Codi estudi: 09M0002361
- Id. mostra: 01

21/ 09/09 VH 09M0002361

Informe Patologia Molecular

MOSTRA: Citología
LOCALITZACIÓ: Pulmón

MATERIAL D'ESTUDI: DNA
TÈCNiques SOL·LICITADES: Estudio de mutaciones de EGFR

TÈCNiques UTILITZADES:

Se ha realizado el protocolo de extracción de ADN a partir de tejido mediante el protocolo de "DNA Tissue Kit" de QIAGEN. Se ha comprobado

la calidad del ADN mediante la amplificación de Actina y posteriormente se han amplificado y secuenciado los exones

RESULTAT:

Presencia de una mutacion en el exon 19 que da lugar a una deleccion de 7 aminoacidos en las posiciones 747-753.

[Año 2016]

PCR RTG

Data informe: 25.04.2016 12:11h
Data darrera versió: 27.07.2016 08:27h

BIOLOGIA MOLECULAR

Estudio Molecular: Mutaciones de EGFR (Dr. Hernández, Dra. Sansano)
Num Dna: 16/3100
Se ha procedido a la valoración del bloque ,la muestra a analizar presenta un 60% de componente tumoral y 0 % de tejido necrótico. Posteriormente se ha realizado el protocolo de extracción de ADN a partir de tejido mediante el protocolo de "DNA Tissue Kit" de QIAGEN. Se ha comprobado la calidad del ADN mediante la amplificación de Actina y posteriormente se ha determinado la presencia de mutaciones del gen EGFR, siguiendo las instrucciones del " EGFR RGQ PCR Kit " mediante PCR a tiempo real. Finalmente se han amplificado y secuenciado los exones 19, 20 y 21 del gen que codifica para el receptor EGFR, bajo el protocolo descrito en el "Clin Cancer Res. 2005;11(16) August 15, 2005"

RESULTADO: Presencia de una mutación doble en el gen EGFR, mostrando una delección en el exón 19 y un cambio puntual en la posición 790 del exón 20.

Resultado Molecular → **EGFR Exon19del** + **EGFR T790M**

Tratamiento indicado: Olmutinib



[Año 2024]

El paciente progresa → Se realiza panel OCA

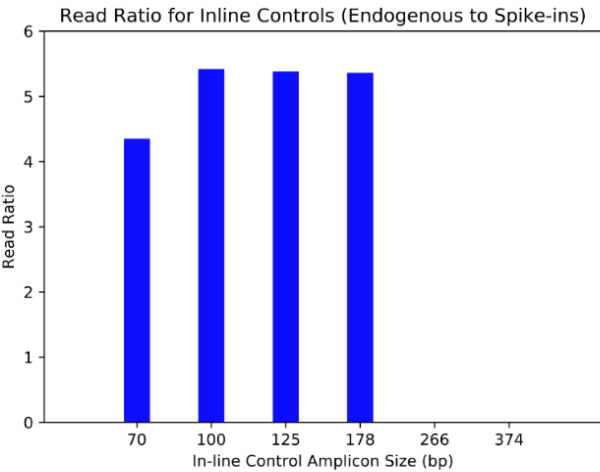
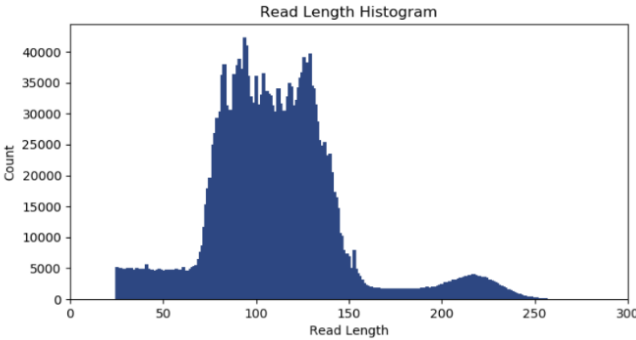
Resultado Molecular → **EGFR Exon19del**

Tratamiento indicado: Erlotinib

Sample QC - DNA



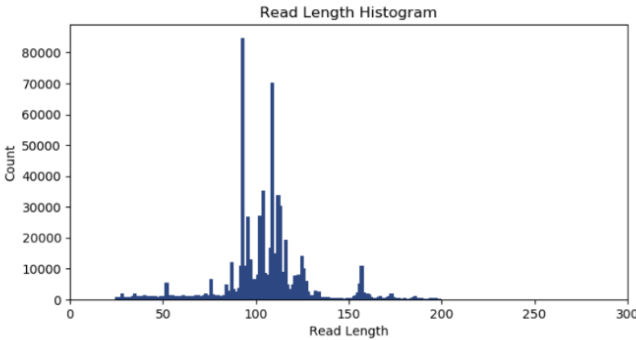
MAPD	<=0.5	0,28
Mapped Reads	>=3000000	2.675.283
Mean AQ20 Read Length (bp)	>=100	93
Mean Read Length (bp)	>=100	111
Uniformity Of Amplicon Coverage	>=80	93,45%



Sample QC - RNA



Mapped Fusion Reads	>=200000	677.344
Mean AQ20 Read Length (bp)	>=75	92
Mean Read Length (bp)	>=60	105



TUMOR 95%

Panel OncoPrint™ Comprehensive Assay v3 GX (Thermo Fisher Scientific) → Panel amplio: 161 genes

VH24P006475A001001D_VH24P006475A002001R QC ✗ Key Findings **Variants** Plugins Reports

All SNVs/Indels Fusions CNVs ☐ Apply Non-Small Cell Lung Cancer Gene List

OncoPrint Extended 5.16_PubL_0.01 Filter Chain Applied
10 of 3,068 SNVs/Indels

User Classification	Variant ID	Key Variant	Locus	OncoPrint Variant Class	OncoPrint Gene Class	Gene	AA Change	Ref	Alt	Type	Call	Effective Read Depth	Nuc Change	Allele Frequency (%)
Classification ▾	.	No	chrX:76940005			ATRX	p.Leu248Pro ✗	A	G	snp	PRESENT (HETEROZYGOUS)	29	c.743T>C	6.9
Classification ▾	.	No	chr13:32907129			BRCA2	p.Ile505Thr ✗	T	C	snp	PRESENT (HETEROZYGOUS)	1997	c.1514T>C	48.2
Classification ▾	.	Yes	chr7:55242470	EGFRExon19Deletion	Gain-of-Function	EGFR	p.Leu747_Ala755delinsSerLysAsp	TAAGAGAAGCAACATCTCCGAAAGC	CGAAAGA	complex	PRESENT (HETEROZYGOUS)	1930	c.2240_2264delTAAGAGAAGCAACATCTCCGAAAGCinsCGAAAGA	66.3
Classification ▾	.	No	chr7:55242494			EGFR	p.Ala755Asp	C	A	snp	PRESENT (HETEROZYGOUS)	1991	c.2264C>A	60.9
Classification ▾	.	Yes	chr7:55249092	Hotspot	Gain-of-Function	EGFR	p.Cys797Ser	GC	CT	mnp	PRESENT (HETEROZYGOUS)	1567	c.2390_2391delGCTinsCT	32.4
Classification ▾	COSM6240	Yes	chr7:55249071	Hotspot	Gain-of-Function	EGFR	p.Thr790Met	C	T	snp	PRESENT (HETEROZYGOUS)	1585	c.2369C>T	17.9
Classification ▾	COSM6493937	Yes	chr7:55249091	Hotspot	Gain-of-Function	EGFR	p.Cys797Ser	T	A	snp	PRESENT (HETEROZYGOUS)	1584	c.2389T>A	8.6
Classification ▾	.	No	chr9:139411786			NOTCH1	p.Pro498Arg ✗	G	C	snp	PRESENT (HETEROZYGOUS)	148	c.1493C>G	9.5
Classification ▾	.	Yes	chr1:120612002	Truncating	Loss-of-Function	NOTCH2NLC**	p.? ✗	CGG	C	del	PRESENT (HETEROZYGOUS)	77	c.-39436CCG>G	26
Classification ▾	.	No	chr7:6026650			PMS2	p.Glu582Asp ✗	T	A	snp	PRESENT (HETEROZYGOUS)	1993	c.1746A>T	22.4

NHC: 1xxxx706

ANÁLISIS NGS: ESTUDIO DE VARIANTES

VH24P006475A001001D_VH24P006475A002001R

QC ✖

Key Findings

Variants

Plugins

Reports

All SNVs/Indels Fusions CNVs

☐ Apply Non-Small Cell Lung Cancer Gene List

OncoPrint Extended 5.16_PubL0.01 Filter Chain Applied
10 of 3,069 SNVs/Indels

Edit Filters

User Classification	Variant ID	Key Variant	Locus	OncoPrint Variant Class	OncoPrint Gene Class	Gene	AA Change	Ref	Alt	Type	Call	Effective Read Depth	Nuc Change	Allele Frequency (%)
Classification	.	Yes	chr7:55242470	EGFRExon19Deletion	Gain-of-Function	EGFR	p.Leu747_Ala755delinsSerLysAsp	TAAGAGAAGCAACATCTCCGAAAGC	CGAAAGA	complex	PRESENT (HETEROZYGOUS)	1930	c.2240_2264del=TAAGAGAAGCAACATCTCCGAAAGCinsCGAAAGA	66.3
Classification	.	No	chr7:55242494			EGFR	p.Ala755Asp	C	A	snp	PRESENT (HETEROZYGOUS)	1991	c.2264C>A	60.9
Classification	.	Yes	chr7:55249092	Hotspot	Gain-of-Function	EGFR	p.Cys797Ser	GC	CT	mnp	PRESENT (HETEROZYGOUS)	1567	c.2390_2391del=GCinsCT	32.4
Classification	COSM6240	Yes	chr7:55249071	Hotspot	Gain-of-Function	EGFR	p.Thr790Met	C	T	snp	PRESENT (HETEROZYGOUS)	1585	c.2369C>T	17.9
Classification	COSM6493937	Yes	chr7:55249091	Hotspot	Gain-of-Function	EGFR	p.Cys797Ser	T	A	snp	PRESENT (HETEROZYGOUS)	1584	c.2389T>A	8.6



EGFR:NM_005228.5:exon 19:c.2240_2264delinsCGAAAGA:p.(Leu747_Ala755delinsSerLysAsp) VAF 66.3%

[EGFRExon19Deletion]



EGFR:NM_005228.5:exon 20:c.2369C>T:p.(Thr790Met) VAF 17.9%

[T790M]



EGFR:NM_005228.5:exon 20:c.2390_2391delinsCT:p.(Cys797Ser) VAF 32.4%

[C797S]



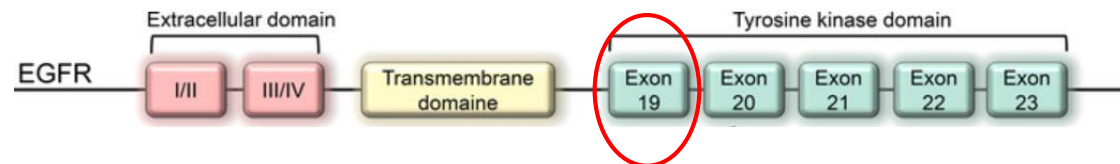
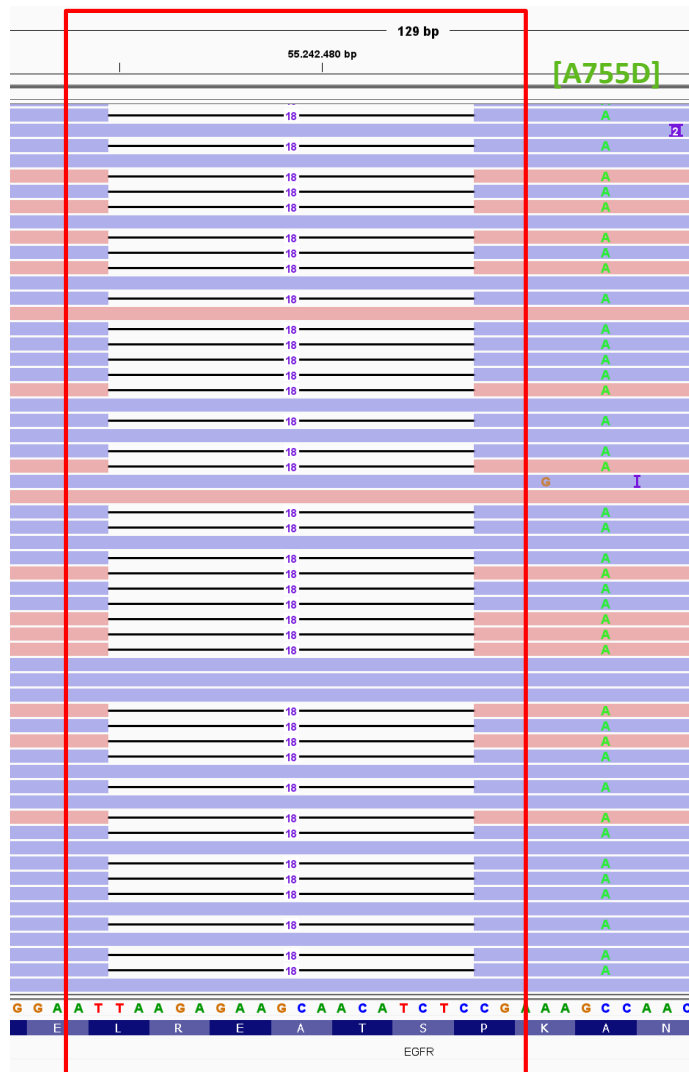
EGFR:NM_005228.5:exon 19:c.2264C>A:p.(Ala755Asp) VAF 60.9%

[A755D]



NHC: 12615706

EGFR:NM_005228.5:exon 19:c.2240_2264delinsCGAAAGA:p.(Leu747_Ala755delinsSerLysAsp) VAF 66.3% [EGFExon19Deletion]



OncoKB

EGFR / L747_A755delinsSKD

EGFR L747_A755delinsSKD

Oncogenic • Likely Gain-of-function • Level 1 • FDA Level 2

EGFR, a receptor tyrosine kinase, is altered by amplification and/or mutation in lung and brain cancers among others.

The EGFR L747_A755delinsSKD alteration has been identified as a statistically significant hotspot and is likely to be oncogenic.

Therapeutic

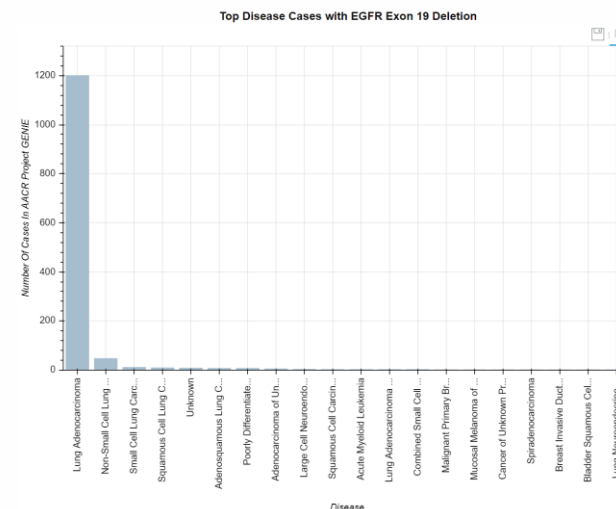
FDA-Recognized Content

A list of the cancer type-specific EGFR alterations that may predict response to a targeted drug and the corresponding OncoKB™ level of evidence assigning their level of [clinical actionability](#).

If you notice any mistakes or omissions, please reach out to us.

Search

Level	Alterations	Level-associated cancer types	Drugs	Citations	Description
1	Exon 19 in-frame deletions (729_761del), L858R	Non-Small Cell Lung Cancer	Afatinib	3	
2	Exon 19 in-frame deletions (729_761del), L858R	Non-Small Cell Lung Cancer	Dacomitinib	2	
3	Exon 19 in-frame deletions (729_761del), L858R	Non-Small Cell Lung Cancer	Erlotinib, Erlotinib + Ramucirumab	2	
4	Exon 19 in-frame deletions (729_761del), L858R	Non-Small Cell Lung Cancer	Gefitinib	6	
5	Exon 19 in-frame deletions (729_761del), L858R	Non-Small Cell Lung Cancer	Osimertinib	4	
6	G719, L861Q, Exon 19 in-frame deletions (729_761del), Exon 19 in-frame insertions (729_761ins), L858R, S768I	Non-Small Cell Lung Cancer	Patritumab Deruxtecan	3	



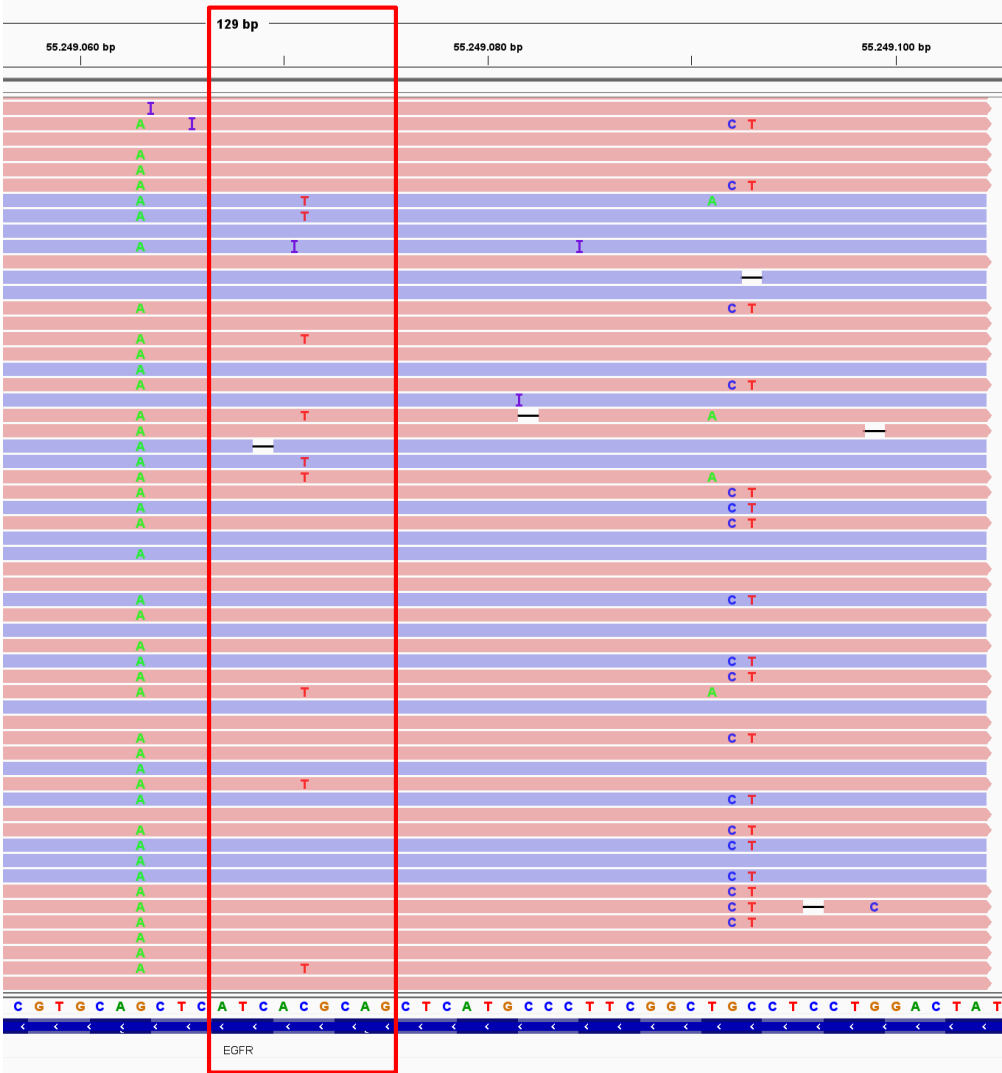
- Mutación oncogénica dominio TK de probable ganancia de función.
- Mayor frecuencia en Adenocarcinoma de pulmón.
- In-frame → Eliminación de la pauta de lectura del exón 19 → Activación constitutiva de la actividad TK.
- Sensibilidad a Inhibidores TKIs de EGFR.

EGFExon19del:

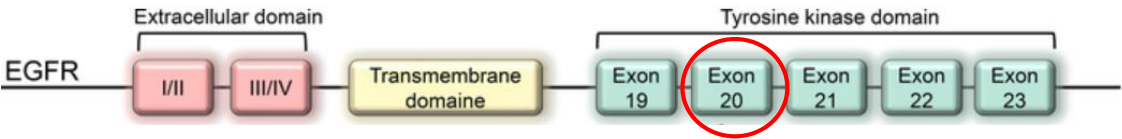
c.2240_2264delTAAGAGAAGCAACATCTCCGAAAGCinsCGAAAGA

NHC: 12615706

EGFR:NM_005228.5:exon 20:c.2369C>T.p.(Thr790Met) VAF 17.9% [T790M]



EGFR T790M



EGFR / T790M

EGFR T790M

OncKB

Oncogenic • Gain-of-function • Level 1 • Level R1 • FDA Level 2

EGFR, a receptor tyrosine kinase, is altered by amplification and/or mutation in lung and brain cancers among others.

The EGFR T790M mutation is known to be oncogenic.

Therapeutic

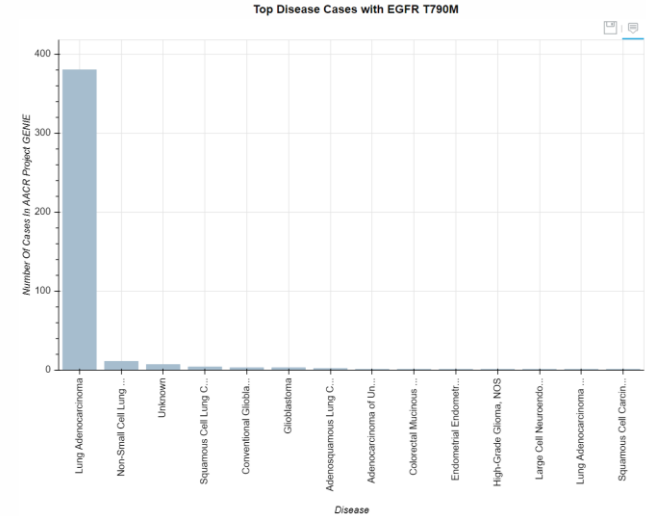
FDA-Recognized Content

A list of the cancer type-specific EGFR alterations that may predict response to a targeted drug and the corresponding OncoKB™ level of evidence assigning their level of [clinical actionability](#).

If you notice any mistakes or omissions, please reach out to us. [✉](#)

Search ...

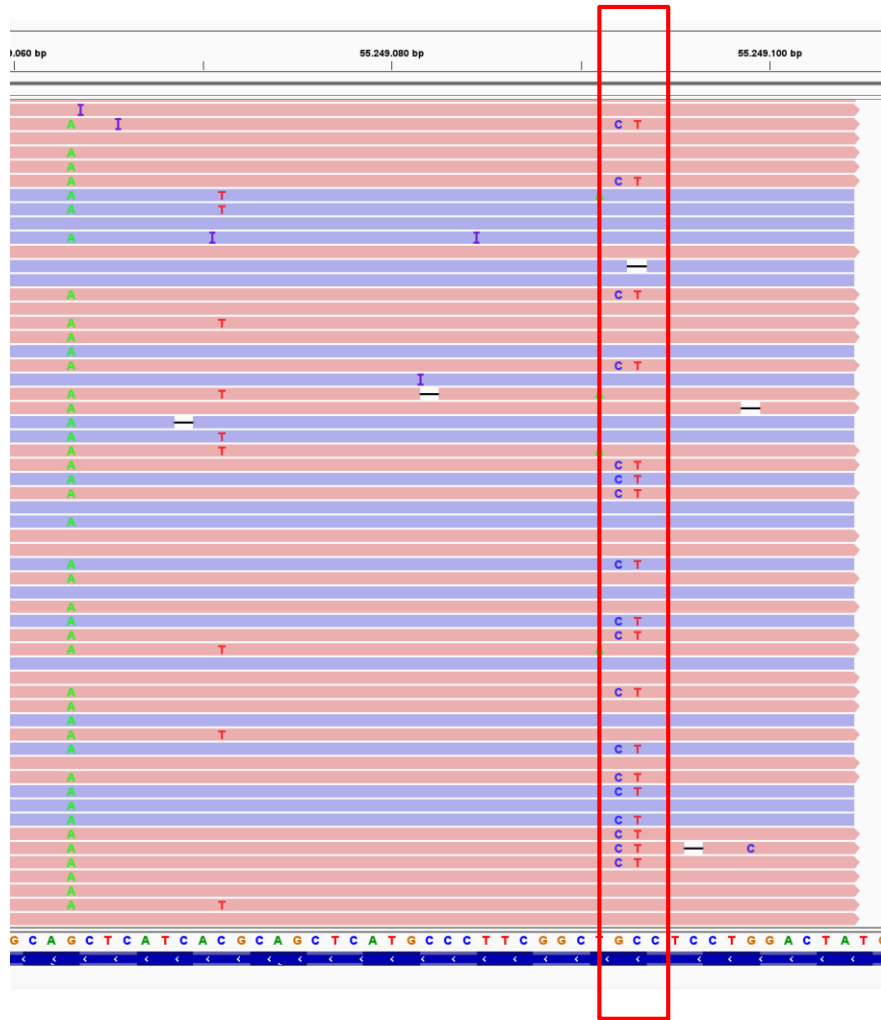
Level ▾	Alterations	Level-associated cancer types ⓘ	Drugs	Citations	Description
1	T790M	Non-Small Cell Lung Cancer	Osimertinib	4	📄
R1	T790M	Non-Small Cell Lung Cancer	Erlotinib, Gefitinib, Afatinib	4	📄



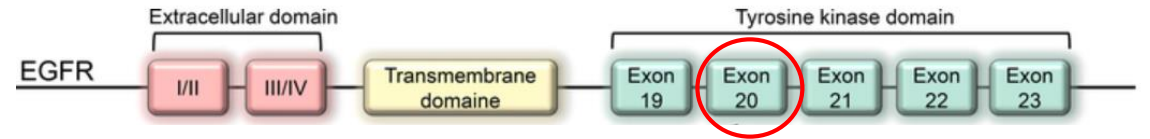
- Mutación oncogénica de ganancia de función.
- Mayor frecuencia en Adenocarcinoma de pulmón.
- Mutación secundaria adquirida en el dominio TK, de resistencia a inhibidores de la tirosina quinasa (TKI) de EGFR de primera y segunda generación.
- Sensibilidad a TKI EGFR de tercera generación.

NHC: 1xxx06

EGFR:NM_005228.5:exon 20:c.2390_2391delinsCT:p.(Cys797Ser) VAF 32.4% [C797S]



EGFR C797S
c.2390_2391delGCinsCT



EGFR / C797S

EGFR C797S

Resistance · Inconclusive · Level R2 · FDA Level 3

EGFR, a receptor tyrosine kinase, is altered by amplification and/or mutation in lung and brain cancers among others.

The EGFR C797S has been found in the context of resistance to a targeted therapy(s).

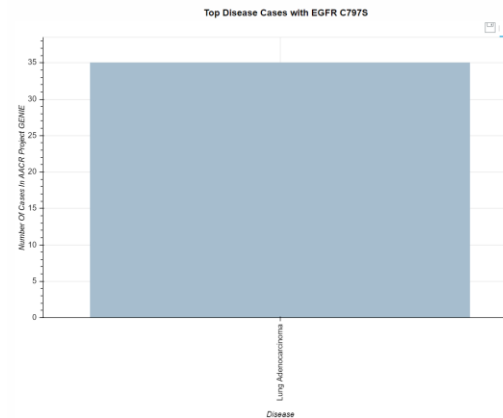
Therapeutic FDA-Recognized Content

A list of the cancer type-specific EGFR alterations that may predict response to a targeted drug and the corresponding OncoKB™ level of evidence assigning their level of [clinical actionability](#).

If you notice any mistakes or omissions, please reach out to us.

Search ...

Level	Alterations	Level-associated cancer types	Drugs	Citations	Description
R2	C797S	Non-Small Cell Lung Cancer	Osimertinib	2	



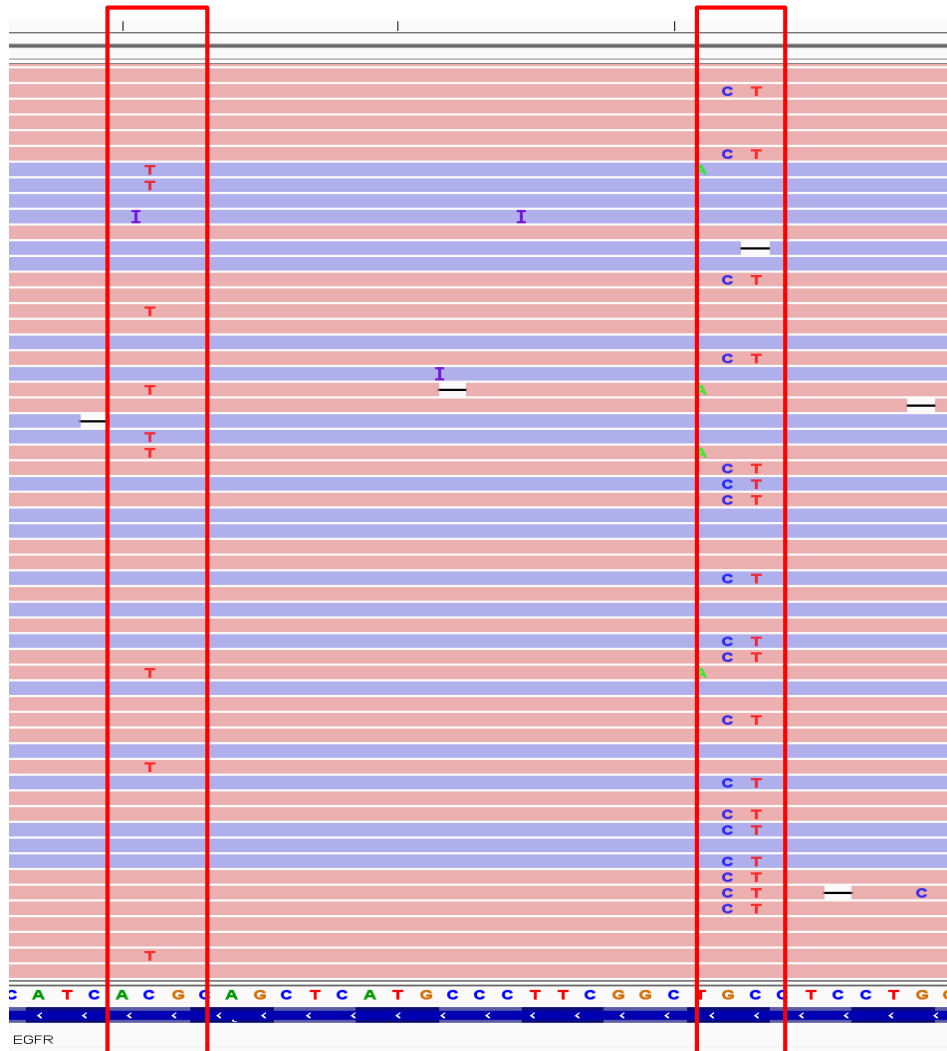
- Mutación en dominio TK de resistencia.
- Entidad tumoral descrita: Adenocarcinoma de pulmón.
- Mutación adquirida de resistencia a inhibidores TKI EGFR de tercera generación.



Importancia CIS/TRANS respecto a T790M

NHC: 1xxxx06

EGFR:NM_005228.5:exon 20:c.2390_2391delinsCT:p.(Cys797Ser) VAF 32.4% [C797S]



EGFR T790M

EGFR C797S

en nuestro caso

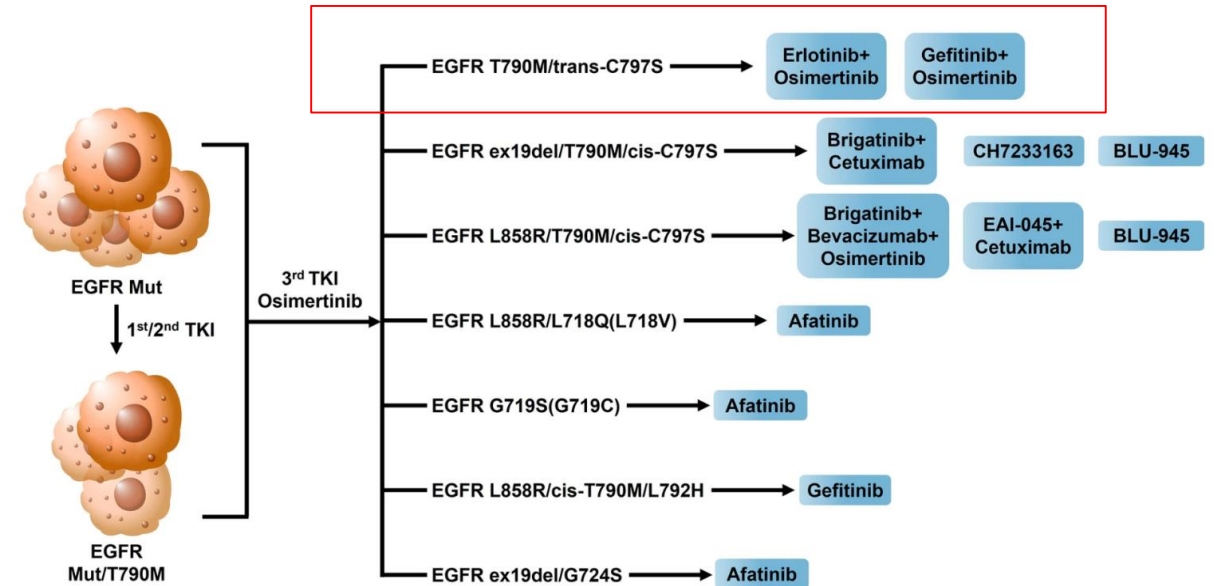
EGFR T790M/trans-C797S, sin embargo...

Review | [Open access](#) | Published: 11 April 2023

Toward the next generation EGFR inhibitors: an overview of osimertinib resistance mediated by EGFR mutations in non-small cell lung cancer

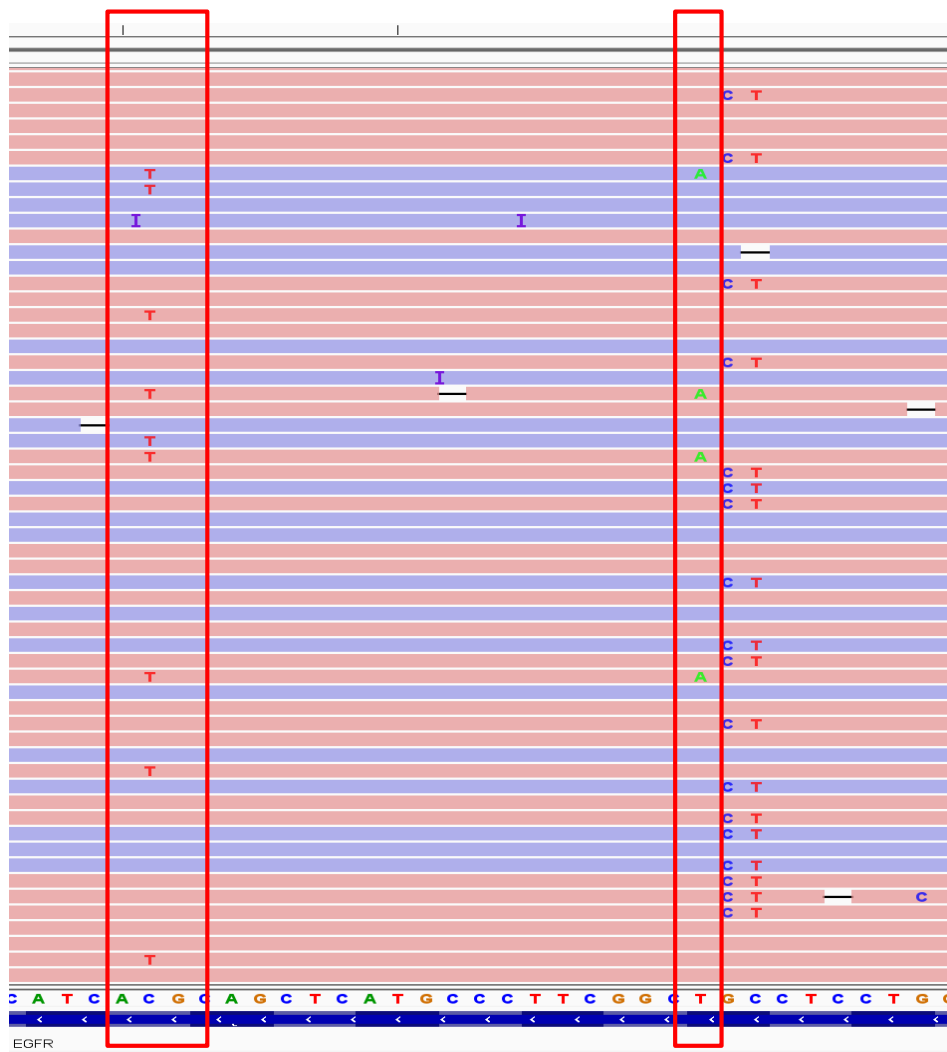
Yufeng Li, Tianyu Mao, Jing Wang, Hongrui Zheng, Ziyi Hu, Pingping Cao, Suisui Yang, Lingyun Zhu, Shunyao Guo, Xinfei Zhao, Yue Tian, Hua Shen & Fan Lin

Cell Communication and Signaling 21, Article number: 71 (2023) | [Cite this article](#)



NHC: 1xxx06

EGFR:NM_005228.5:exon 20 | c.2389T>A | p.Cys797Se [C797S]



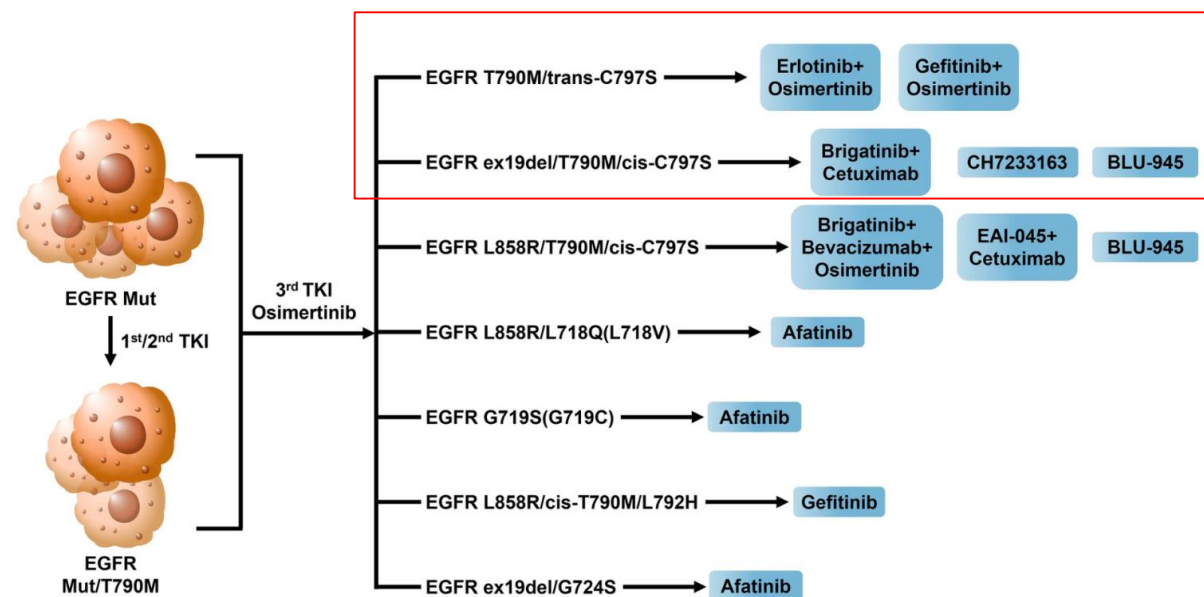
EGFR T790M

EGFR C797S
c.2389T>A

está apareciendo

EGFR T790M/cis-C797S

¿Concomitancia EGFR T790M/cis-trans-C797S?



NHC: 1xxxx06

EGFR:NM_005228.5:exon 20:c.2390_2391delinsCT:p.(Cys797Ser) VAF 32.4% [C797S]

EGFR:NM_005228.5:exon 20 | c.2389T>A | p.Cys797Se [C797S]

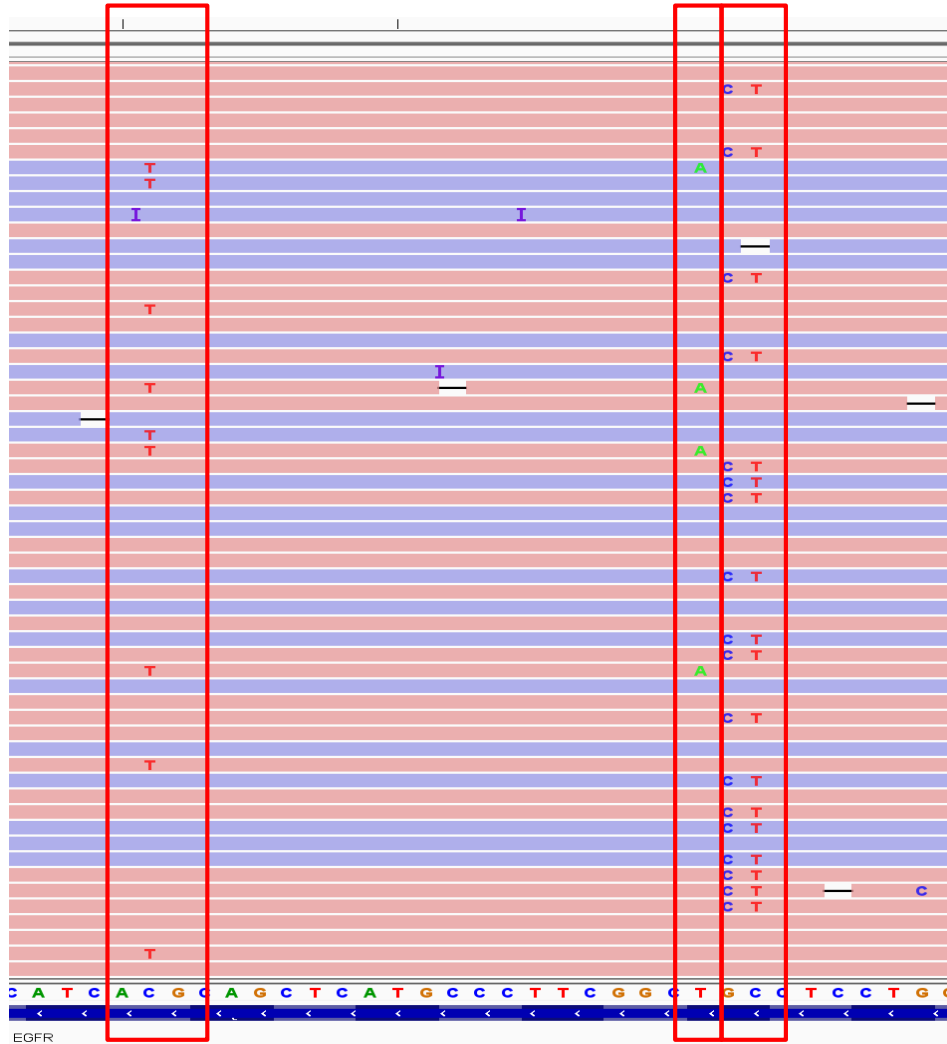
¡EGFR T790M/cis-trans-C797S!

The concomitant EGFR T790M/C797S in trans and cis in three osimertinib-resistant lung adenocarcinoma patients.

Authors: Chunling Liu, Jidong Li, Hailong Liu, Rong Du, Nan Fang, Jingbo Zhang, Yu Tang, Jianwei Wang, and Qixi Wu | [AUTHORS INFO & AFFILIATIONS](#)

Publication: Journal of Clinical Oncology • Volume 37, Number 15_suppl • https://doi.org/10.1200/JCO.2019.37.15_suppl.e20105

We firstly reported three cases with concomitant EGFR T790M/C797S in trans and EGFR T790M/C797S in cis mutation in the word. Physicians tried either the EGFR TKI inhibitor combination therapy or VEGFR inhibitor, but neither of them had an effect on these patients. All three patients had very poor prognosis and died within two months of the gene test, indicating that the concomitant of the cis and trans mutation could be a very important prognostic prediction factor.



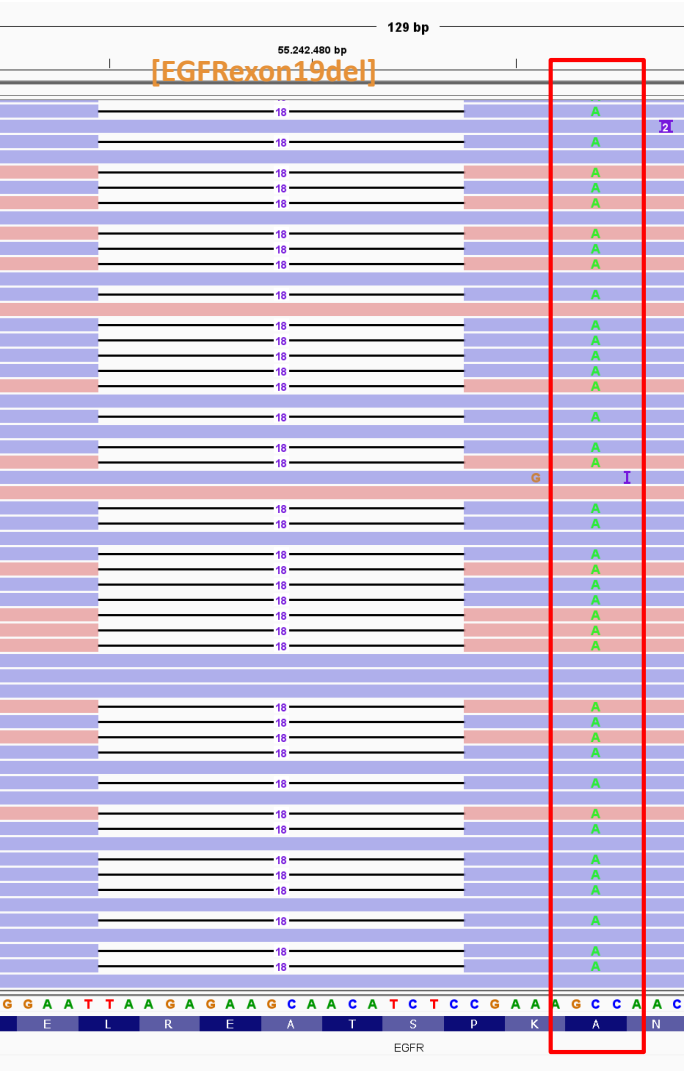
EGFR T790M

EGFR C797S
c.2389T>A

EGFR C797S
c.2390_2391delGinsCT

NHC: 1xxxx06

EGFR:NM_005228.5:exon 19:c.2264C>A:p.(Ala755Asp) VAF 60.9% [A755D]



EGFR A755D

OncKB

EGFR / A755D

EGFR A755D

Inconclusive ⓘ · Inconclusive ⓘ

EGFR, a receptor tyrosine kinase, is altered by amplification and/or mutation in lung and brain cancers among others.

There is conflicting and/or weak data describing the biological significance of the EGFR A755D mutation.



- Alteración de significado incierto [VUS].
- Descrita en cáncer de pulmón de célula pequeña.
- Funcionalmente no caracterizada.
- Bibliografía....

> BMC Cancer. 2021 Apr 30;21(1):482. doi: 10.1186/s12885-021-08228-2.

Efficacy and acquired resistance for EGFR-TKI plus thoracic SBRT in patients with advanced EGFR-mutant non-small-cell lung cancer: a propensity-matched retrospective study

Xia Wang ^{# 1 2}, Zhimin Zeng ^{# 1 2}, Jing Cai ¹, Peng Xu ^{1 2}, Pingan Liang ¹, Yuxi Luo ¹, Anwen Liu ³

Affiliations + expand

PMID: 33931014 PMCID: PMC8086057 DOI: 10.1186/s12885-021-08228-2

Mechanisms of acquired resistance

showed that T790M mutations were detected in 64.3% (18/28) of patients, followed by TP53 mutations in 28.6% (8/28), BRAF mutations in 3.6% (1/28), ATM mutations in 3.6% (1/28), Met amplification in 3.6% (1/28), mTOR mutation in 3.6% (1/28), KRAS mutations in 3.6% (1/28), PTEN mutations in 3.6% (1/28), EGFR 19 p.A755D mutations in 3.6% (1/28), RB1 mutations in 3.6% (1/28) and PIK3CA mutations in 3.6% (1/28). Approximately 78.6% (22/28) of patients in the

Consensuamos reportarla como VUS y que aparezca en el informe ...

Molecular VH24P006475A001001- PM OCA Surt al informe

Normal **B I U** μ m 2 3 α β λ κ

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA
Origen de la mostra: Nòdul a pulmó dret (Bloc: VH-24-B-006526A001)
Tipus de mostra: FFPE
Indicació: Adenocarcinoma. Pacient amb mutació EGFR en progressió que necessita BAG de lesió pulmonar per a estudi de mutacions de resistència.
Referència DNA: VH24P006475A001, Mostra Valorable
Referència RNA: VH24P006475A002, Mostra Valorable
Contingut tumoral: 95%

RESULTATS

1. Variants ACCIONABLES
EGFR:NM_005228.5:exon 19:c.2240_2264delinsCGAAAGA:p.(Leu747_Ala755delinsSerLysAsp) VAF 66.3% [EGFRExon19Deletion]
EGFR:NM_005228.5:exon 20:c.2369C>T:p.(Thr790Met) VAF 17.9% [T790M]
EGFR:NM_005228.5:exon 20:c.2390_2391delinsCT:p.(Cys797Ser) VAF 32.4% [C797S]

2. ALTRES alteracions patogèniques
No s'han detectat alteracions patogèniques

3. Variants DE SIGNIFICAT INCERT
EGFR:NM_005228.5:exon 19:c.2264C>A:p.(Ala755Asp) VAF 60.9%

Añadiríamos la C797S en CIS...

2. ALTRES alteracions patogèniques
No s'han detectat alteracions patogèniques

3. Variants DE SIGNIFICAT INCERT
EGFR:NM_005228.5:exon 19:c.2264C>A:p.(Ala755Asp) VAF 60.9%

En funció del coneixement actual no es pot determinar la rellevància clínica de les variants de significat incert, per tant, no es poden tenir en compte en el maneig clínic del pacient. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

COMENTARIS I RECOMANACIONS
La mutació L747_A755delinsSKD detectada a l'exó 19 del gen EGFR és oncogènica i de guany de funció. S'associa a sensibilitat a tractament amb inhibidors TKI d'EGFR.

La mutació T790M de l'exó 20 d'EGFR es produeix al domini tirosina quinasa d'EGFR i és oncogènica i de guany de funció. L'aparició d'EGFR T790M generalment passa com una mutació secundària adquirida després d'una exposició perllongada a inhibidors de la tirosina quinasa (TKI) d'EGFR de primera i segona generació, i se la reconeix com una mutació de resistència a aquests inhibidors. La mutació EGFR T790M s'associa amb sensibilitat a TKI EGFR de tercera generació.

La mutació C797S de l'exó 20 d'EGFR es troba al domini tirosina quinasa d'EGFR i és una mutació de resistència a inhibidors TKI EGFR de tercera generació.

Els resultats obtinguts es presentaran en el comitè molecular de tumors.

IMPORTANTE ANOTACIÓN DE DISCORDANCIA DE RESULTADOS GUARDANT vs BX

GUARDANT 2024:

PD
Guardant: TP53:NM_000546.5:exon5:c.421T >C:p.C141R 0.11%

OCA BIOPSIA 2024:

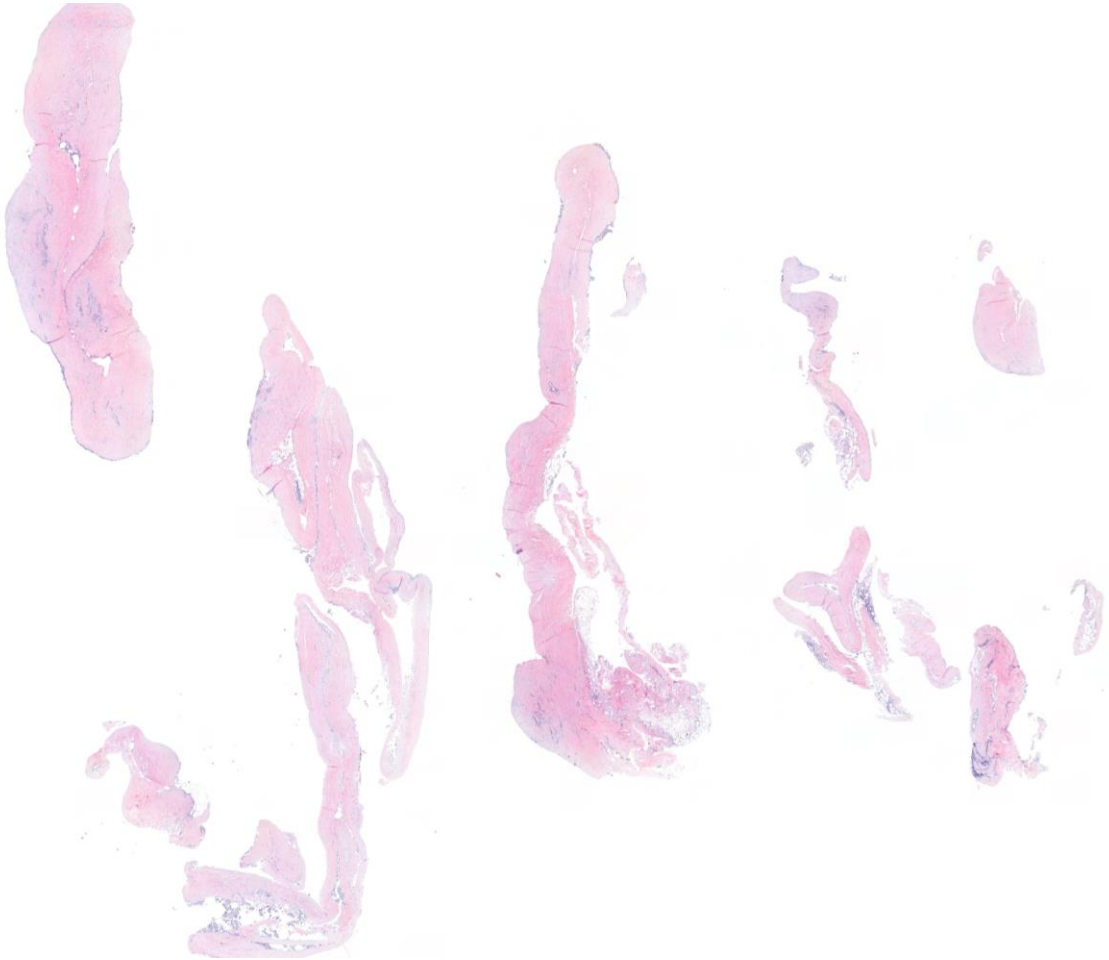
Variant ID	Key Variant	Locus	Oncomine Variant Class	Gene	AA Change	Ref	A...	T...	Call	Effective Read Depth	Nuc Change	Allele Frequency (%)
COSM43901	No	chr17:7578509		TP53	p.Cys141Arg	A	G	snp	ABSENT	687	c.421T>C	0

Comité Molecular Inter-hospitalario

Viernes 20 Enero 2023

Agenda

Caso 01 VH22P29436 HUVH



Normal + B I U ≡ ≡ ≡ ≡ ≡ μm ° ° α β λ κ

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Origen de la mostra: pulmó
Tipus de mostra: FFPE
Indicació: Càncer de pulmó
Referència DNA: VH22P029436A001, Mostra Valorable
Referència RNA: VH22P029436A002, Mostra Valorable
Contingut tumoral: 20%

RESULTATS

1. Variants ACCIONABLES

EGFR NM_005228.5: c.2237_2255delinsT, p.Glu746_Ser752delinsVal VAF 14,5%

2. ALTRES alteracions patogèniques

BRAF NM_004333.6: c.1799T>A, p.Val600Glu VAF 7,9%

3. Variants DE SIGNIFICAT INCERT

No s'han detectat alteracions de significat incert

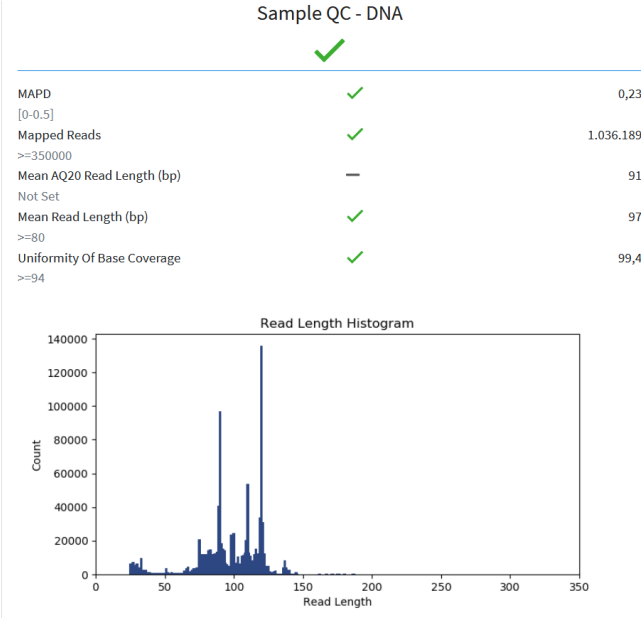
En funció del coneixement actual no es pot determinar la rellevància clínica de les variants de significat incert, per tant, no es poden tenir en compte en el maneig clínic del pacient. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

COMENTARIS i RECOMANACIONS

La delecció detectada a l'exó 19 del gen EGFR s'associa a sensibilitat a tractament amb inhibidors EGFR-TKIs. D'altra banda, la mutació V600E detectada a l'exó 15 del gen BRAF s'associa a resistència a tractament amb inhibidors EGFR-TKIs però s'associa a sensibilitat a tractament amb inhibidors de la tirosina cinasa BRAF. Els resultats obtinguts es presentaran en el comitè molecular de tumors

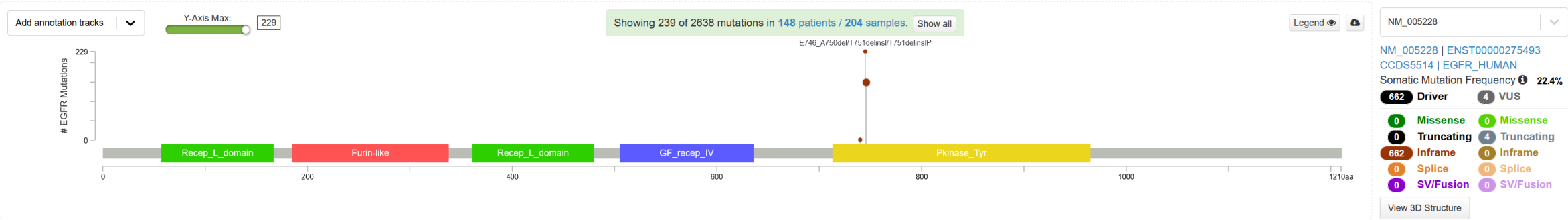
METODOLOGIA

Panell NGS: Oncomine Precision Assay (ThermoFisher Scientific)



EGFR

BRAF



239 Mutations: includes 90 duplicate mutations in patients with multiple samples (page 10 of 10)

EGFR E746_S752delinsV in lung adenocarcinoma

Oncogenic

Gain-of-function

EGFR, a receptor tyrosine kinase, is altered by amplification and/or mutation in lung and brain cancers among others.

The EGFR E746_S752delinsV alteration is known to be oncogenic.

The EGFR tyrosine kinase inhibitors osimertinib, dacomitinib, afatinib, erlotinib, and gefitinib are FDA-approved for the treatment of patients with non-small cell lung cancer harboring an EGFR exon 19 deletion such as E746_S752delinsV.

Biological Effect

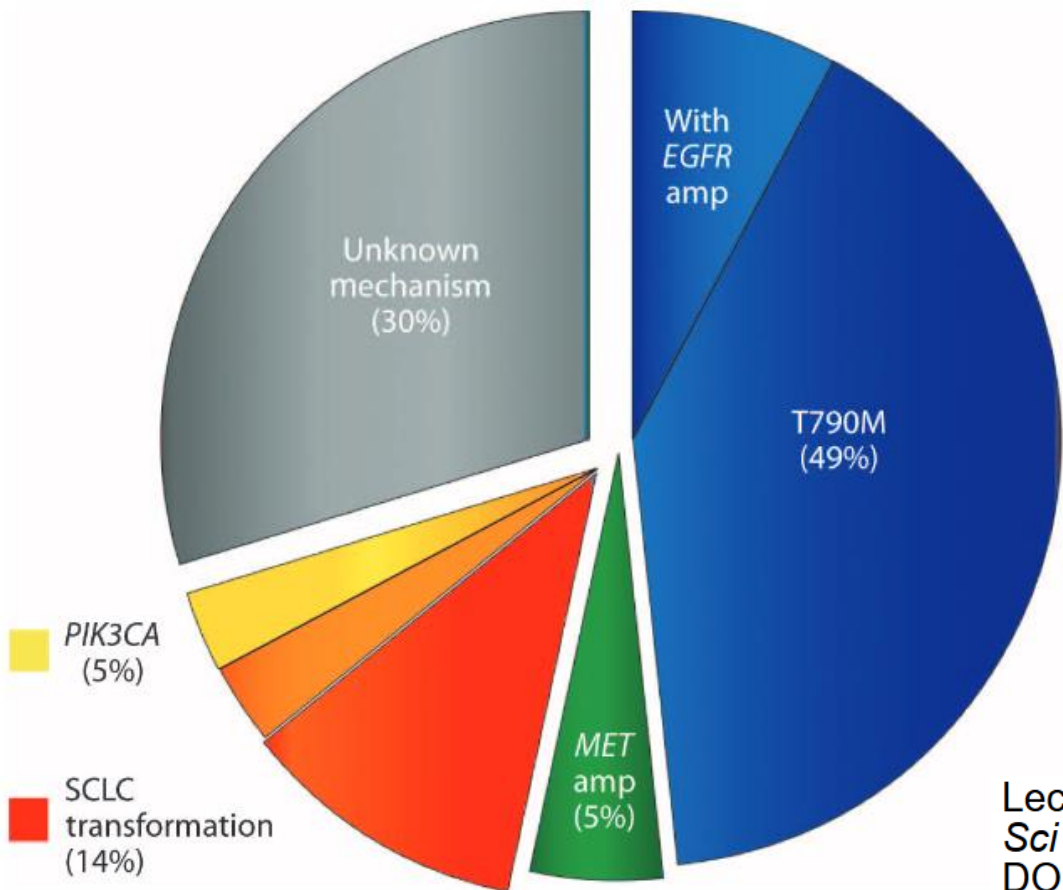
Therapeutic Implications

Level	Alteration(s)	Drug(s)	Level-associated cancer type(s)	
1	Exon 19 in-frame deletions, L858R	Erlotinib	Non-Small Cell Lung Cancer	
1	Exon 19 in-frame deletions, L858R	Erlotinib + Ramucirumab	Non-Small Cell Lung Cancer	
1	Exon 19 in-frame deletions, L858R	Afatinib	Non-Small Cell Lung Cancer	
1	Exon 19 in-frame deletions, L858R	Gefitinib	Non-Small Cell Lung Cancer	
1	Exon 19 in-frame deletions, L858R	Osimertinib	Non-Small Cell Lung Cancer	
4	Exon 19 in-frame	Dacomitinib	Non-Small Cell Lung Cancer	

Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors

Lecia V. Sequist,^{1,2,*†} Belinda A. Waltman,^{2,*} Dora Dias-Santagata,^{2,3,*} Subba Digumarthy,^{2,4} Alexa B. Turke,^{1,2} Panos Fidiias,^{1,2} Kristin Bergethon,³ Alice T. Shaw,^{1,2} Scott Gettinger,⁵ Arjola K. Cosper,¹ Sara Akhavanfard,^{2,3} Rebecca S. Heist,^{1,2} Jennifer Temel,^{1,2} James G. Christensen,⁶ John C. Wain,^{1,2,7} Thomas J. Lynch,⁵ Kathy Vernovsky,¹ Eugene J. Mark,^{2,3} Michael Lanuti,^{1,2,7} A. John Iafrate,^{2,3} Mari Mino-Kenudson,^{2,3} Jeffrey A. Engelman^{1,2†}

Mecanismos de resistencia anti EGFR



Lecia V. Sequist *et al.*
Sci Transl Med **3**, 75ra26 (2011);
DOI: 10.1126/scitranslmed.3002003

Treatment Options for EGFR T790M-Negative EGFR Tyrosine Kinase Inhibitor-Resistant Non-Small Cell Lung Cancer

Salvatore Corallo¹ · Ettore D'Argento¹ · Antonia Strippoli¹ · Michele Basso¹ · Santa Monterisi¹ · Sabrina Rossi² · Alessandra Cassano¹ · Carlo M. Barone¹

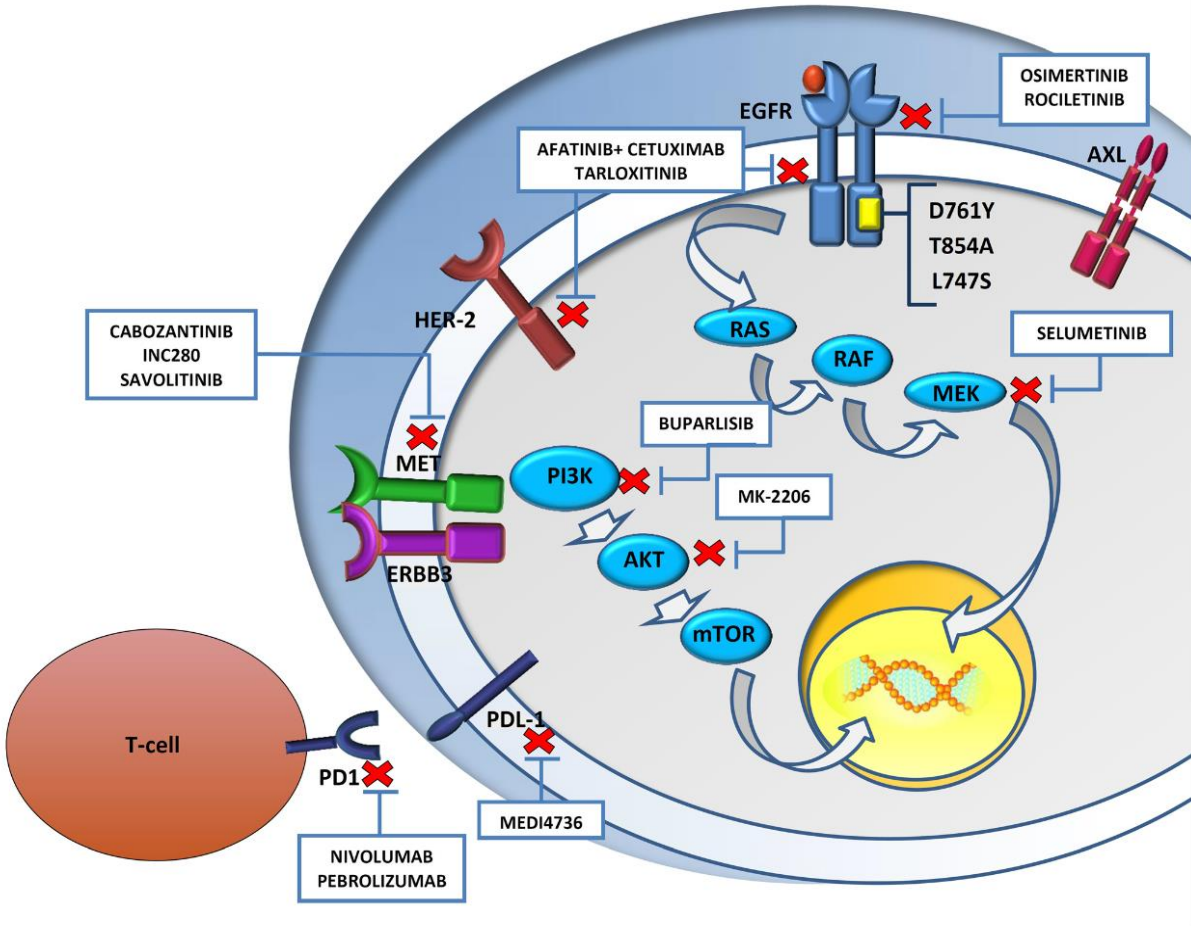


Fig. 1 Mechanisms of acquired resistance to EGFR-TKIs and targeted therapies being evaluated in clinical trials

Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor *BRAF* gene mutations but lack mutations in *KRAS*, *NRAS*, or *MEK1*

Kadoaki Ohashi^a, Lecia V. Sequist^{b,c}, Maria E. Arcila^d, Teresa Moran^e, Juliann Chmielecki^f, Ya-Lun Lin^a, Yumei Pan^a, Lu Wang^d, Elisa de Stanchina^g, Kazuhiko Shien^h, Keisuke Aoe^{h,i}, Shinichi Toyooka^h, Katsuyuki Kiura^k, Lynnette Fernandez-Cuesta^l, Panos Fidas^{b,c}, James Chih-Hsin Yang^{m,n}, Vincent A. Miller^o, Gregory J. Riely^o, Mark G. Kris^o, Jeffrey A. Engelman^{b,c}, Cindy L. Vnencak-Jones^p, Dora Dias-Santagata^{q,r}, Marc Ladanyi^d, and William Pao^{a,1}

Table 2. RAS signaling pathway gene mutations in tumor samples from 212 patients with EGFR-mutant lung cancer and acquired resistance to EGFR TKIs

Institution	EGFR T790M	NRAS	KRAS	BRAF	MEK1
MSKCC*	57/103	0/103	0/103	0/103	0/103
Vanderbilt-Ingram Cancer Center†	8/8	0/8	0/8	1/8	0/8
Massachusetts General Hospital‡	42/84	0/84	0/84	1/84	0/35
Others§	n/d	0/17	n/d	n/d	n/d
Total	107/195 (54.9%)	0/212	0/195	2/195 (1.0%)	0/146

n/d, no data.
*Sixty-eight MSKCC patient samples were assessed by a mass spectrometry-based (Sequenom) assay (ref. 39), and 35 samples were assessed using a SNaPshot-based assay (ref. 32).
†Eight samples were analyzed at Vanderbilt-Ingram Cancer Center by the SNaPshot assay.
‡Eighty-four samples were analyzed at Massachusetts General Hospital by SNaPshot assay (ref. 38).
§Seventeen samples were analyzed for NRAS mutations by direct sequencing at other institutions (nine patients at Okayama University, Japan, five patients at National Taiwan University Hospital, and three patients at the Max Planck Institute, Cologne, Germany).

1% de BRAF V600E en resistencias a EGFR TKI



Audit of Molecular Mechanisms of Primary and Secondary Resistance to Various Generations of Tyrosine Kinase Inhibitors in Known Epidermal Growth Factor Receptor-Mutant Non-small Cell Lung Cancer Patients in a Tertiary Centre

M. Suryavanshi *, J. Jaipuria †, S. Mattoo *, S. Dhandha *, M. Khatri *

* Molecular Diagnostics, Rajiv Gandhi Cancer Institute and Research Centre, New Delhi, India

† Department of Urooncology, Rajiv Gandhi Cancer Institute and Research Centre, New Delhi, India

Abstract

Aims: Presently, three generations of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are approved against oncogene addicted EGFR-mutant non-small cell lung cancer (NSCLC). Patients with actionable EGFR mutations invariably develop resistance. This resistance can be intrinsic (primary) or acquired (secondary).

Materials and methods: This was a retrospective study carried out between January 2016 and April 2021 analysing 486 samples of NSCLC for primary and secondary resistance to first- (erlotinib, gefitinib), second- (afatinib) and/or third-generation (osimertinib) TKIs in EGFR-mutant NSCLCs by next generation sequencing (NGS). Tissue NGS was carried out using the ThermoFisher Ion Torrent™ Oncomine™ Focus 52 gene assay; liquid biopsy NGS was carried out using the Oncomine Lung Cell-Free Total Nucleic Acid assay. All cases were previously tested for a single EGFR gene with the Therascreen® EGFR RGQ PCR kit.

Results: The results were divided into four groups: (i) group 1: primary resistance to first- and/or second-generation TKIs. This group, with 21 cases, showed EGFR exon 20 insertions, dual, complex mutations and variant of unknown significance, *de novo* MET gene amplification besides other mutations. (ii) Group 2: primary resistance to third-generation TKIs. This group showed two cases, with one showing dual EGFR mutation (L858R and E709A) and EGFR gene amplification. (iii) Group 3: secondary resistance to first- and second-generation TKIs. This group had 27 cases, which were previously reported negative for EGFR T790M by single gene testing. Significant findings were MET gene amplification in four cases, with one also showing MET exon 14 skipping mutation. Three cases showed small cell change and one showed loss of primary mutation. (iv) Group 4: secondary resistance to third-generation TKIs. The latter group was further subgrouped into group 4A: secondary resistance to osimertinib (third-generation TKI) when offered as second-line therapy after first- and second-generation TKIs on detection of T790M mutation. This group had 15 cases. EGFR T790M mutation was lost in 10 (10/15; 67%) cases and was retained in five cases. Patients with T790M loss experienced early resistance (6.9 months versus 12.6 months mean, $P = 0.0024$) compared with cases that retained T790M. Two cases gained MET amplification as the resistance mechanisms. Other mutations that were found when EGFR T790M was lost were in FGFR3, KRAS, PIK3CA, CTNNB1, BRAF genes. One case had EML4-ALK translocation. Two cases showed driver EGFR deletion 19, retained T790M and C797S mutation in Cis form. Group 4B: secondary resistance to osimertinib (when given as first-line therapy) in EGFR-mutant NSCLC. This group had three cases. The duration of osimertinib treatment ranged from 11 to 17 months. Two patients showed additional C797S mutation along with primary EGFR mutation.

Conclusion: This study shows the wide spectrum of primary and secondary EGFR resistance mechanisms to first, second and third generation of TKIs and helps us to identify newer therapeutic targets that could carry forward the initial advantage offered by EGFR TKIs.

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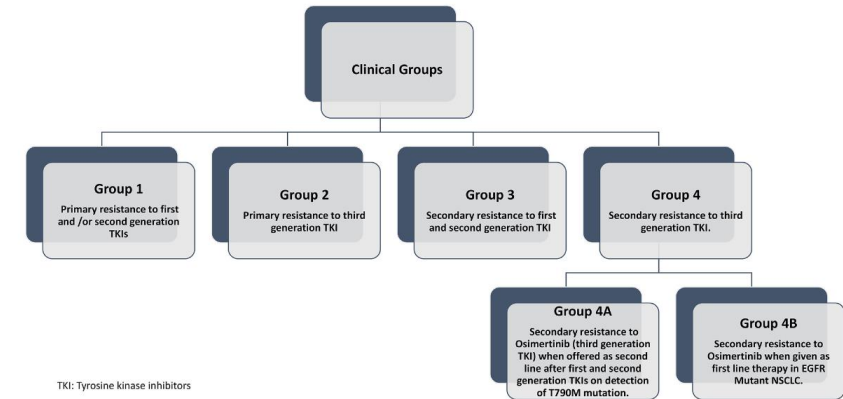


Fig 1. Clinical groups describing primary and secondary resistance to first-, second- and third-generation tyrosine kinase inhibitors (TKIs).

27 casos frente a Erlotinib----- No BRAF V600E

Tratamiento dual: EGFR TKI + BRAFinh

> JCO Precis Oncol. 2021 Jan 11;5:PO.20.00315. doi: 10.1200/PO.20.00315. eCollection 2021.

Erlotinib and Trametinib in Patients With *EGFR*-Mutant Lung Adenocarcinoma and Acquired Resistance to a Prior Tyrosine Kinase Inhibitor

Jia Luo ¹, Alex Makhnin ¹, Yosef Tobin ¹, Linda Ahn ¹, Sara A Hayes ², Afsheen Iqbal ¹, Kenneth Ng ¹, Maria E Arcila ³, Gregory J Riely ¹, Mark G Kris ¹, Helena A Yu ¹

Futuros pasos

> [Bioorg Chem](#). 2022 Mar;120:105616. doi: 10.1016/j.bioorg.2022.105616. Epub 2022 Jan 19.

Optimization and SAR investigation of novel 2,3-dihydropyrazino[1,2-a]indole-1,4-dione derivatives as EGFR and BRAF^{V600E} dual inhibitors with potent antiproliferative and antioxidant activities

Hesham A M Gomaa¹, Mohamed E Shaker², Sami I Alzarea³, O M Hendawy⁴, Fatma A M Mohamed⁵, Ahmed M Gouda⁶, Asmaa T Ali⁷, Martha M Morcoss⁸, Mostafa H Abdelrahman⁹, Laurent Trembleau¹⁰, Bahaa G M Youssif¹¹

Affiliations + expand

PMID: 35078049 DOI: [10.1016/j.bioorg.2022.105616](#)

Abstract

Using a single drug to treat cancer with dual-targeting is an unusual approach when compared to other drug combinations. Dual-targeting agents were developed as a result of insufficient efficacy and drug resistance when single-targeting agents were used. As a result, the 2,3-dihydropyrazino[1,2-a]indole-1,4-dione derivatives 13-22 have been developed as dual EGFR and BRAF^{V600E} inhibitors. The target compounds were synthesized and tested in vitro against four cancer cell lines, with compounds 15, and 19-22 demonstrating potent antiproliferative activity. In vitro studies revealed that these compounds have dual inhibitory effect on EGFR and BRAF^{V600E}. Compounds 15, and 19-22 exhibited inhibitions of EGFR with IC₅₀ ranging from 32 nM to 63 nM which were superior to erlotinib (IC₅₀ = 80 ± 10 nM). Compounds 20, 21 and 22 showed promising inhibitory activity of BRAF^{V600E} (IC₅₀ = 55, 45 and 51 nM, respectively) and were found to be potent inhibitors of cancer cell proliferation (GI₅₀ = 51, 35 and 44 nM, respectively). Compounds 20, 21 and 22 showed good antioxidant activity comparable to the reference Trolox. Lastly, the best active dual inhibitors were docked inside EGFR and BRAF^{V600E} active sites to clarify their binding modes.

Keywords: Antiproliferative; BRAF(V600E); EGFR; Indole; Melanoma cell.

> [Arch Pharm \(Weinheim\)](#). 2022 Dec 16;e2200464. doi: 10.1002/ardp.202200464.

Online ahead of print.

Discovery of new cyanopyridine/chalcone hybrids as dual inhibitors of EGFR/BRAF^{V600E} with promising antiproliferative properties

Hesham A Abou-Zied¹, Eman A M Beshr², Hesham A M Gomaa³, Yaser A Mostafa⁴, Bahaa G M Youssif⁴, Alaa M Hayallah⁴,⁵, Mohamed Abdel-Aziz²

Affiliations + expand

PMID: 36526595 DOI: [10.1002/ardp.202200464](#)

Abstract

As dual EGFR and BRAF^{V600E} inhibitors, 2-(3-cyano-4,6-bis(aryl)-2-oxo-1,2-dihydropyridine-1-yl)-N-(4-cinnamoylphenyl) acetamide derivatives 8-20 were developed. Compounds 8, 12, and 13 showed strong antiproliferative activity when the target compounds were synthesized and tested in vitro against four cancer cell lines. These hybrids have a dual inhibition activity on EGFR and BRAF^{V600E}, according to in vitro studies. The EGFR was inhibited by compounds 8, 12, and 13 with IC₅₀ values between 89 and 110 nM, which were equivalent to those of erlotinib (IC₅₀ = 80 nm). Compound 13 was found to be an effective inhibitor of the proliferation of cancer cells (GI₅₀ = 0.72 µM) and demonstrated hopeful inhibitory activity of BRAF^{V600E} (IC₅₀ = 58 nm), which is superior to erlotinib (IC₅₀ = 65 nm). Compound 13 caused apoptosis and showed cell cycle arrest in the G0/G1phase in a study on the MCF-7 cell line. The new compounds can fit tightly into the active sites of EGFR and BRAF^{V600E} kinases, according to molecular docking analyses.

Keywords: antiproliferative; apoptosis; docking; hybridization; kinases.

Comité Molecular Inter-hospitalario

Viernes 10 Febrero 2023

Agenda

Caso 01 NHC 11836931 VH23P000191 HUVH

Caso 02 NHC 17900375 VH22P26854 Althaia

VH-22-P-26854
INFORME ANATOMIA PATOLÒGICA

Es rep Htx+bloc de parafina procedent del H.Althaia, Xarxa Assistencial de Manresa, amb número de referència B-220014808,per estudi de Biomarcadors en tumors sòlids, mitjançant seqüenciació massiva (NGS)

Patòleg LANDOLFI., STEFANIA

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT
SEQÜENCIACIÓ MASSIVA (NGS)

ESTUDIO DE BIOMARCADORES EN TUMORES SOLIDOS MEDIANTE SECUENCIACIÓN MASIVA (NGS)

INFORMACIÓN MUESTRA

Origen de la muestra: GIST
Tipo de muestra: FFPE
Indicación: Tumor Gastrointestinal
Referencia DNA: VH22P026854A001, Muestra Valorable
Referencia RNA: VH22P026854A002, Muestra Valorable
Contenido tumoral: 80%

RESULTADOS

1. Variantes ACCIONABLES

KIT NM_000222.2: c.1679T>A, p.(Val560Asp) VAF 61,6%

2. OTRAS alteraciones patogénicas

FGFR3. Amplificación del número de copias (CNV). Número de copias: 7.08

CDKN2A. Pérdida del número de copias (CNV). Número de copias: 0

3. Variantes DE SIGNIFICADO INCIERTO

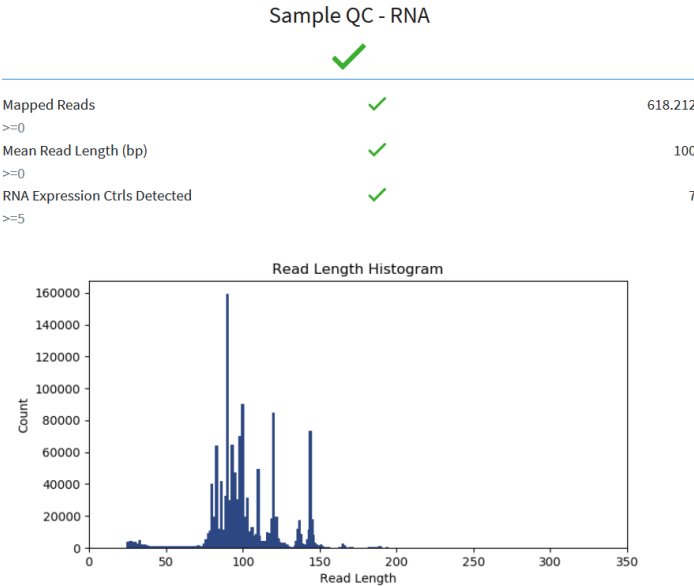
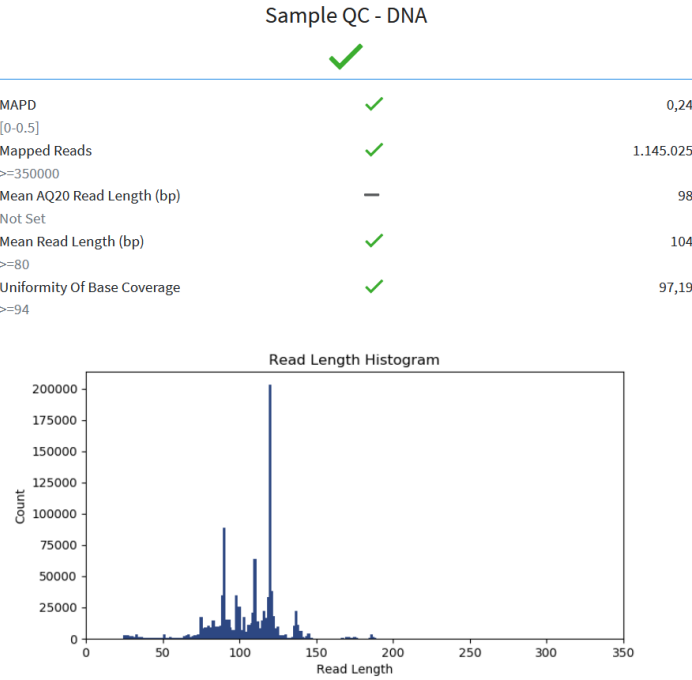
No se han detectado otras variantes de significado incierto

En función del conocimiento actual no se puede determinar la relevancia clínica de las variantes de significado incierto, por lo tanto, no se pueden tener en cuenta en el manejo clínico del paciente. La aparición de nueva información científica podría cambiar en el futuro esta interpretación.

COMENTARIOS y RECOMENDACIONES

La alteración encontrada en el exon 11 del gen KIT esta asociada a sensibilidad frente a tratamientos con inhibidores tirosina quinasa específicos del receptor KIT.

La interpretación de los resultados moleculares y sus implicaciones clinico-patológicas se discutiran en el comite molecular correspondiente.



Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
VH22P026854A1D_VH22P026854A2R	28 NOV 2022	Male	FFPE	Solid Tumor	Colorectal Cancer	Stage IV	40

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity
100.00%	99.17%	4340	97.19%	91.41%	97.60%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

CDKN2A

CDKN2A

CNV

FGFR3

FGFR3

CNV

KIT

COSM1257

AA Change: p.V560D

Allele Frequency: 0.616

SNV/Indel

All

SNVs/Indels

Fusions

CNVs

Variant ID	Oncomine Gene Class	Gene	AA Change	Allele Frequency	Allele Frequency (%)	Call	Coverage	Nuc Change	Effective Read Depth
COSM1257	Gain-of-Function	KIT	p.V560D	0.616	61.6	PRESENT (HETEROZYGOUS)	7803	c.1679T>A	7803

C-KIT EXON 11

Primer Forward gagtgcctaatgactgagacaataattattaaaggtgatctatcccccttctcccca

cag aaa ccc atg tat gaa

Q K P M Y E

549 550 551 552 553 554

gta cag tgg aag gtt gag gag ata aat gga aac aat tat gtt tac ata gac cca aca

V Q W K V V E E I N G N N Y V Y I D P T

555 557 559 560

568

572

caa ctt cct tat gat cac

Q L P Y D H

579

aatgggagtttcccagaaacaggctgagtttggtcagtatgaaacag

gggctttccatgtcaccttt Primer Reverse



KIT(NM_000222.3):V560D

hg38

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Editions

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Community

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Demo

chr4-54727447-T-A (KIT:p.V560D)

Link a publication

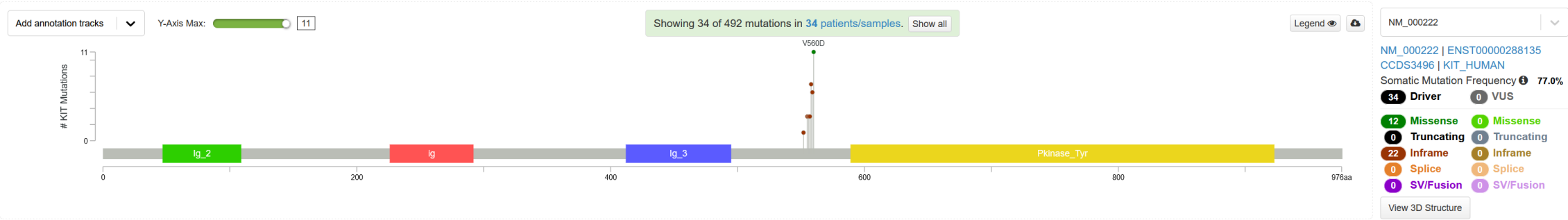
General Information SNV KIT(NM_000222.3):c.1679T>A (p.Val560Asp)	PharmGKB No data available	ACMG Classification Pathogenic 10 points = 10 P - 0 B	Frequencies exomes: not found genomes: not found (cov:31.5)	Pathogenicity Scores 1010
Genes KIT	Publications Variant: 5 Gene: 287	ClinVar Pathogenic ☆☆☆ Submissions: 1	MitoMap No data available	ClinGen No data available
Community Contributions	Transcripts NM_000222.3 - missense MANE Select	Uniprot Variants No data available	Conservation Scores phyloP100: 7.859	Region Browser

⊕ V560D Evidence

7 total, showing 7

EID	Disease	Drugs
⊕ EID4123	⊙ Gastrointestinal Stromal...	⊙ Sunitinib
⊕ EID4124	⊙ Gastrointestinal Stromal...	⊙ Sunitinib
⊕ EID4122	⊙ Gastrointestinal Stromal...	⊙ Sunitinib
⊕ EID2464	⊙ Gastrointestinal Stromal...	⊙ Imatinib
⊕ EID4127	⊙ Gastrointestinal Stromal...	⊙ Regorafenib ⊙ Imatinib ⊙ Ponatinib ⊙ Sunitinib
⊕ EID4077	⊙ Cancer	⊙ Sunitinib
⊕ EID5348	⊙ Cancer	⊙ Imatinib Mesylate

KIT



KIT V560D in gastrointestinal stromal tumors

OncogenicGain-of-function

KIT, a receptor tyrosine kinase, is recurrently mutated in gastrointestinal stromal tumors.

The KIT V560D mutation is known to be oncogenic.

The multikinase KIT and PDGFRA inhibitors imatinib, sunitinib, regorafenib and ripretinib are FDA-approved for the treatment of patients with gastrointestinal stromal tumors (GISTs) harboring KIT exon 11 alterations, such as KIT V560D.

Biological EffectTherapeutic Implications

Level	Alteration(s)	Drug(s)	Level-associated cancer type(s)	
1	V560D and 65 other alterations	Imatinib	Gastrointestinal Stromal Tumor	
1	V560D and 65 other alterations	Sunitinib	Gastrointestinal Stromal Tumor	
1	V560D and 65 other alterations	Regorafenib	Gastrointestinal Stromal Tumor	
1	V560D and 65 other alterations	Ripretinib	Gastrointestinal Stromal Tumor	

VH22P026854A1D_VH22P026854A2R

QC 

Key Findings

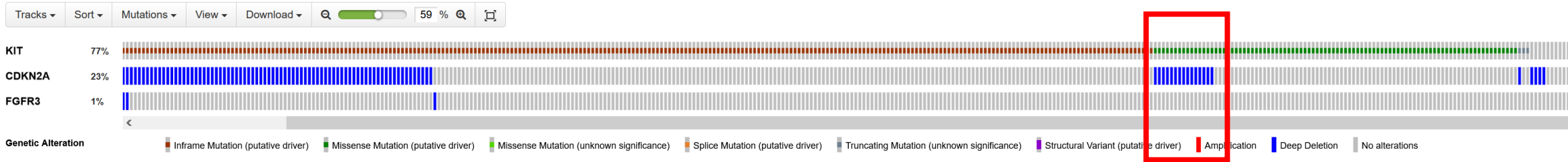
Variants

Plugins

Reports

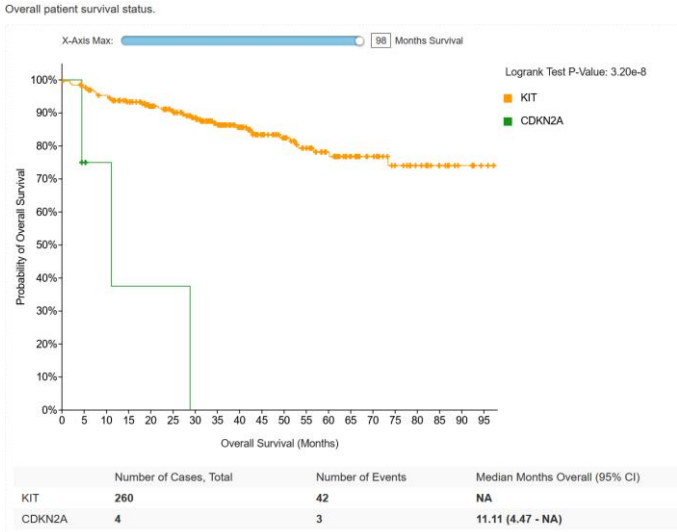
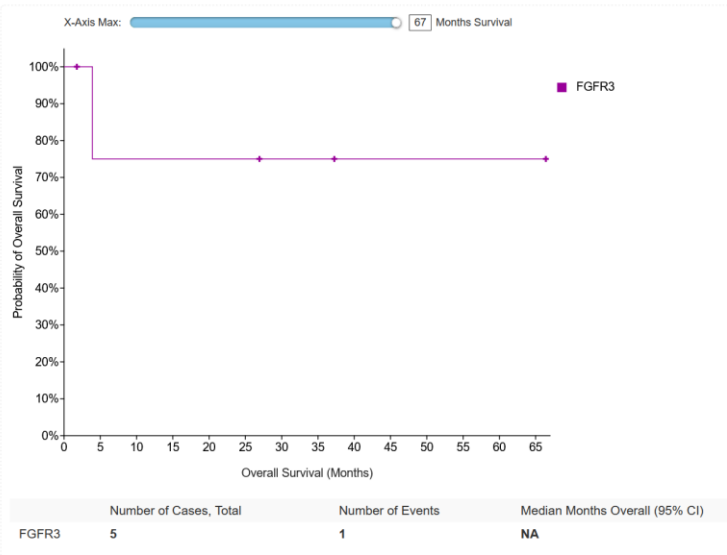
- All
- SNVs/Indels
- Fusions
- CNVs

Variant ID	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	Copy Number	Call	CNV Confidence	CNV Ratio
FGFR3	chr4:1800932	Amplification	Gain-of-Function	FGFR3	7.08	PRESENT (GAIN)	5%:5.72, 95%:8.64	2.02
CDKN2A	chr9:21968174	Deletion	Loss-of-Function	CDKN2A	0	PRESENT (LOSS)	5%:0, 95%:0	0.22



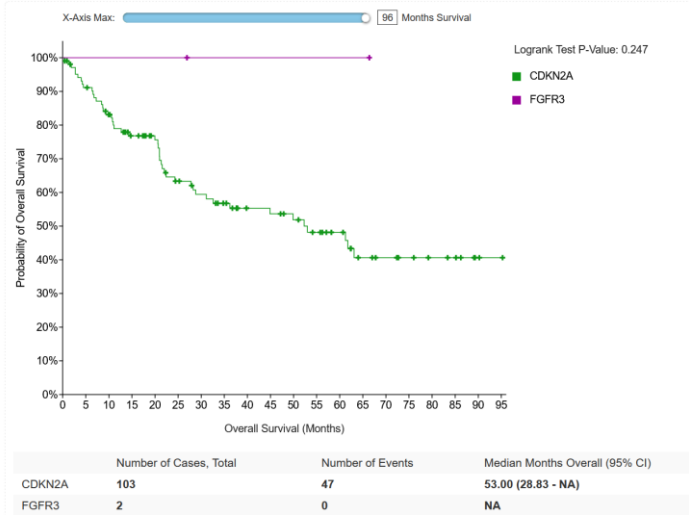
Overall

Overall patient survival status.



Overall

Overall patient survival status.



RESEARCH ARTICLE

FGFR-Mediated Reactivation of MAPK Signaling Attenuates Antitumor Effects of Imatinib in Gastrointestinal Stromal Tumors

Fang Li¹, Hung Huynh², Xiaoyan Li¹, David A. Ruddy¹, Youzhen Wang¹, Richard Ong², Pierce Chow², Shumei Qiu¹, Angela Tam¹, Daniel P. Rakiec¹, Robert Schlegel¹, John E. Monahan¹, and Alan Huang¹

ABSTRACT

Activating mutations in either KIT or PDGFRA are present in approximately 90% of gastrointestinal stromal tumors (GIST). Although treatment with the KIT and PDGFR inhibitor imatinib can control advanced disease in about 80% of GIST patients, the beneficial effect is not durable. Here, we report that [ligands from the FGF family reduced the effectiveness of imatinib in GIST cells, and FGF2 and FGFR1 are highly expressed in all primary GIST samples examined](#). The combination of KIT and FGFR inhibition showed increased growth inhibition in imatinib-sensitive GIST cell lines and improved efficacy in patient-derived GIST xenografts. In addition, inhibition of MAPK signaling by imatinib was not sustained in GIST cells. An ERK rebound occurred through activation of FGF signaling, and was repressed by FGFR1 inhibition. Downregulation of Sprouty proteins played a role in the imatinib-induced feedback activation of FGF signaling in GIST cells.

SIGNIFICANCE: We here show that FGFR-mediated reactivation of the MAPK pathway attenuates the antiproliferation effects of imatinib in GISTs. The imatinib-induced ERK rebound can be repressed by the FGFR inhibitor BGJ398, and combined KIT and FGFR inhibition leads to increased efficacy *in vitro* and in patient-derived xenografts. *Cancer Discov*; 5(4): 438–51. ©2015 AACR.

Published in final edited form as:

Cancer Res. 2015 March 1; 75(5): 880–891. doi:10.1158/0008-5472.CAN-14-0573.

Crosstalk between KIT and FGFR3 Promotes Gastrointestinal Stromal Tumor Cell Growth and Drug Resistance

Nathalie Javidi-Sharifi^{1,2}, Elie Traer^{1,2}, Jacqueline Martinez¹, Anu Gupta³, Takehiro Taguchi⁵, Jennifer Dunlap^{1,6}, Michael C. Heinrich^{1,2,7}, Christopher L. Corless^{1,6}, Brian P. Rubin^{3,4,8}, Brian J. Druker^{1,2,9}, and Jeffrey W. Tyner^{1,10,*}

Abstract

Kinase inhibitors such as imatinib have dramatically improved outcomes for GIST patients, but many patients develop resistance to these treatments. While in some patients this event corresponds with mutations in the GIST driver oncogenic kinase KIT, other patients development resistance without KIT mutations. In this study, we address this patient subset in reporting a functional dependence of GIST on the FGF receptor FGFR3 and its crosstalk with KIT in GIST cells. Addition of the FGFR3 ligand FGF2 to GIST cells restored KIT phosphorylation during imatinib treatment, allowing sensitive cells to proliferate in the presence of the drug. FGF2 expression was increased in imatinib-resistant GIST cells, the growth of which was blocked by RNAi-mediated silencing of FGFR3. Moreover, combining KIT and FGFR3 inhibitors synergized to block the growth of imatinib-resistant cells. [Signaling crosstalk between KIT and FGFR3 activated the MAPK pathway to promote resistance to imatinib](#). Clinically, an immunohistochemical analysis of tumor specimens from imatinib-resistant GIST patients revealed a relative increase in FGF2 levels, with a trend towards increased expression in imatinib-naïve samples consistent with possible involvement in drug resistance. [Our findings provide a mechanistic rationale to evaluate existing FGFR inhibitors and multi-kinase inhibitors that target FGFR3 as promising strategies to improve treatment of GIST patients with de novo or acquired resistance to imatinib](#).

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Michael C. Heinrich^{1†} , Janice Patterson^{1†}, Carol Beadling¹, Yuexiang Wang², Maria Debiec-Rychter³, Barbara Dewaele³, Christopher L. Corless¹, Anette Duensing⁴, Chandrajit P. Raut⁵, Brian Rubin⁶, Tamas Ordog⁷, Matt van de Rijn⁸, Jerry Call⁹, Thomas Mühlenberg¹⁰, Jonathan A. Fletcher^{2†} and Sebastian Bauer^{10,11‡}

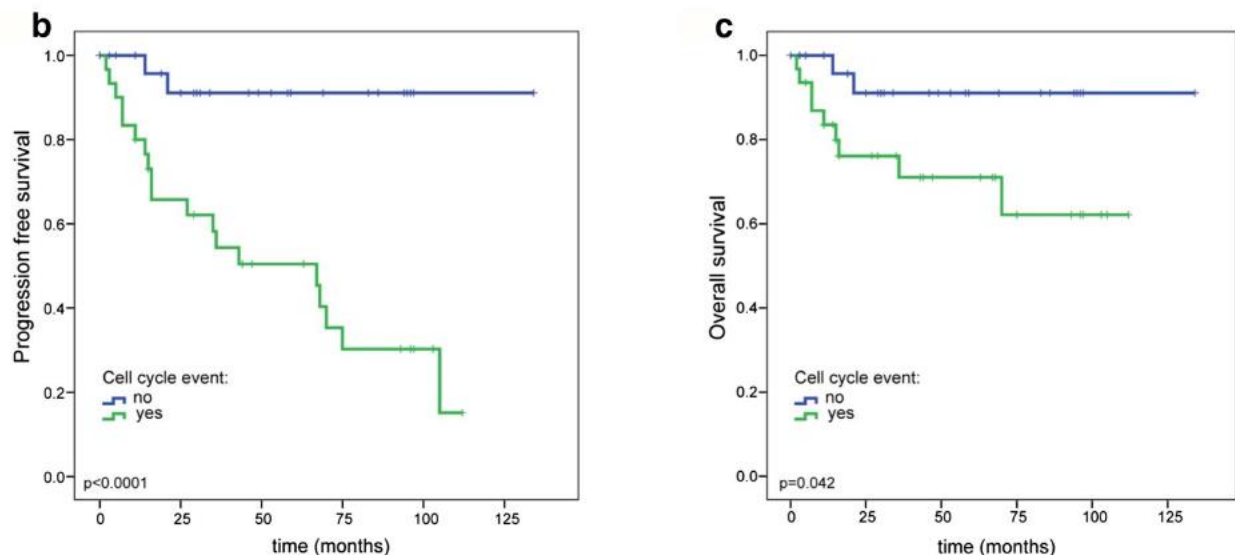


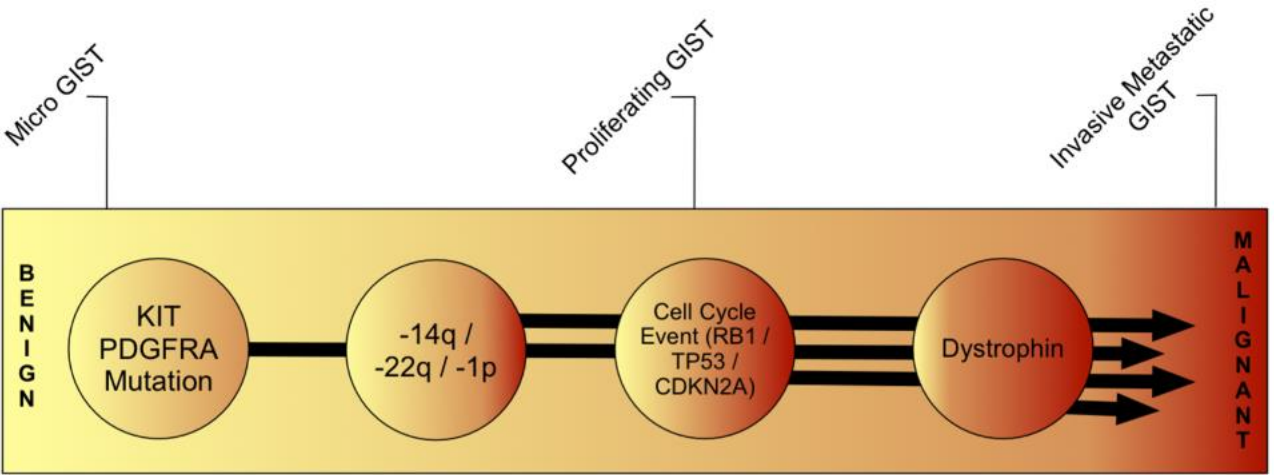
Fig. 4 a Systematic screen for cell cycle-related genetic events in GIST with very low, low, intermediate and high risk of relapse according to the AFIP classification using Ion Torrent sequencing, MLPA and FISH analyses. Kaplan–Meier curves showing relapse-free (b) and overall survival (c) for patients with (green line) or without (blue line) a cell cycle-related event involving either *TP53*, *CDKN2A*, or *RB1*

RESEARCH

Open Access

Genomic aberrations in cell cycle genes predict progression of *KIT*-mutant gastrointestinal stromal tumors (GISTs)

Michael C. Heinrich^{1*†}, Janice Patterson^{1†}, Carol Beadling¹, Yuexiang Wang², Maria Debiec-Rychter³, Barbara Dewaele³, Christopher L. Corless¹, Anette Duensing⁴, Chandrajit P. Raut⁵, Brian Rubin⁶, Tamas Ordog⁷, Matt van de Rijn⁸, Jerry Cal⁹, Thomas Mühlenberg¹⁰, Jonathan A. Fletcher^{2‡} and Sebastian Bauer^{10,11‡}



Cell cycle-related genetic event	Very low risk	Low risk	Intermediate risk	High risk < 6 mitoses	High risk ≥ 6 mitoses
Any	17%	35%	42%	67%	86%
CDKN2A	17%	7%	25%	50%	48%
RB1	0	14%	17%	33%	38%
TP53	0	14%	29%	17%	28%

Fig. 5 Model of genetic progression of GIST from benign to invasive/metastatic tumors

Activity and Safety of Palbociclib in Patients with Advanced Gastrointestinal Stromal Tumors Refractory to Imatinib and Sunitinib: A Biomarker-driven Phase II Study

Maud Toulmonde¹, Jean-Yves Blay³, Olivier Bouche⁴, Olivier Mir⁵, Nicolas Penel⁶, Nicolas Isambert⁷, Florence Duffaud⁸, Emmanuelle Bompas⁹, Thomas Esnaud², Romain Boidot¹⁰, Damien Geneste¹¹, François Ghiringhelli¹⁰, Carlo Lucchesi¹¹, Carine A. Bellera², François Le Loarer¹², and Antoine Italiano¹

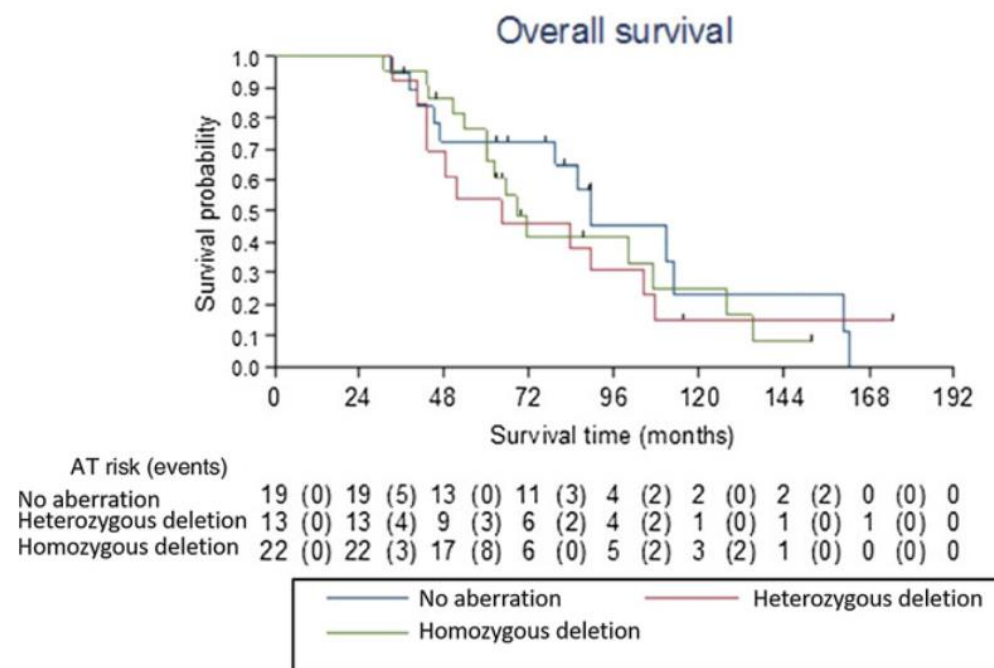


Figure 3. OS according to *CDKN2A* status ($N = 54$ patients with interpretable results for the array-CGH analysis).

Caso 03 NHC 20593976 Biopsia 2021



MUTATION TEST RESULTS

Patient initials	A.F.A.
Biopsy number	M21-3227
Original block	21B-14957 A4
Analysis requested by	Paolo Nuciforo (Molecular Pathology Lab)
Results sent to	Dr. Iranzo
Sample tissue origin	lung
Primary origin	lung
Date	15-11-2021
Time	13:30

Molecular Oncology Lab informed a sample tumor cellularity of 80% (macrodissection).

Results

Variants

Pathogenic/Likely pathogenic variants

Variant	MAF
ATM:NM_000051.3:exon11:c.1673del:p.G558fs	25%
KEAP1:NM_203500.2:exon3:c.997G>T:p.G333C	26%
KRAS:NM_004985.5:exon2:c.34G>T:p.G12C	39%
LZTR1:NM_006767.4:exon8:c.741C>G:p.S247R	22%
TP53:NM_000546.5:exon6:c.646G>T:p.V216L	19%

VUS

Variant	MAF
BRD4:NM_058243.2:exon16:c.3367G>T:p.E1123X	18%
GRM3:NM_000840.2:exon2:c.34T>A:p.L12I	12%
KMT2D:NM_003482.3:exon39:c.11156G>A:p.R3719K	52%
LRP1B:NM_018557.2:exon41:c.6494G>A:p.R2165Q	27%
MAPK7:NM_139033.2:exon4:c.1391del:p.P464fs	22%
NOTCH2:NM_024408.4:exon19:c.3143G>A:p.R1048H	46%
NOTCH4:NM_004557.4:exon3:c.439C>T:p.P147S	23%
PDGFRA:NM_006206.6:exon4:c.449G>C:p.C150S	12%
PTPRS:NM_002850.4:exon5:c.383A>G:p.D128G	25%
SHQ1:NM_018130.3:exon5:c.505A>G:p.I169V	47%



TBX3:NM_016569.4:exon4:c.839C>T:p.A280V	14%
TSHR:NM_000369.3:exon5:c.412C>T:p.L138F	19%

MAF: mutant allele fraction

VUS: variant of unknown significance

Tumor mutational burden

TMB: 12.69 mutations/megabase

TMB $\geq$ 13 is considered high.

Copy number alterations

Gain regions

No significant gain regions were identified.

Loss regions

No significant loss regions were identified.

Cancer Genomics Core Lab
Ana Vivancos, PhD

07-12-2021

Caso 03 NHC 20593976 VH22P27025 HUVH

muestra ID	VH22P027025A001001		
Tipo de muestra	Tejido FFPE		
ID de Cartucho	59391280		
Tipo Test	KRAS_IVD/3.0	Versión de TTP	3.3
ID del lote	00005939	Fecha expir.	15 jun. 2023
N.º de serie del Instrumento	SER1430		
Versión software Instrumento	27.0		
N.º serie de la Consola	C00001018		
Versión de software de la Consola	4.4.0.630		
Solicitud de Test completada	29 nov. 2022 (16:21)		
Test iniciado	29 nov. 2022 (16:21)		
Prueba finalizada	29 nov. 2022 (18:30)		
Estado Test	Resultado publicado: Automático, 29 nov. 2022 (18:30)		
Operador	Sandra Valera		

Resultado del Test (1) Dispositivo médico de diagnóstico in vitro. Para uso en procedimientos de diagnóstico.

Idylla KRAS Mutation Test

GENOTIPO KRAS	MUTACIÓN DETECTADA EN EL CODÓN 12 DE KRAS
Mutación	G12C
Proteína	p.Gly12Cys
Cambio nucleotídico	c.34G>T

(1) La información relativa al propósito planteado, al rendimiento clínico y a la interpretación clínica del ensayo aplicado está disponible en las instrucciones de uso.

VH-22-P-27025
INFORME ANATOMIA PATOLÒGICA

ANÀLISI MUTACIONAL DEL GEN KRAS

ANÁLISIS MUTACIONAL DEL GEN KRAS

Información de la muestra:

Origen de la muestra: Pulmón

Tipo de muestra: FFPE

Contenido tumoral: 60%

Resultado:

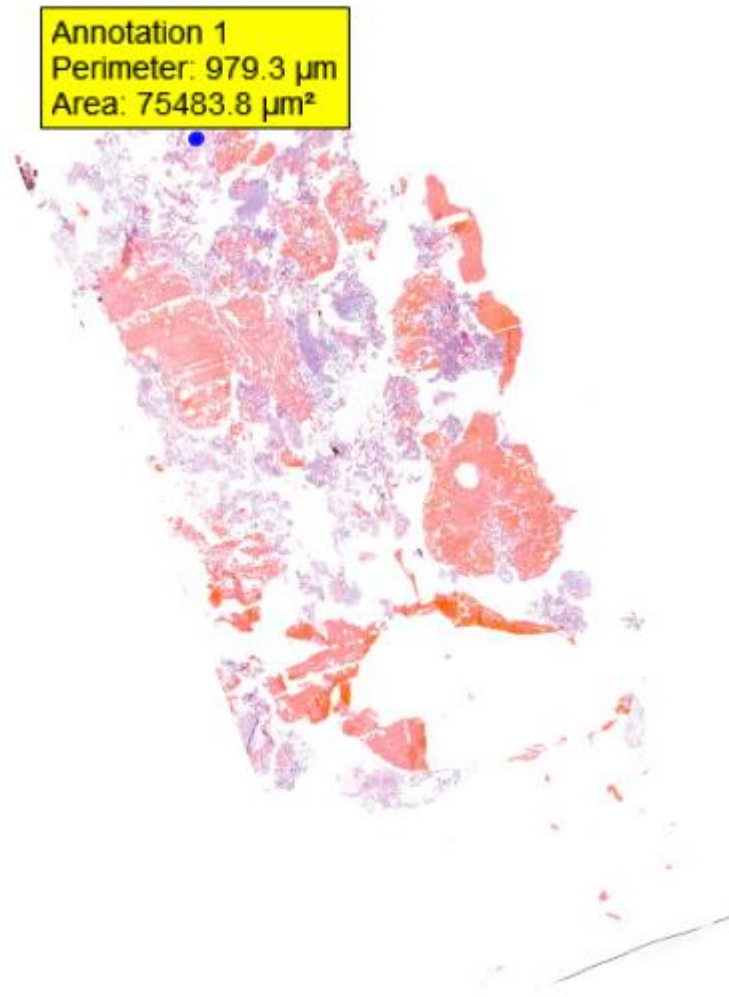
KRAS: PRESENCIA de una mutación en el exón 2 del gen KRAS(p.G12C)

Metodología:

PCR a tiempo real usando el test “Idylla KRAS Mutation Test” (Biocartis) que identifica 21 mutaciones en los exones 2, 3 y 4 del gen KRAS.

Patòleg	SANSANO VALERO, IRENE
Biòleg	SESE FAUSTINO, MARTA
Biòleg	HERNANDEZ LOSA, JAVIER

Caso 02 NHC: 14524938; VH22P24435 Hospital Vall d'Hebron



Molecular VH22P024435A001001- NGSPULMÒ DNA/RN Surt al informe

Normal μm α β λ κ

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Origen de la mostra: ÒS
Tipus de mostra: FFPE
Indicació: Càncer de pulmó
Referència DNA: VH22P024435A001, Mostra Valorable
Referència RNA: VH22P024435A002, Mostra Valorable
Contingut tumoral: 38%

RESULTATS

- Variants ACCIONABLES**
No s'han detectat alteracions accionables
- ALTRES alteracions patogèniques**
KIF5B(15) - RET(12)
Nombre de lectures: 1.103
Locus: chr10:32317356 - chr10:43612032
- Variants DE SIGNIFICAT INCERT**
No s'han detectat alteracions de significat incert

En funció del coneixement actual no es pot determinar la rellevància clínica de les variants de significat incert, per tant, no es poden tenir en compte en el maneig clínic del pacient. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

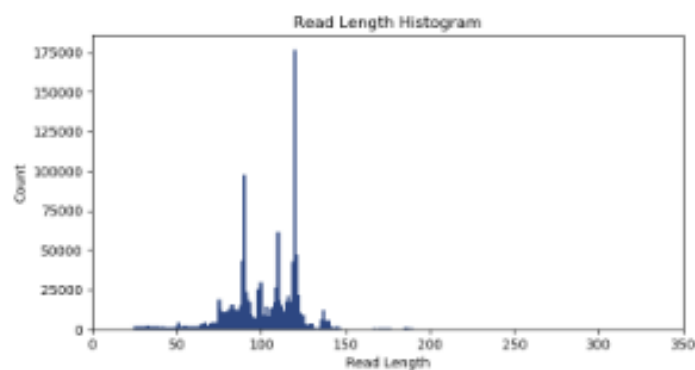
COMENTARIS I RECOMANACIONS

Els resultats obtinguts es presentaran en el comitè molecular de tumors

Sample QC - DNA



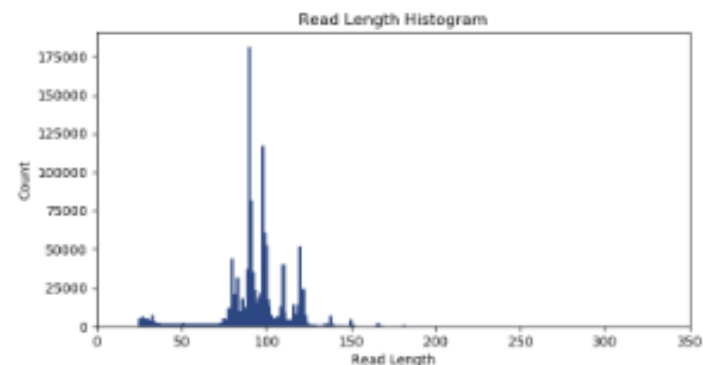
MAPD	✓	0,19
[0-0.5]		
Mapped Reads	✓	1.248.245
>=350000		
Mean AQ20 Read Length (bp)	—	93
Not Set		
Mean Read Length (bp)	✓	101
>=80		
Uniformity Of Base Coverage	✓	99,4
>=94		



Sample QC - RNA



Mapped Reads	✓	536.939
>=0		
Mean Read Length (bp)	✓	93
>=0		
RNA Expression Ctrls Detected	✓	7
>=5		



Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
22P024435A1D_22P024435A2R	03 NOV 2022	Female	FFPE	Solid Tumor	Non-Small Cell Lung Cancer	Stage IV	30

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity of A
100.00%	100.00%	4673	99.40%	93.27%	99.60%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

OncoPrint Extended (5.16) filter chain and Non-Small Ce

RET
KIF5B-RET.K15R12.COSF1232.1

Fusion

RET
RET

Fusion

ALK

BRAF

All

SNVs/Indels

Fusions

CNVs

☐ Gene List Only

OncoPrint Extended (5.16) Filter Chain Applied
2 of 1,007 Fusions

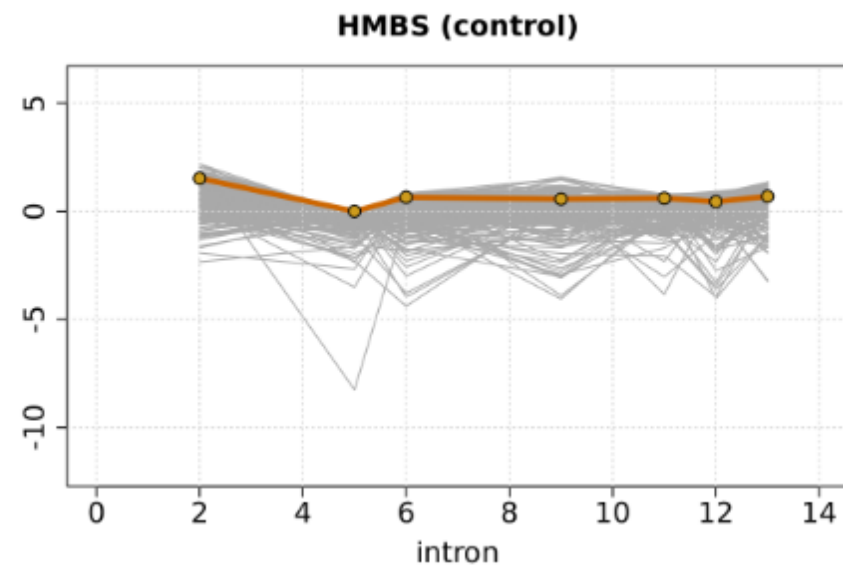
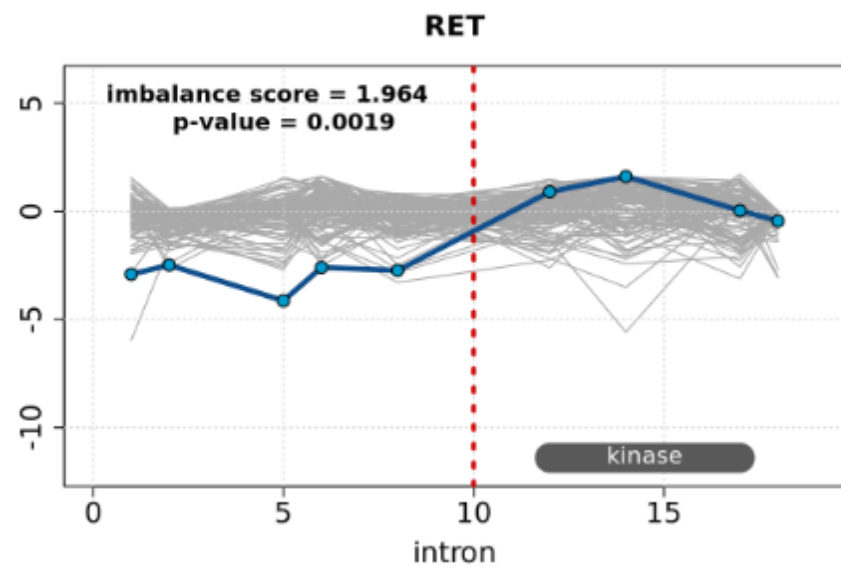
Edit Filters

Export

Columns

Visualization

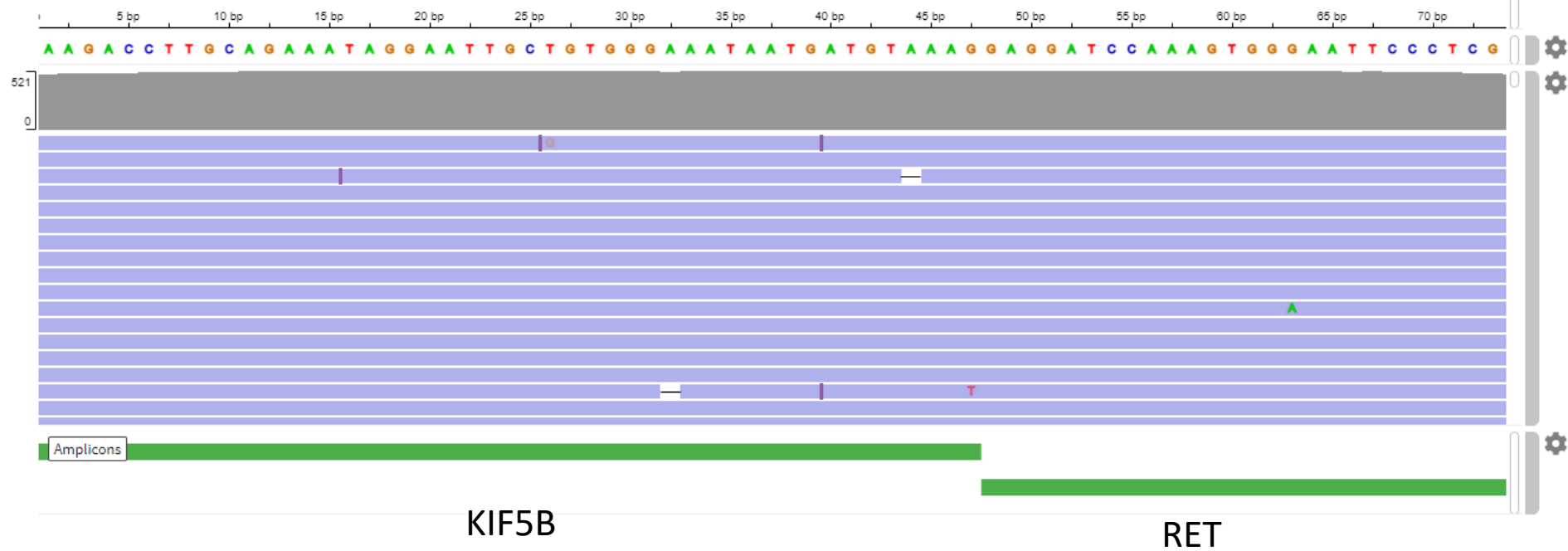
User Classification	Variant ID	Key Variant	Locus	OncoPrint Variant Class	OncoPrint Gene Class	Genes (Exons)
Classification	KIF5B-RET.K15R12.COSF1232.1	Yes	chr10:32317356 - chr10:43612032	Fusion	Gain-of-Function	KIF5B(15) - RET(12)
Classification	RET	Yes	chr10:43607628	ExpressionImbalance	Gain-of-Function	RET



Gene Browser 74 bp

[View in IGV](#)

Cursor Guide Center Line Track Labels ☐ ☒



muestra ID	VH22P024435A002002		
Tipo de muestra	Tejido FFPE		
ID de Cartucho	57150076		
Tipo Test	GeneFusion_RUO/1.0	Versión de TTP	1.6
ID del lote	00005715	Fecha expir.	12 nov. 2022

N.º de serie del Instrumento	SER1430
Versión software Instrumento	27.0
N.º serie de la Consola	C00001018
Versión de software de la Consola	4.4.0.630
Solicitud de Test completada	04 nov. 2022 (12:43)
Test iniciado	04 nov. 2022 (12:43)
Prueba finalizada	04 nov. 2022 (15:40)
Estado Test	Resultado publicado: Automático, 04 nov. 2022 (15:40)
Operador	Rosa Somoza

Resultado del Test Solo para uso en investigación. No para uso en procedimientos de diagnóstico.

Idylla™ GeneFusion Assay

OVERALL GENEFUSION RESULT	DETECTED
ALK FUSION	
ALK Specific Fusion	Not Detected
ALK Expression Imbalance	Not Detected
Cq of the 5' ALK	36.6
ΔCq ALK Expression Imbalance (3' - 5')	-4.2
ROS1 FUSION	
ROS1 Specific Fusion	Not Detected
ROS1 Expression Imbalance	Not Detected
Cq of the 5' ROS1	31.7
ΔCq ROS1 Expression Imbalance (3' - 5')	0.7
RET FUSION	
RET Specific Fusion	Detected
Cq RET Specific Fusion	28.9
RET Expression Imbalance	Not Detected
Cq of the 5' RET	30.7
ΔCq RET Expression Imbalance (3' - 5')	-0.7
MET EXON 14 SKIPPING	

Idylla™ GeneFusion Panel*



IVD

Detection of

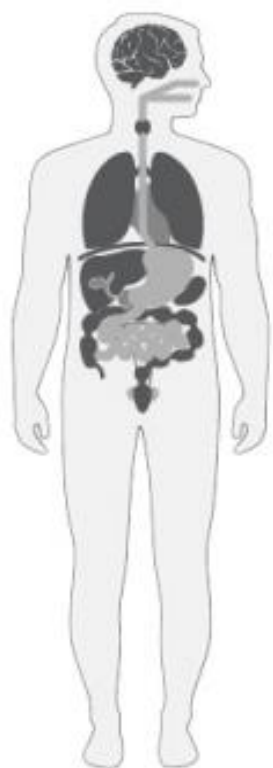
- ALK - ROS1 - RET fusion transcripts
- MET exon 14 skipping transcripts
- Indication of the presence of ALK, RET, ROS1 based on expression imbalance assay

in FFPE sections of tumor tissue from NSCLC patients, incl. resected samples and core needle biopsies



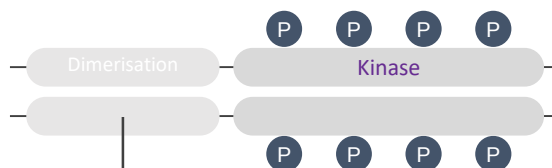
RET alterations: Mutations + Fusions

RET fusions are found in many tumour types



Non-small cell lung cancer (NSCLC) (2%)
Papillary and other thyroid cancers (10%-20%)

Pancreatic cancer (<1%)
 Salivary gland cancer (<1%)
 Spitz tumours (<1%)
 Colorectal cancer (<1%)
 Ovarian cancer (<1%)
 Myeloproliferative disorders (<1%)
 Many others (<1%)



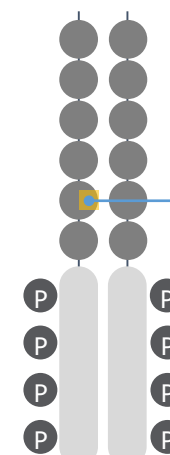
KIF5B (most common in lung cancer)
CCDC6* or *NCOA4 (most common in thyroid cancer)

RET-activating mutations are found in medullary thyroid cancer (MTC)



MTC
Sporadic (>60%)
Hereditary (>90%)

Activation by ligand-independent dimerisation



Covalent disulphide bonds in cysteine-rich region

Kinase domain mutation

Direct kinase activation



Most common mutation: *RET* M918T

- ♦ RET=rearranged during transfection.
- ♦ Drilon A, et al. Oral presentation. ASCO 2018. Abstract 102.

ESMO Recommendations on the standard methods to detect RET alterations



REVIEW

ESMO recommendations on the standard methods to detect *RET* fusions and mutations in daily practice and clinical research

C. Belli^{1†}, F. Penault-Llorca^{2†}, M. Ladanyi³, N. Normanno⁴, J.-Y. Scoazec^{5,6}, L. Lacroix^{7,8,9}, J. S. Reis-Filho¹⁰, V. Subbiah¹¹, J. F. Gainor¹², V. Endris¹³, M. Repetto^{1,14}, A. Drilon^{15,16†}, A. Scarpa¹⁷, F. André¹⁸, J.-Y. Douillard¹⁹ & G. Curigliano^{1,14*}

Table 1. Summary of main features, strengths and weaknesses of all available techniques to detect *RET* rearrangements

Method	Sensitivity	Specificity	Detection of partner	Detection of expression	Screening
IHC	Moderate ^a	Moderate ^b	No	Yes	No
FISH	High	High	No/Yes ^c	No	Rare circumstances
RT-PCR	Moderate/high ^d	High	Yes/No ^e	Yes	Rare circumstances
DNA-seq NGS	Moderate ^f	High/moderate ^g	Yes	No	Yes
RNA-seq NGS	High	High	Yes	Yes ^h	Yes

DNA-seq NGS, DNA sequencing by next-generation sequencing; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; RNA-seq NGS, RNA sequencing by next-generation sequencing; RT-PCR, reverse transcription polymerase chain reaction.

^a False positive up to 40%.

^b False negative up to 40%.

^c In case of the use of specific fusion partner probe.

^d In settings with many possible fusion partners, risk of lower sensitivity.

^e Does not allow the detection of novel partners.

^f False positive: detected rearrangements by DNA-based assays may not result in fusions, so correlation with RNA-based confirmation of predicted fusion transcript is needed.

^g False negative: some introns involved in rearrangements may be inadequately covered for technical reasons.

^h Indication on the in-frame nature of the fusion (functionality).

→ ESMO recommends NGS (preferably RNA-based) for RET testing

- FISH, RT-PCR or liquid biopsy could be also used, but in case of negative results NGS should be considered.*
- MTC patients should be tested for germline RET mutations, as well as for somatic mutations in metastatic tumors (using tissue from metastatic site)*

Comité Molecular Inter-hospitalario

Viernes 03 Marzo 2023

Agenda

Caso 01 NHC 16051658 VH23P003557 HUAV



ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Origen de la mostra: gangli limfàtic

Tipus de mostra: PAAF

Indicació: Càncer de pulmó

Referència DNA: VH23P003557A001, Mostra Valorable

Referència RNA: VH23P003557A002, Mostra Valorable

Contingut tumoral: 60%

RESULTATS

1. Variants ACCIONABLES

EGFR NM_005228.5: c.2292_2293insCATCAC, p.Tyr764_Val765insHisHis VAF 42,4%

2. ALTRES alteracions patogèniques

CTNNB1 NM_001904.4: c.133T>G, p.Ser45Ala VAF 53,8%

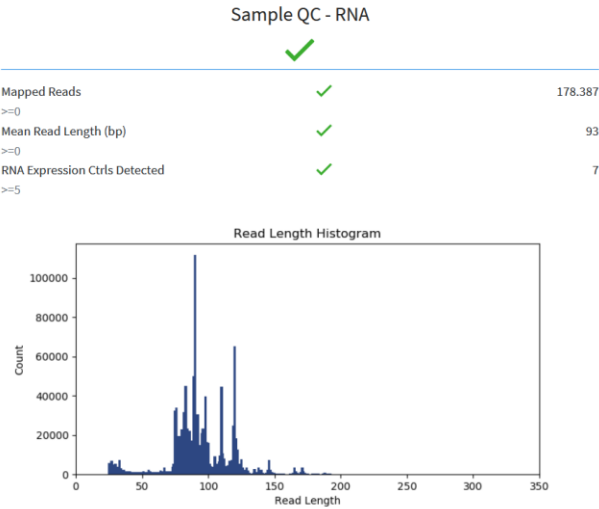
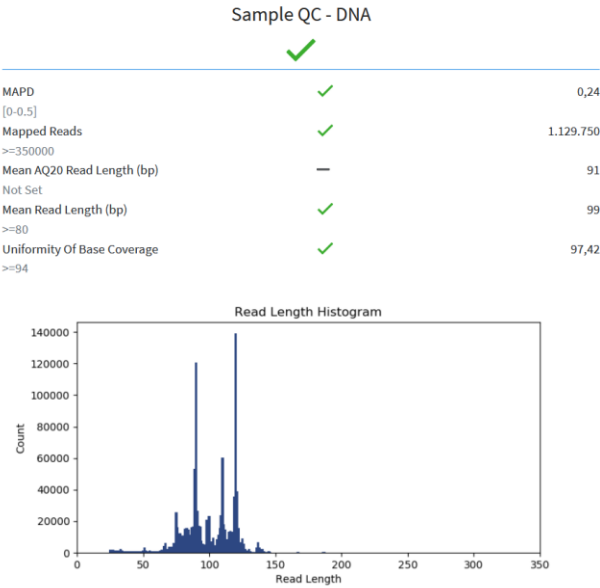
EGFR. Amplificació del nombre de còpies (CNV). Nombre de còpies: 6,93

CDKN2A. Reducció del nombre de còpies (CNV). Nombre de còpies: 0

3. Variants DE SIGNIFICAT INCERT

No s'han detectat alteracions de significat incert

Caso 01 NHC 16051658 VH23P003557 HUAV



VH23P003557A001001D_VH23P003557A003001R

QC ✓

Key Findings

Variants

Plugins

Reports

Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
VH23P003557A001001D_VH23P003557A003001R	15 FEB 2023	Female	FFPE	Solid Tumor	Non-Small Cell Lung Cancer	Stage IV	45

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x 100.00%	Target base coverage at 350x 100.00%	Average Base Coverage Depth 4139	Uniformity Of Base Coverage 97.42%	Percent Reads on Target 95.18%	Uniformity of Amplicon Coverage 98.00%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

EGFR
EGFRvIII.E1E8.Del1

Fusion

EGFR
EGFR

CNV

MET
MET

CNV

EGFR
AA Change: p.Y764_V765insHH
Allele Frequency: 0.424

SNV/Indel

ALK

NTRK3

RET

ROS1

Oncomine Variants (5.16) Filter Chain Applied 5 of 3,178 Variants Edit Filters													
All	SNVs/Indels	Fusions	CNVs	☐ Gene List Only									
User Classification	Variant ID	Key Variant	Type	Oncomine Gene Class	Oncomine Variant Class	Gene	Locus	AA Change	Allele Frequency	Allele Frequency (%)	Ref		
Classification	COSM5685	Yes	snp	Gain-of-Function	Hotspot	CTNNB1	chr3:41266136	p.S45A	0.538	53.8	T		
Classification	EGFRvIII.E1E8.Del1	Yes	RNAExonVariant	Gain-of-Function	RNAExonVariant	EGFR	chr7:55087058 - chr7:55223523						
Classification	EGFR	Yes	CNV	Gain-of-Function	Amplification	EGFR	chr7:55211070						
Classification	MET	Yes	CNV	Gain-of-Function	Amplification	MET	chr7:116371891						
Classification	CDKN2A	Yes	CNV	Loss-of-Function	Deletion	CDKN2A	chr9:21968174						

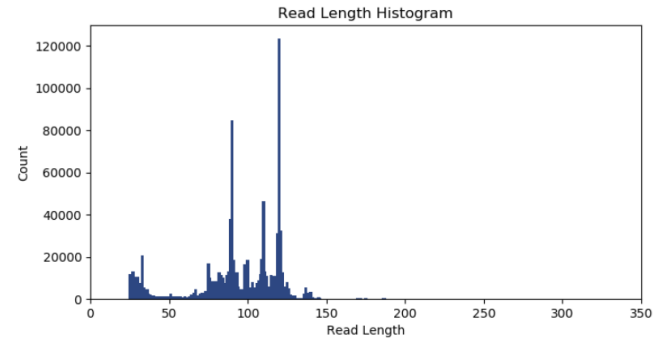
Nueva extraccion de ADN



Sample QC - DNA



MAPD	✓	0,28
[0-0.5]		
Mapped Reads	✓	941.081
>=350000		
Mean AQ20 Read Length (bp)	—	88
Not Set		
Mean Read Length (bp)	✓	93
>=80		
Uniformity Of Base Coverage	✓	98,02
>=94		



VH23P003557A004D_21_4571R QC ✓ Key Findings **Variants** Plugins Reports

Oncomine Variants (5.16) Filter Chain Applied 1 of 2,161 SNVs/Indels Edit Filters											
All	SNVs/Indels	Fusions	CNVs	☐ Gene List Only							
User Classification	Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	AA Change	Allele Frequency	Allele Frequency (%)	Ref	Alt
Classification ▾	COSM5685	Yes	chr3:41266136	Hotspot	Gain-of-Function	CTNNB1	p.S45A	0.524	52.4	T	G

Oncomine Variants (5.16) Filter Chain Applied 3 of 14 CNVs Edit Filters											
All	SNVs/Indels	Fusions	CNVs	☐ Gene List Only							
User Classification	Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	Copy Number	Call	P-Value	CNV Confidence	CNV Rati
Classification ▾	EGFR	Yes	chr7:55211070	Amplification	Gain-of-Function	EGFR	6.13	PRESENT (GAIN)	4.53e-14	5%:4.99, 95%:7.46	1.93
Classification ▾	MET	Yes	chr7:116371891	Amplification	Gain-of-Function	MET	4.93	PRESENT (GAIN)	9.737e-7	5%:3.78, 95%:6.3	1.66
Classification ▾	CDKN2A	Yes	chr9:21968174	Deletion	Loss-of-Function	CDKN2A	0	PRESENT (LOSS)	0	5%:0, 95%:0	0.39

Muestra 02

VH23P003557A004D_21_4571R QC  [Key Findings](#) **[Variants](#)** [Plugins](#) [Reports](#)

All SNVs/Indels Fusions CNVs		☐ Gene List Only Oncomine Variants (5.16) Filter Chain Applied 1 of 2,161 SNVs/Indels Edit Filters											
User Classification	Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	AA Change	Allele Frequency	Allele Frequency (%)	Ref	Alt		
Classification ▼	COSM5685	Yes	chr3:41266136	Hotspot	Gain-of-Function	CTNNB1	p.S45A	0.524	52.4	T	G		

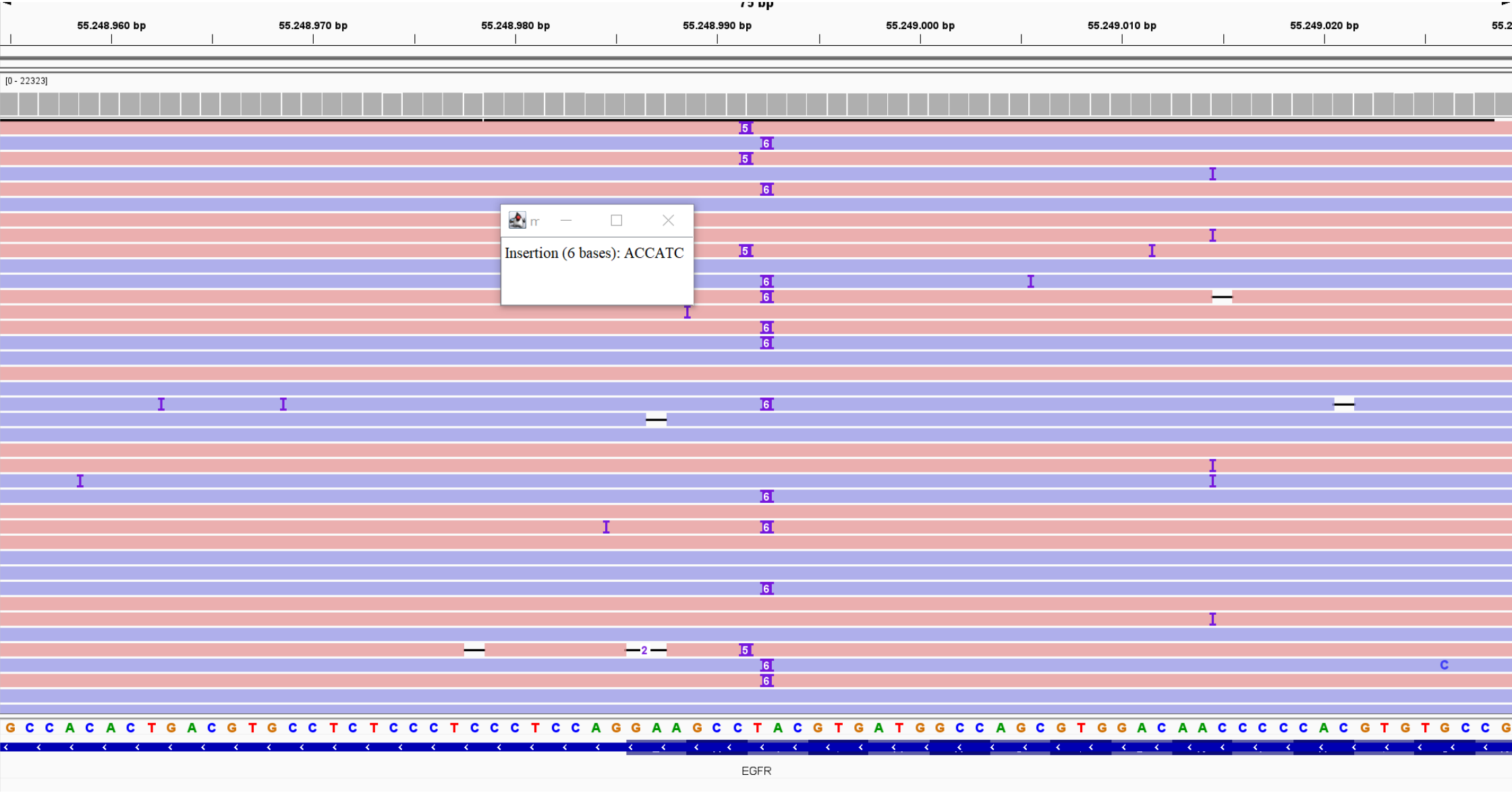
VH23P003557A004D_21_4571R QC  [Key Findings](#) **[Variants](#)** [Plugins](#) [Reports](#)

All SNVs/Indels Fusions CNVs		☐ Gene List Only Oncomine Extended (5.16) Filter Chain Applied 3 of 2,161 SNVs/Indels Edit Filters											
User Classification	Variant ID	Locus	Gene	AA Change	Allele Frequency	Allele Frequency (%)	Ref	Alt	Type	Call	Coverage		
Classification ▼	.	chr3:41266143	CTNNB1	p.S47T	0.524	52.4	G	C	snp	PRESENT (HETEROZYGOUS)	4791		
Classification ▼	COSM5685	chr3:41266136	CTNNB1	p.S45A	0.524	52.4	T	G	snp	PRESENT (HETEROZYGOUS)	4802		
Pathogenic ▼ x	.	chr7:55248992	EGFR	p.Y764_V765insHH	0.422	42.2	T	TACCATC	ins	PRESENT (HETEROZYGOUS)	11052		

Muestra 01

VH23P003557A001001D_VH23P003557A003001R QC  [Key Findings](#) **[Variants](#)** [Plugins](#) [Reports](#)

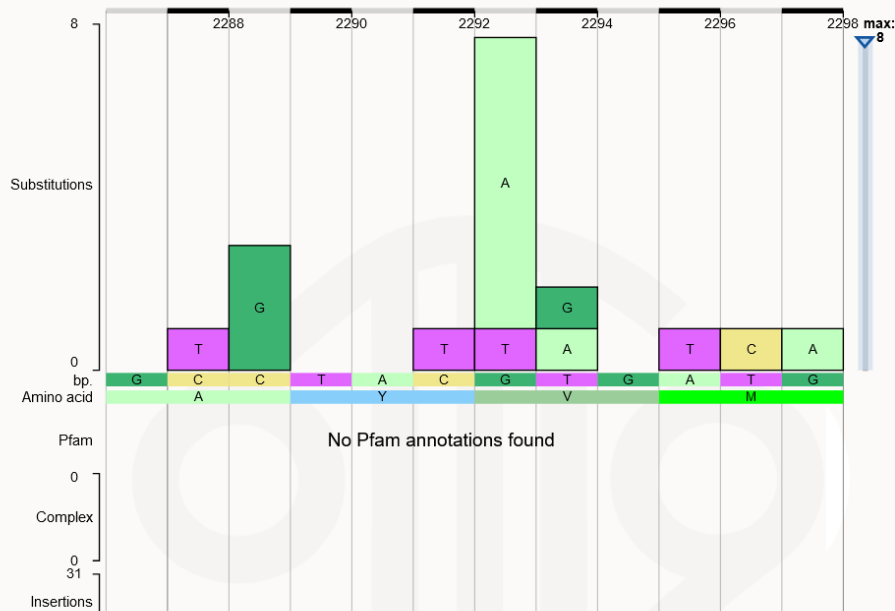
All SNVs/Indels Fusions CNVs		☐ Gene List Only Oncomine Extended (5.16) Filter Chain Applied 3 of 2,157 SNVs/Indels Edit Filters											
User Classification	Variant ID	Locus	Gene	AA Change	Allele Frequency	Allele Frequency (%)	Ref	Alt	Type	Call	Coverage		
Classification ▼	.	chr3:41266143	CTNNB1	p.S47T	0.538	53.8	G	C	snp	PRESENT (HETEROZYGOUS)	6437		
Classification ▼	COSM5685	chr3:41266136	CTNNB1	p.S45A	0.538	53.8	T	G	snp	PRESENT (HETEROZYGOUS)	6451		
Classification ▼	.	chr7:55248992	EGFR	p.Y764_V765insHH	0.424	42.4	T	TACCATC	ins	PRESENT (HETEROZYGOUS)	8054		



Genomic Mutation ID  COSV51766421
Legacy Identifier  COSM6962256
Gene name [EGFR](#)
AA mutation p.Y764_D770dup (Insertion - In frame)
CDS mutation c.2290_2310dup (Insertion)
Nucleotides inserted TACGTGATGGCCAGCGTGGAC
Genomic coordinates GRCh38, [7:55181319..55181320](#), [view](#)
CDD [n/a](#)
HomoloGene [n/a](#)
Ever confirmed somatic? Yes
Remark [n/a](#)
Recurrent [n/a](#)
Drug resistance [n/a](#)
Alternative Ids  [133407213{EGFR_ENST00000454757}](#)

Gene view

The gene view histogram is a graphical view of mutations across EGFR. These mutations are displayed in the histogram to highlight the region of interest, or by using the sliders in the filters panel to the left.

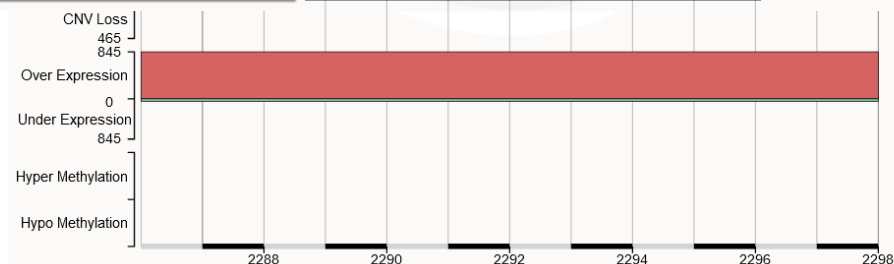


c.2290_2310dup (p.Y764_D770dup)

Position: 2290
Mutation count: 1

c.2296_2309dup (p.D770Efs*61)

Position: 2296
Mutation count: 1



Comité Molecular Inter-hospitalario

Viernes 24 Febrero 2023

Agenda

Caso 01 NHC 17134618 VH23P004331 HUVH

Caso 02 NHC 12249472 VH23P002732 HUVH

Caso 03 NHC 14663495 VH23P002950 Althaia

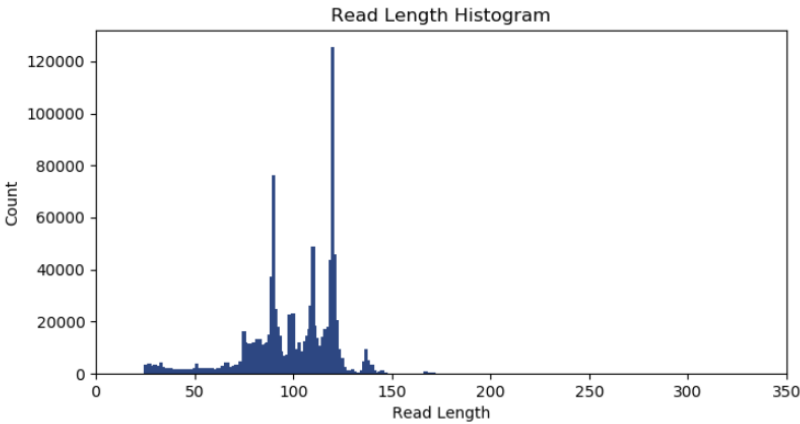
Caso 02 NHC 12249472 VH23P002732 HUVH

DNA:
KRAS NM_033360.4 c.35G>A p.G12D VAF 9,1
RET NM_020975.6 c.2410G>A p.V804M VAF 50,9
RNA: NO VAL
50% Contenido Tumoral

Sample QC - DNA



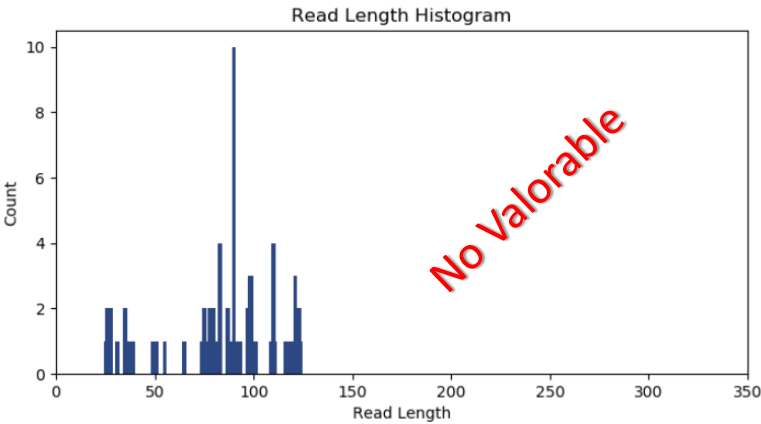
MAPD	✓	0,26
[0-0.5]		
Mapped Reads	✓	1.121.631
>=350000		
Mean AQ20 Read Length (bp)	—	88
Not Set		
Mean Read Length (bp)	✓	98
>=80		
Uniformity Of Base Coverage	✓	98,88
>=94		



Sample QC - RNA



Mapped Reads	✓	9
>=0		
Mean Read Length (bp)	✓	84
>=0		
RNA Expression Ctrls Detected	✗	0
>=5		



VH23P002732A001002D_VH23P002732A003001R

QC

Key Findings

Variants

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All

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Fusions

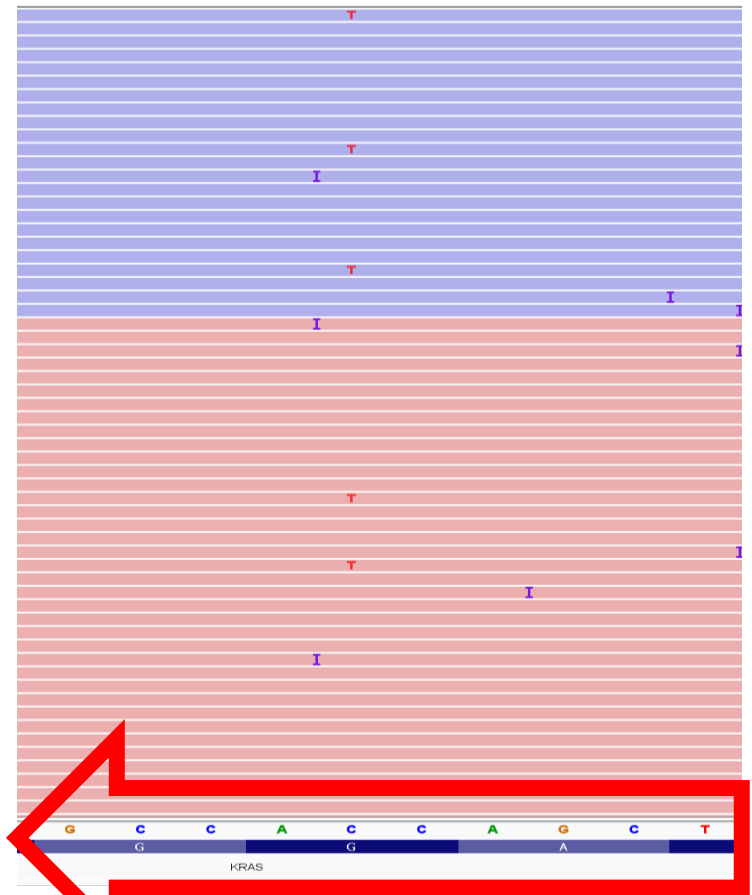
CNVs

☐ Gene List Only

Oncomine Extended (5.16) Filtered 3 of 3

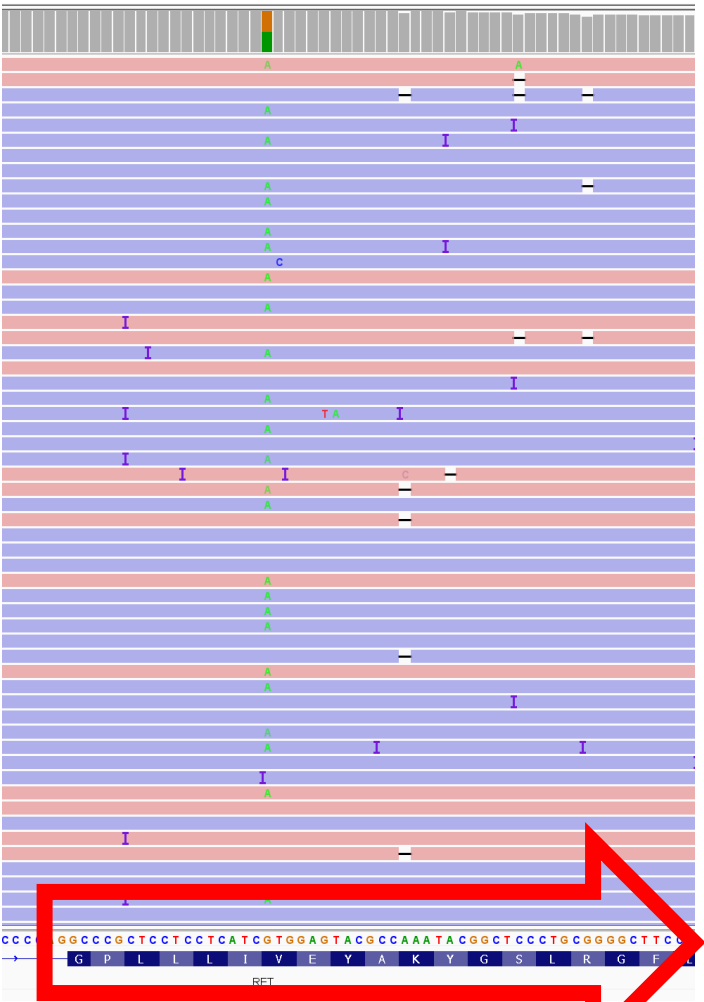
Variant ID	Locus	Gene	AA Change	Allele Frequency (%)	Ref	Alt	Call	Coverage	Nuc Change
.	chr12:25398283	KRAS	p.G12D	9.1	AC	AT	PRESENT (HETEROZYGOUS)	5041	c.35G>A
COSM521	chr12:25398284	KRAS	p.G12D	9	C	T	PRESENT (HETEROZYGOUS)	5047	c.35G>A
OM3195	chr10:43614996	RET	p.V804M	50.9	G	A	PRESENT (HETEROZYGOUS)	1500	c.2410G>A

KRAS G12D



GGT>GAT

RET V804M



GTG>ATG

chr10-43119548-G-A (RET:p.V804M)

Submit to ClinVar

Link publication

Classify

Share

API Link

Favorites

General Information SNV RET(NM_020975.6):c.2410G>A (p.Val804Met)	AMP Classification No data available	Cancer Samples Summary Samples: 1 Sources: 1	cBioPortal Samples: 1 Type: Bowel Cancer, NOS	ACMG Classification Pathogenic 15 points = 15 P - 0 B	Conservation Scores phyloP100: 9.988	Structural Variants
Genes RET	JAX CKB No data available	Cosmic No data available	PharmGKB No data available	ClinVar Pathogenic ☆☆☆ Submissions: 30	Uniprot Variants Pathogenic	Beacon Network
Community Contributions	CIVIC No data available	ICGC Somatic No data available	Region Browser	LOVD No data available	Pathogenicity Scores 5122	Protein Viewer
Publications Variant: 116 Gene: 2162	DoCM No data available	Cancer Hotspots No data available	Expression Data No data available	Frequencies exomes: f = 0.000125 genomes: f = 0.0000986 (cov:33.7)	OMIM No data available	
Transcripts NM_020975.6 - missense MANE Select	PMKB No data available	GDC No data available	GWAS No data available	MitoMap No data available	ClinGen	

Variant

Explain

Chromosome

chr10

UCSC genome browser

Mastermind

TraP Score

Position

43119548

REF Sequence

G

ALT Sequence

A

Variant type

SNV

Cytoband

10q11.21-10q11.21

HGVS

RET(NM_020975.6):c.2410G>A
(p.Val804Met)

RS ID

rs79658334 | dbSNP

Gene symbol

RET

Somatic *RET* fusions and mutations associated with oncogenesis

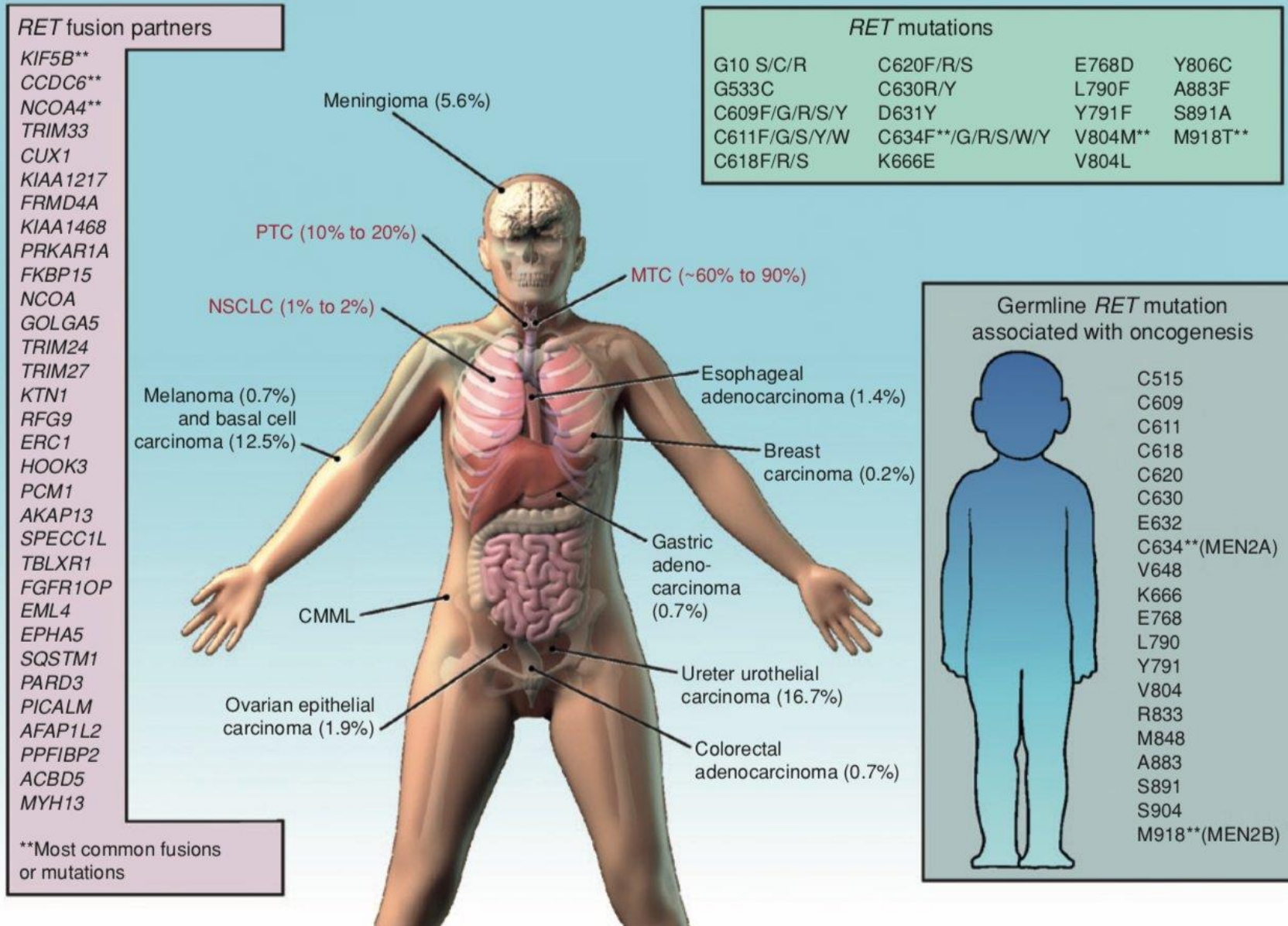
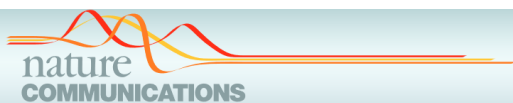


Figure 1. Frequency and distribution of *RET* fusions and *RET* mutations across malignancies. Visual art © 2019 The University of Texas MD Anderson Cancer Center. Red text indicates the most prevalent *RET*-dependent malignancies. CMML, chronic myelomonocytic leukemia.

Letter to the Editor

Emergence of a *RET* V804M Gatekeeper Mutation During Treatment With Vandetanib in *RET*-Rearranged NSCLC

Ibiayi Dagogo-Jack MD, Sara E. Stevens NP, Jessica J. Lin MD, Rebecca Nagy MS, Lorin Ferris BA, Alice T. Shaw MD, PhD, Justin F. Gainor MD

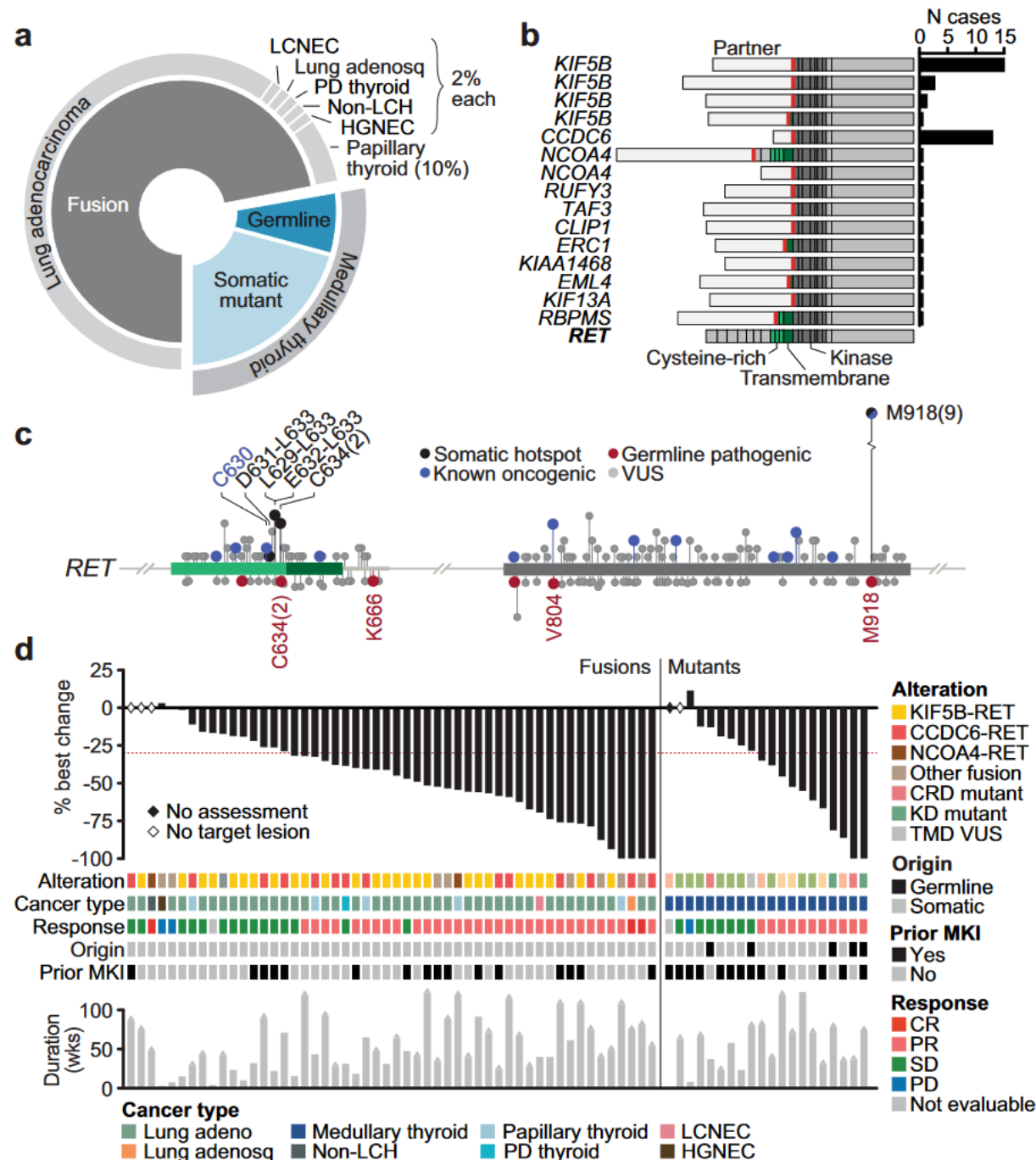
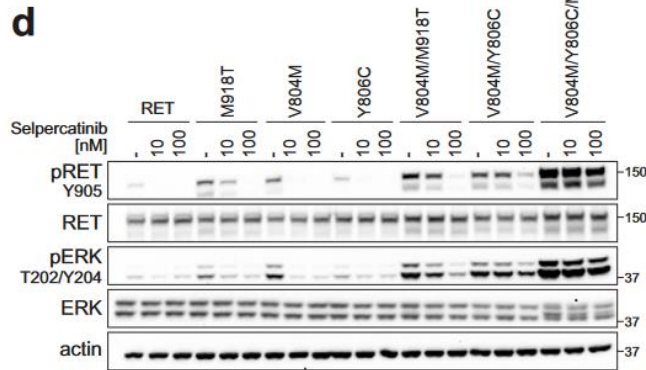
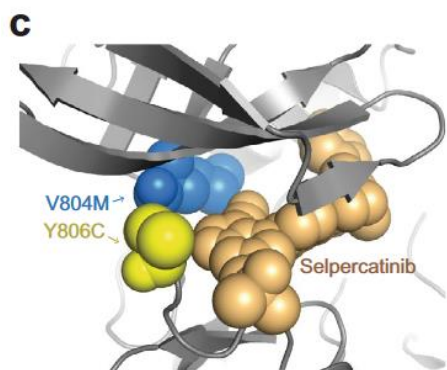


ARTICLE

<https://doi.org/10.1038/s41467-022-28848-x>

OPEN

The evolution of *RET* inhibitor resistance in *RET*-driven lung and thyroid cancers



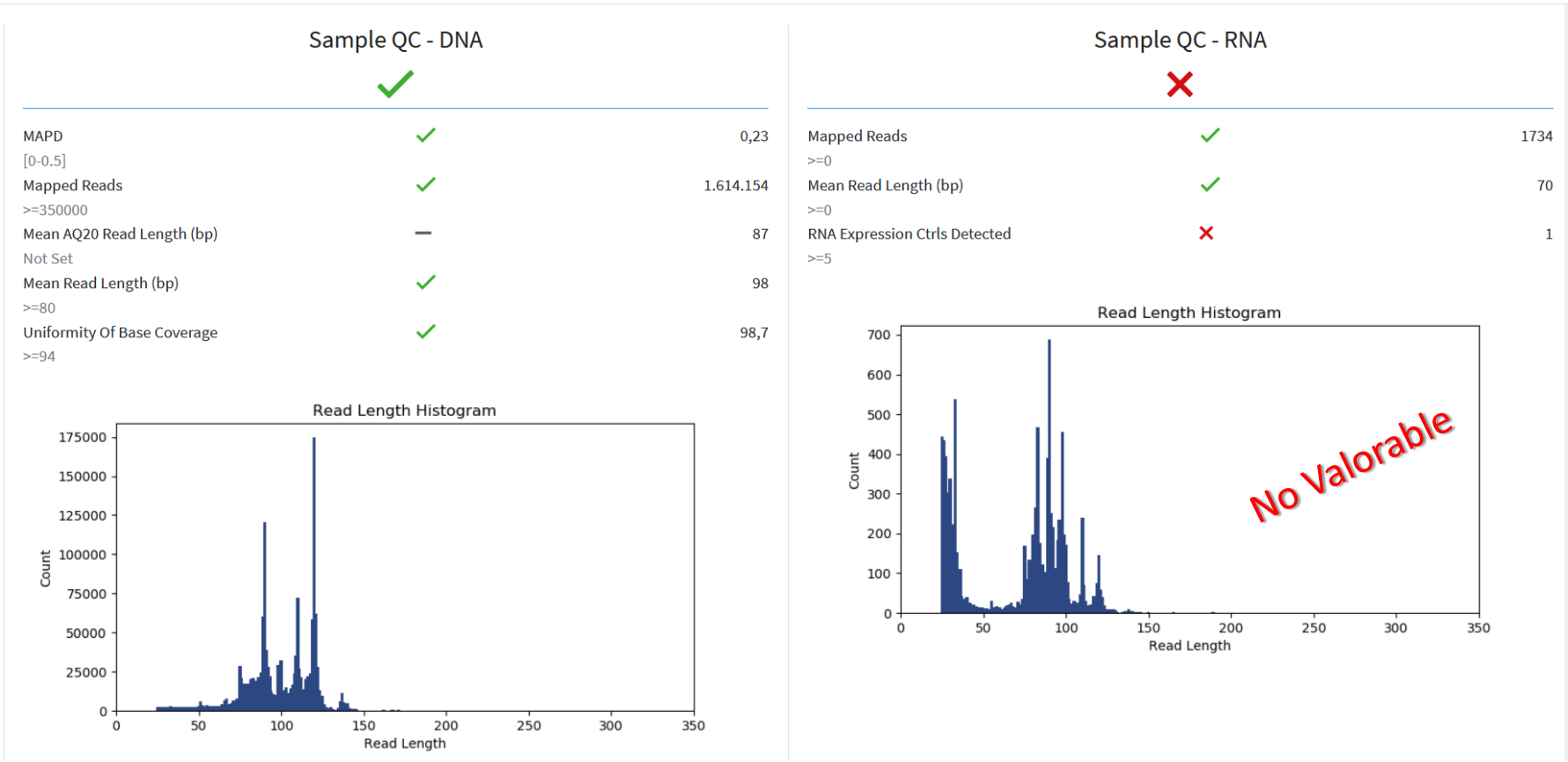
Caso 03 NHC 14663495 VH23P002950 Althaia

Dx: Adenocarcinoma Diferenciación Enteroblástica

DNA:
KRAS NM_033360.4 c.35G>T p.G12V VAF 40,6
TP53 NM_000546.5 c.586C>T p.R196* VAF 85,8
EGFR AMP 5,5
MET AMP 4,7

RNA: NO VAL

20% Contenido Tumoral



VH23P002950A001001D_VH23P002950A003001R

QC

×

Key Findings

Variants

Plugins

Reports

All

SNVs/Indels

Fusions

CNVs

☐ Gene List Only

Onco

Key Variant	Locus	Oncomine Variant Class	Gene	AA Change	Allele Frequency (%)	Ref	Alt	Type	Call	Coverage	Nuc Change
Yes	chr12:25398283	Hotspot	KRAS	p.G12V	40.6	AC	AA	snp	PRESENT (HETEROZYGOUS)	4614	c.35G>T
Yes	chr12:25398284	Hotspot	KRAS	p.G12V	40.6	C	A	snp	PRESENT (HETEROZYGOUS)	4618	c.35G>T
Yes	chr17:7578263	Truncating	TP53	p.R196*	85.8	G	A	snp	PRESENT (HETEROZYGOUS)	4939	c.586C>T

VH23P002950A001001D_VH23P002950A003001R

QC

×

Key Findings

Variants

Plugins

Reports

All

SNVs/Indels

Fusions

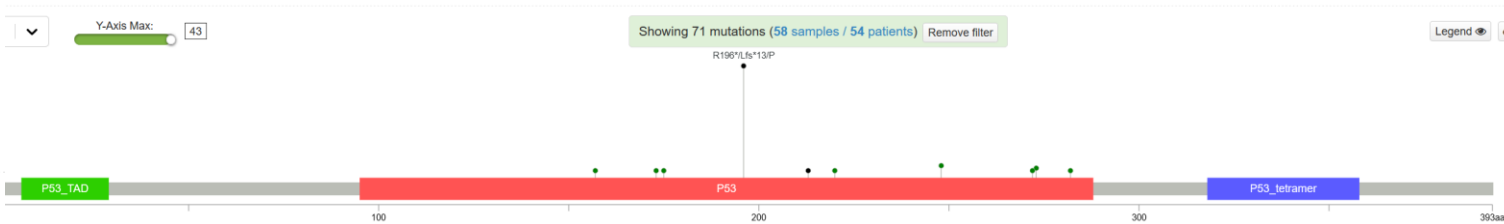
CNVs

☐ Gene List Only

Variant ID	Locus	Oncomine Variant Class	Gene	Copy Number	Call	P-Value	CNV Confidence	CNV Ratio
EGFR	chr7:55211070	Amplification	EGFR	5.5	PRESENT (GAIN)	0.00002524	5%:4, 95%:7.19	1.35
MET	chr7:116371891	Amplification	MET	4.7	PRESENT (GAIN)	0.004762	5%:3.05, 95%:6.6	1.27

No Informado

Overall



TP53 R196* in colon adenocarcinoma

Likely Oncogenic

Likely Loss-of-function

TP53, a tumor suppressor in the DNA damage pathway, is the most frequently mutated gene in cancer.

The TP53 R196* mutation is likely oncogenic.

There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with TP53 R196* mutant colon adenocarcinoma.

Biological Effect

Truncating mutations of TP53 occur throughout the gene and lead to the production of several C-terminally truncated protein forms. These alterations are predicted to be inactivating and are associated with poor prognosis (📄). Experimental studies have revealed that truncating mutations promote cancer cell proliferation, survival and metastasis, since ectopic expression of these mutations in melanoma cells increased cell motility and tumor formation in vivo. This was due in part to aberrant localization of truncated proteins to the mitochondria, regulating genes involved in cell survival, including CypD (📄).

The information above is intended for research purposes only and should not be used as a substitute for professional diagnosis and treatment.

TP53 R196*

hg38

Search

Editions

About

Community

News

Demo

Javier Hernand...

chr17-7674945-G-A (TP53:p.R196*)

Submit to ClinVar

Link publication

Classify

Share

API Link

Favorites

General Information SNV TP53(NM_000546.6):c.586C>T (p.Arg196Ter)	AMP Classification No data available PREMIUM	Cancer Samples Summary Samples: 1203 Sources: 5	cBioPortal Samples: 711 Type: Colorectal Cancer, Breast Can...	ACMG Classification Pathogenic 17 points = 17 P - 0 B	IARC TP53 Germline Samples: 65 Type: Breast Cancer, NOS, Bone Can...	ClinGen No data available New
Genes TP53	JAX CKB No data available	Cosmic No data available PREMIUM	IARC TP53 Somatic Samples: 244 Type: Cancer of Unknown Primary, B...	ClinVar Pathogenic ☆☆☆ Submissions: 17	Conservation Scores phyloP100: 2.23	GWAS No data available
Community Contributions	CIVIC No data available	ICGC Somatic No data available	PharmGKB No data available PREMIUM	LOVD No data available PREMIUM New	Uniprot Variants No data available	Structural Variants New
Publications Variant: 205 Gene: 2129	DoCM No data available	Cancer Hotspots Samples: 131 Type: Colorectal Cancer, Esophagog...	Region Browser New	Frequencies exomes: f = 0.00000398 genomes: not found (cov:32.4)	Pathogenicity Scores 151	Beacon Network
Transcripts NM_000546.6 - nonsense MANE Select	PMKB No data available	GDC Samples: 52 Type: Glioma, Infiltrating Ductal Carc...	Expression Data No data available	MitoMap No data available	OMIM No data available PREMIUM New	Protein Viewer

Variant

Explain

Chromosome

chr17

UCSC genome browser

Mastermind

TraP Score

Position

7674945

REF Sequence

G

ALT Sequence

A

Variant type

SNV

Cytoband

17p13.1-17p13.1

HGVS

TP53(NM_000546.6):c.586C>T (p.Arg196Ter)

RS ID

rs397516435 | dbSNP

Gene symbol

TP53

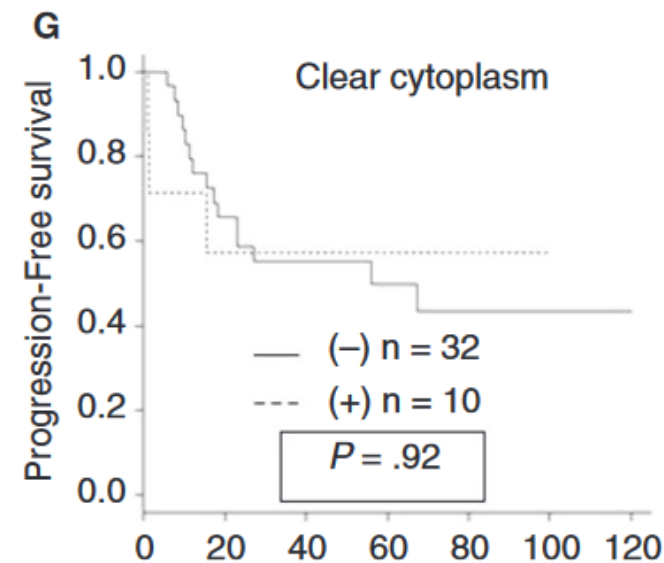
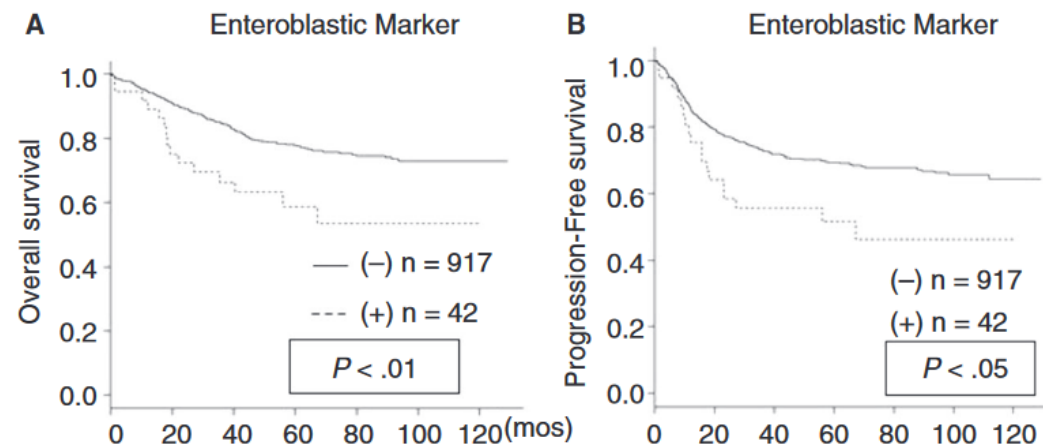
Molecular and clinicopathological features of colorectal adenocarcinoma with enteroblastic differentiation

Yuya Yamashiro,¹ Tsuyoshi Saito,^{1,2}  Takuo Hayashi,¹  Takashi Murakami,³ 
Yuka Yanai,¹ Sho Tsuyama,¹ Yoshiyuki Suehara,^{2,4} Kazuya Takamochi⁵ & Takashi Yao¹

52% casos TP53 mutado

Table 4. Genetic alterations in CAED

Case no.	Clear cytoplasm	TP53 mutation	p53 overexpression	CNV of ERBB2	HER2 ^b	MSI ^c
1 ^a	+	p.Pro152Leu c.455C>T	+	None	+ (IHC 3+)	–
2 ^a	+	p.Val157Phe c.469G>T	+	None	–	–
3 ^a	+	p.Arg273Cys c.817C>T	+	None	–	+
4 ^a	+	p.Arg282Trp c.844C>T	+	None	–	–
	+	p.Ile195Thr c.584T>C				
5 ^a	+	p.Arg196Ter c.586C>T	–	None	–	–
6 ^a	+	p.Arg273Cys c.817C>T	+	×3	–	+
	+	p.Ala161Thr c.481G>A				
7 ^a	+	p.Tyr163Cys c.488A>G	+	×3	–	–
8	+	p.Ser241Phe c.722C>T	+	NA	–	–
9	+	p.Val173Ala c.518T>C	+	NA	–	–
10 ^a	+	None	–	×3	–	–
11 ^a	–	None	+	×3	–	–
12 ^a	–	p.Gly245Val c.734G>T	+	None	–	–



Comité Molecular Inter-hospitalario

Viernes 18 Noviembre 2022

Agenda

Caso 01 NHC: 17756078 VH22P24894 Althaia

Caso 02 NHC: 17906477 VH22P24921 Althaia

1 / Case: VH22P024921A001001 Patient: DOMINIQUE ANDRE VINCENT

Integration HE (HEMATOXILINA-EOSINA)

DOMINIQUE ANDRE VINCENT 10/19/1963

VH22P024921A001001

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

ESTUDI DE BIOMARCADORS EN TUMORS SÒLIDS MITJANÇANT SEQÜENCIACIÓ MASSIVA (NGS)

INFORMACIÓ MOSTRA

Centre sol·licitant: Althaia, Xarxa Assistencial Manresa

Referència origen: 2b12821a1

Origen de la mostra: pulmó

Tipus de mostra: FFPE

Indicació: Càncer de pulmó

Referència DNA: VH22P024921A003, Mostra Valorable

Referència RNA: VH22P024921A002, Mostra NO Valorable

Contingut tumoral: 40%

RESULTATS

1. Variants ACCIONABLES

EGFR NM_005228.5: c.2300_2308dup, p.Ala767_Val769dup VAF 29%

2. ALTRES alteracions patogèniques

CTNNB1 NM_001904.4: c.101G>A, p.Gly34Glu VAF 8,2%

GNAS NM_000516.6: c.602G>A, p.Arg201His VAF 10,4%

KRAS. Amplificació del nombre de còpies (CNV). Nombre de còpies: 21,96

3. Variants DE SIGNIFICAT INCERT

No s'han detectat alteracions de significat incert

En funció del coneixement actual no es pot determinar la rellevància clínica de les variants de significat incert, per tant, no es poden tenir en compte en el maneig clínic del pacient. L'aparició de nova informació científica podria canviar en el futur aquesta interpretació.

COMENTARIS i RECOMANACIONS

S'ha detectat una inserció a l'exó 20 del gen EGFR. Les insercions a l'exó 20 del gen EGFR s'associen a resistència a tractament amb EGFR-TKIs.

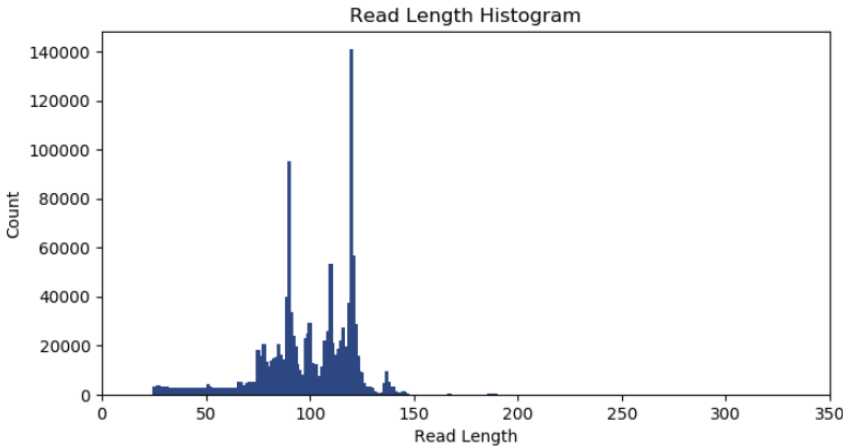
Els resultats obtinguts es presentaran en el comitè molecular de tumors

Caso 02 NHC: 17906477 VH22P24921 Althaia

Sample QC - DNA



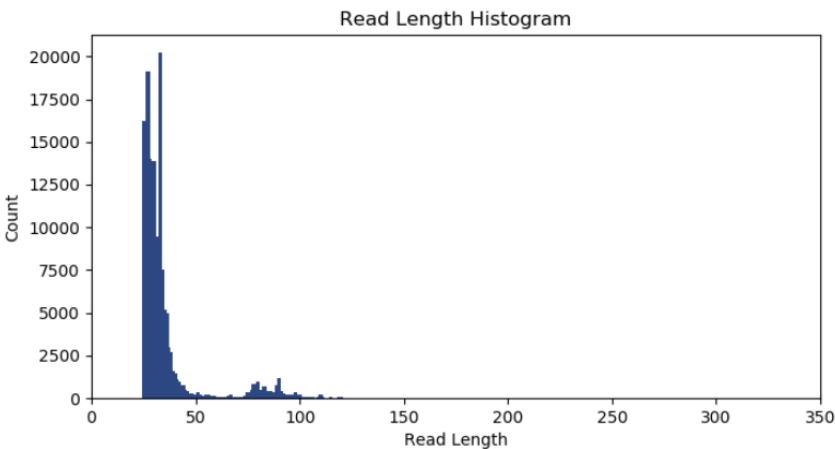
MAPD	✓	0,2
[0-0.5]		
Mapped Reads	✓	1.376.735
>=350000		
Mean AQ20 Read Length (bp)	—	86
Not Set		
Mean Read Length (bp)	✓	98
>=80		
Uniformity Of Base Coverage	✗	92,95
>=94		



Sample QC - RNA



Mapped Reads	✓	1816
>=0		
Mean Read Length (bp)	✓	34
>=0		
RNA Expression Ctrls Detected	✗	2
>=5		



Sample Details

Sample Name	Collection Date	Gender	Sample Type	Application Category	Cancer Type	Cancer Stage	%Cellularity
22P024921A3D_22P024921A2R	08 NOV 2022	Unknown	FFPE	Solid Tumor	Non-Small Cell Lung Cancer	Stage IV	45

Key Metrics

Target Coverage		Amplicon Summary			
Target base coverage at 100x	Target base coverage at 350x	Average Base Coverage Depth	Uniformity Of Base Coverage	Percent Reads on Target	Uniformity of Amplicon Coverage
100.00%	99.39%	4792	92.95%	94.08%	93.60%

Key Variants

Key Variants Detected

Other Variants Detected

None Detected

Not Assayed

EGFR

COSM12376

AA Change: p.A767_V769dup

Allele Frequency: 0.29

SNV/Indel

EGFR

EGFR

CNV

KRAS

KRAS

CNV

ALK

BRAF

RET

ROS1

All

SNVs/Indels

Fusions

CNVs

☐ Gene List Only

Variant ID	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	AA Change	Allele Freque... (%)	Coverage	Nuc Change	Effective Read Depth
COSM12376	chr7:55248998	EGFRExon20Insertion	Gain-of-Function	EGFR	p.A767_V769dup	29	4817	c.2308_2309insCCAGCGTGG	4817
COSM27895	chr20:57484421	Hotspot	Gain-of-Function	GNAS	p.R201H	10.4	4351	c.602G>A	4351
COSM5671	chr3:41266104	Hotspot	Gain-of-Function	CTNNB1	p.G34E	8.2	4750	c.101G>A	4750

Results

/

2022_11_08_OPA_GBM

/

OPA DNA Fus_V4

/

22P024921A3D_22P024921A2R

All

SNVs/Indels

Fusions

CNVs

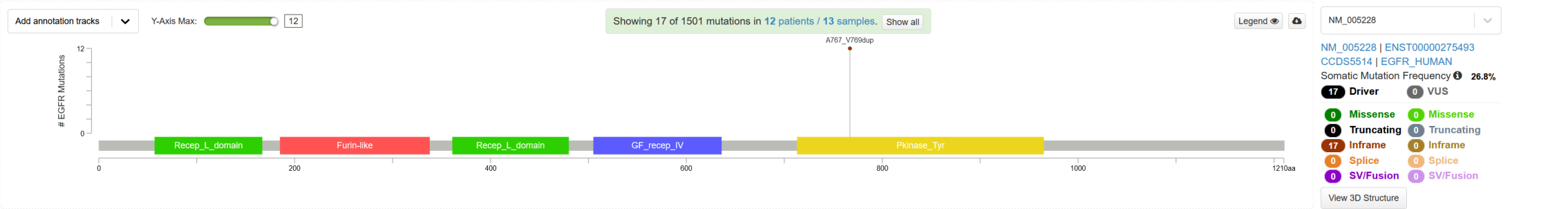
☐ Gene List Only

Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Gene	Copy Number	Call	P-Value	CNV Confidence	CNV Ratio
Yes	chr12:25362832	Amplification	Gain-of-Function	KRAS	21.96	PRESENT (GAIN)	0	5%:18.89, 95%:25.46	5.49
Yes	chr7:55211070	Amplification	Gain-of-Function	EGFR	4.24	PRESENT (GAIN)	3.96e-11	5%:3.6, 95%:4.96	1.5

EGFR

GNAS

CTNNB1



17 Mutations: includes 5 duplicate mutations in patients with multiple samples (page 1 of 1)

Study of Origin	Sample ID	Cancer Type Detailed	Protein Change	Annotation	Mutation Type	Copy #	COSMIC	Allele Freq (T)
Lung Adenocarcinoma (Broad, Cell 2012)	LUAD-RT-S01702	Lung Adenocarcinoma	A767_V769dup		IF ins	Gain		
Lung Adenocarcinoma (TCGA, PanCanc...	TCGA-44-5645-01	Lung Adenocarcinoma	A767_V769dup		IF ins	Gain		0.25
Lung Adenocarcinoma (MSK, NPJ Preci...	P-0028377-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.25
Lung Adenocarcinoma (MSK, NPJ Preci...	P-0029324-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.22
Lung Adenocarcinoma (MSK, NPJ Preci...	P-0031010-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Amp		0.41
Lung Adenocarcinoma (MSK, NPJ Preci...	P-0032644-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.30
Lung Adenocarcinoma (OncoSG, Nat G...	A187	Lung Adenocarcinoma	A767_V769dup		IF ins	Amp		0.50
Non-Small Cell Cancer (MSK, Cancer Di...	P-0002451-T01-IM3	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		
Non-Small Cell Cancer (MSK, Cancer Di...	P-0002451-T02-IM5	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		
Non-Small Cell Cancer (MSK, Cancer Di...	P-0003282-T01-IM5	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		
Non-Small Cell Cancer (MSK, Cancer Di...	P-0004120-T01-IM5	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		
Non-Small Cell Cancer (MSK, Cancer Di...	P-0005286-T01-IM5	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		
Lung Adenocarcinoma (MSK, J Thorac ...	P-0022353-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.05
Lung Adenocarcinoma (MSK, J Thorac ...	P-0028377-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.25
Lung Adenocarcinoma (MSK, J Thorac ...	P-0029324-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.22
Lung Adenocarcinoma (MSK, J Thorac ...	P-0031010-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Amp		0.41
Lung Adenocarcinoma (MSK, J Thorac ...	P-0032644-T01-IM6	Lung Adenocarcinoma	A767_V769dup		IF ins	Diploid		0.30

Showing 1-17 of 17 Mutations: includes 5 duplicate mutations in patients with multiple samples

EGFR A767_V769dup in lung adenocarcinoma

Oncogenic

Gain-of-function

EGFR, a receptor tyrosine kinase, is altered by amplification and/or mutation in lung and brain cancers among others.

The EGFR A767_V769dup alteration is known to be oncogenic.

Non-small cell lung cancers (NSCLC) harboring EGFR exon 20 insertions are unlikely to respond to the EGFR tyrosine kinase inhibitors (TKIs) erlotinib, afatinib and gefitinib. The EGFR-MET bispecific antibody amivantamab and the EGFR TKI mobocertinib are FDA-approved for the treatment of patients with NSCLC harboring EGFR exon 20 insertion mutations such as A767_V769dup.

R1	Exon 20 in-frame insertions (excluding A763_Y764insFQEA)	Erlotinib	Non-Small Cell Lung Cancer	
R1	Exon 20 in-frame insertions (excluding A763_Y764insFQEA)	Gefitinib	Non-Small Cell Lung Cancer	
R1	Exon 20 in-frame insertions (excluding A763_Y764insFQEA)	Afatinib	Non-Small Cell Lung Cancer	
1	Exon 20 in-frame insertions	Amivantamab	Non-Small Cell Lung Cancer	
1	Exon 20 in-frame insertions	Mobocertinib	Non-Small Cell Lung Cancer	
3A	Exon 20 in-frame insertions	Pozotinib	Non-Small Cell Lung Cancer	
3A	Exon 20 in-frame insertions	CLN-081	Non-Small Cell Lung Cancer	

GNAS R201H

This section shows a general overview of the selected mutation. It describes the source of the mutation i.e. gene name, sequence level. You can see more information on our [help pages](#).

Genomic Mutation ID  COSV55670349

Legacy Identifier  COSM27895

Gene name [GNAS](#)

AA mutation p.R844H (Substitution - Missense, position 844, R→H)

CDS mutation c.2531G>A (Substitution, position 2531, G→A)

SNP No

Nucleotides Inserted n/a

Genomic coordinates GRCh38, [20:58909366..58909366](#), view [Ensembl contig](#) 

CDD [NP_536351.1](#) 

HomoloGene n/a

Ever confirmed somatic? Yes

FATHMM prediction Pathogenic (score 0.99)

Remark n/a

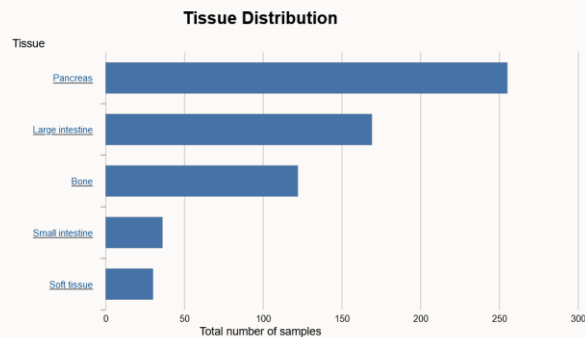
Recurrent n/a

Drug resistance n/a

Alternative Ids  [107633294{GNAS_ENST00000354359}](#), [115707959{GNAS_ENST00000354359}](#), [115807496{GNAS_ENST00000371102}](#), [115615870{GNAS_ENST00000371102}](#)

Tissue distribution

This section displays the distribution of mutated samples and tissue types (top 5). You can see more information on o



varsome COSM27895 hg38 Search Editions About Community News Demo Javier Hernandez

chr20-58909366-G-A (GNAS.p.R201H) [Link a publication](#) [Classify](#) [Copy Shortlink](#) [API Link](#) [Submit to](#)

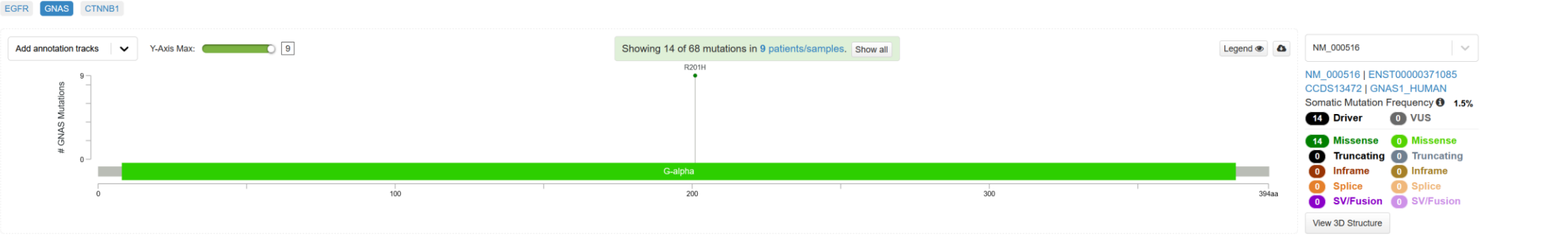
General Information SNV GNAS(ENST00000371085.8);c.602G>A (p.Arg201His)	PharmGKB No data available	PREMIUM ACMG Classification Pathogenic 23 points = 23 P - 0 B	Frequencies exomes: fails QC genomes: f = 0.0000131 (cov:31.0)	Pathogenicity Scores 16 4	Expression Data Top: Pituitary Tissues: 54	Beacon Network
Genes GNAS	Publications Variant: 76 Gene: 302	ClinVar Pathogenic ★★★★☆ Submissions: 6	MitoMap No data available	ClinGen No data available	GWAS No data available	Protein Viewer No data available
Community Contributions	Transcripts ENST00000371085.8 - missense MANE Select	Uniprot Variants Pathogenic	Conservation Scores phyloP100: 9.471	Region Browser	Structural Variants	OMIM No data available

Variant Explains

Chromosome	Position	REF Sequence	ALT Sequence	Variant type	Cytoband	HGVSp	RS ID	Gene symbol
chr20	58909366	G	A	SNV	20q13.32	GNAS(ENST00000371085.8);c.602G>A (p.Arg201His)	rs121913495 dbSNP	GNAS

[UCSC genome browser](#) [Mastermind](#)

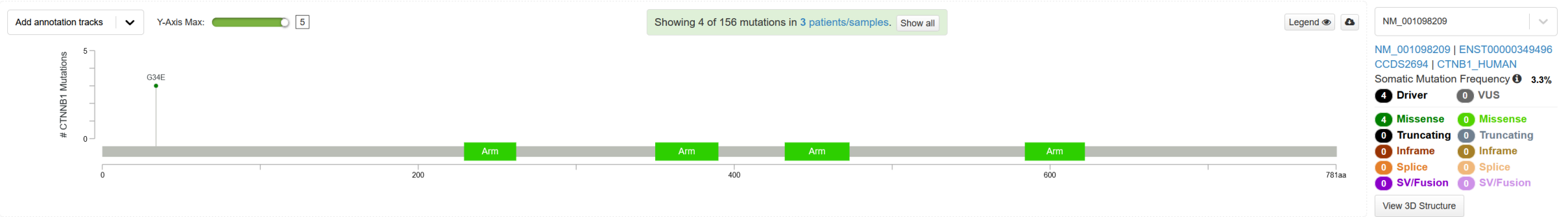
GNAS R201H



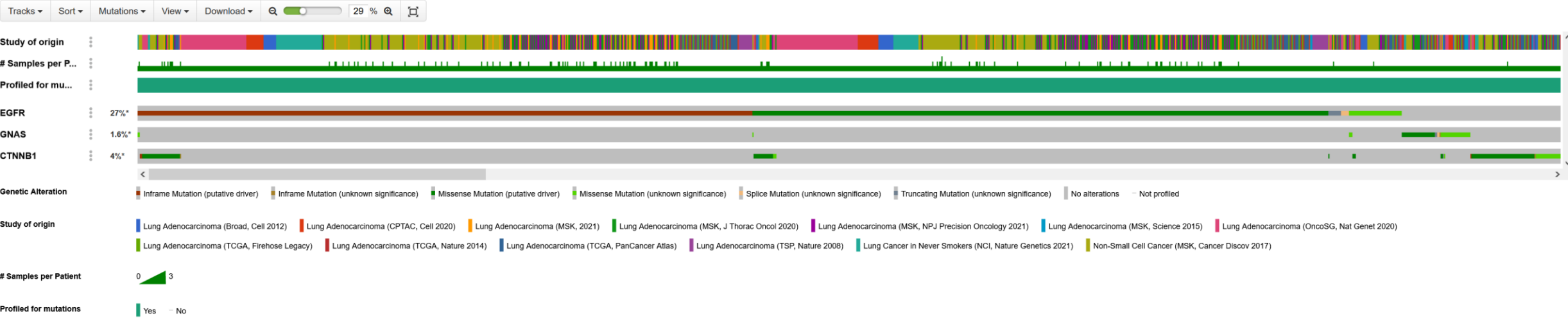
GNAS R201H in lung adenocarcinoma	
Oncogenic	Gain-of-function
GNAS, an intracellular signaling protein, is mutated in various cancers. The GNAS R201H mutation is known to be oncogenic. There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with GNAS R201H mutant lung adenocarcinoma.	
 Biological Effect	
GNAS(R201H) and Kras(G12D) cooperate to promote murine pancreatic tumorigenesis recapitulating human intraductal papillary mucinous neoplasm. Taki K et al. Oncogene. 2016 PMID: 26257060	
Clinical Benefit from Trametinib in a Patient with Appendiceal Adenocarcinoma with a <i>GNAS</i> R201H Mutation. Ang C et al. Case Rep Oncol. NaN PMID: 28868010	
GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Landis CA et al. Nature. 1989 PMID: 2549426	

CTNNB1 G34E

EGFRGNASCTNNB1



CTNNB1 G34E in lung adenocarcinoma	
Likely Oncogenic	Gain-of-function
CTNNB1 (β-catenin), a transcriptional activator, is recurrently mutated in various cancers including endometrial and hepatocellular cancers. The CTNNB1 G34E mutation is likely oncogenic. There are no FDA-approved or NCCN-compendium listed treatments specifically for patients with CTNNB1 G34E mutant lung adenocarcinoma.	
 Biological Effect	
Wnt/Wingless pathway activation and chromosome 6 loss characterize a distinct molecular subgroup of medulloblastomas associated with a favorable prognosis. Clifford SC et al. Cell Cycle. NaN PMID: 17172831	
beta-Catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee. Ellison DW et al. J Clin Oncol. 2005 PMID: 16258095	
Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Miyoshi Y et al. Cancer Res. 1998 PMID: 9635572	



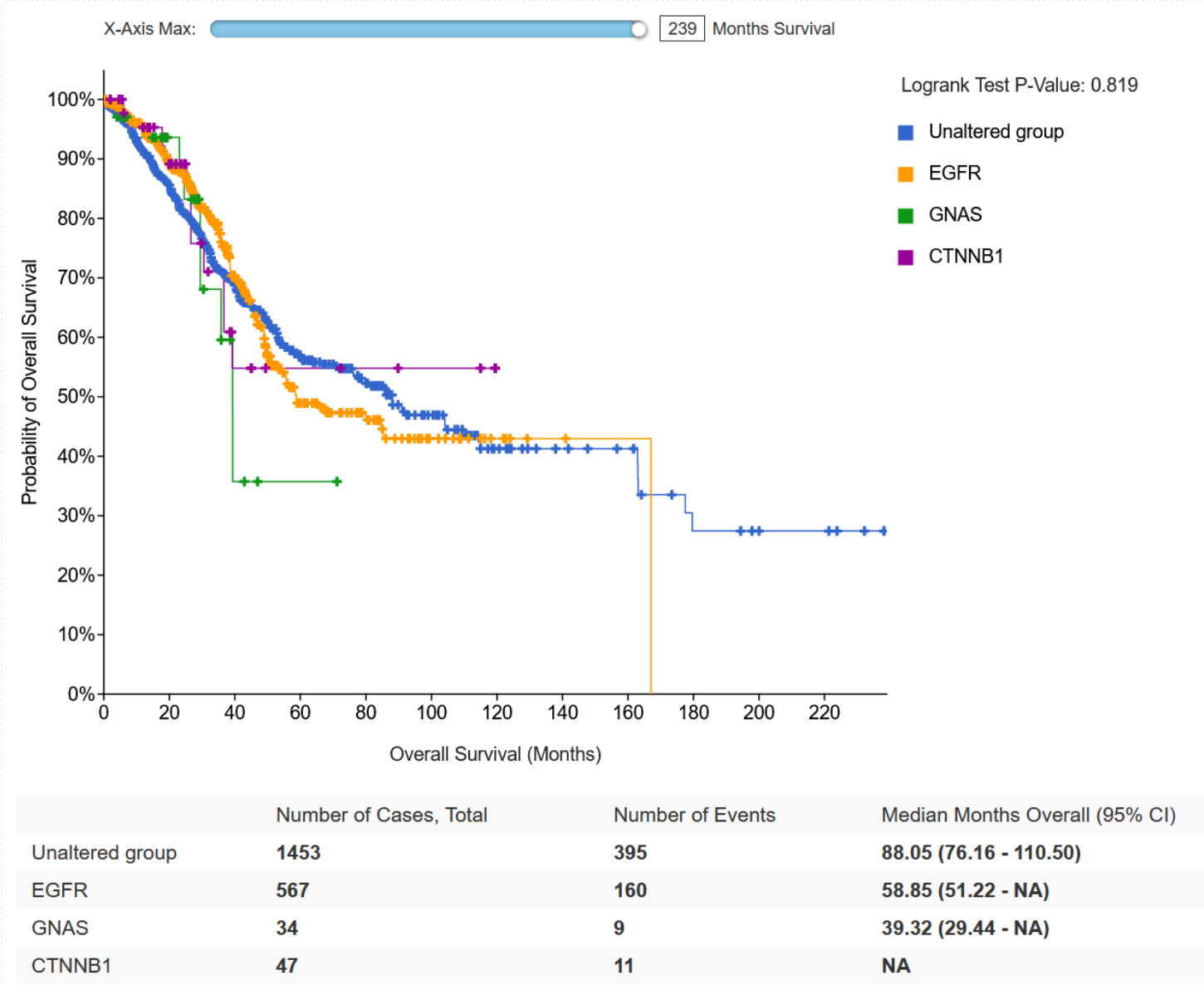
The analysis tested 3 pairs between the 3 tracks in the OncoPrint.

☒ Mutual exclusivity ☒ Co-occurrence ☐ Significant only

A	B	Neither	Both	p-Value	q-Value ^	Tendency
EGFR	CTNNB1	2879	67	<0.001	<0.001	Co-occurrence
EGFR	GNAS	2903	6	<0.001	<0.001	Mutual exclusivity
GNAS	CTNNB1	3969	4	0.214	0.214	Co-occurrence

Showing 1-3 of 3

Overall



Response to trametinib in a nonsmall cell lung cancer patient with osimertinib resistance harboring GNAS R201C and R201H mutations: a case report

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Affiliations + expand

PMID: 35946511 PMCID: PMC9481290 DOI: 10.1097/CAD.0000000000001342

[Free PMC article](#)

Abstract

Osimertinib, an orally administered third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, is widely approved for the first-line and second-line treatment of advanced non-small-cell lung cancer (NSCLC) with EGFR mutations. However, the rapid development of osimertinib resistance renders the unsustainable treatment benefit. Patients with EGFR -mutated NSCLC who develop osimertinib resistance, especially those acquiring relatively rare and 'off-target' resistance mutations, still lack effective therapeutic options for postosimertinib therapy. Herein, we reported a 73-year-old woman diagnosed with T1N3M1 lung adenocarcinoma harboring EGFR L858R mutation, who acquired two GNAS mutations (R201C and R201H) and lost the EGFR L858R mutation after progression on icotinib and osimertinib. The patient was subsequently treated with trametinib and there was no obvious tumor increase. Our study revealed that GNAS R201 can confer the osimertinib resistance in EGFR -positive NSCLC, and present the first report of the prevalence of GNAS R201C and R201H mutants in NSCLC which response to trametinib treatment. Our case suggests that trametinib could be a treatment option in NSCLC patients harboring GNAS -activating mutations.

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Audit of Molecular Mechanisms of Primary and Secondary Resistance to Various Generations of Tyrosine Kinase Inhibitors in Known Epidermal Growth Factor Receptor-Mutant Non-small Cell Lung Cancer Patients in a Tertiary Centre

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Affiliations + expand

PMID: 35810049 DOI: 10.1016/j.clon.2022.06.003

Abstract

Aims: Presently, three generations of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are approved against oncogene addicted EGFR-mutant non-small cell lung cancer (NSCLC). Patients with actionable EGFR mutations invariably develop resistance. This resistance can be intrinsic (primary) or acquired (secondary).

Materials and methods: This was a retrospective study carried out between January 2016 and April 2021 analysing 486 samples of NSCLC for primary and secondary resistance to first- (erlotinib, gefitinib), second- (afatinib) and/or third-generation (osimertinib) TKIs in EGFR-mutant NSCLCs by next generation sequencing (NGS). Tissue NGS was carried out using the ThermoFischer Ion Torrent Oncomine™ Focus 52 gene assay; liquid biopsy NGS was carried out using the Oncomine Lung Cell-Free Total Nucleic Acid assay. All cases were previously tested for a single EGFR gene with the Therascreen® EGFR RGQ PCR kit.

GNAS R201H en resistencia a Osimertinib

CTNNB1 en resistencia a Osimertinib

KRAS* and *EGFR* Amplifications Mediate Resistance to Rociletinib and Osimertinib in Acquired Afatinib-Resistant NSCLC Harboring Exon 19 Deletion/T790M in *EGFR

Kaori Nakatani ¹, Toshimitsu Yamaoka ², Motoi Ohba ³, Ken-Ichi Fujita ³, Satoru Arata ^{3 4}, Sojiro Kusumoto ⁵, Iori Taki-Takemoto ¹, Daisuke Kamei ¹, Shinichi Iwai ¹, Junji Tsurutani ³, Tohru Ohmori ^{3 5}

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PMID: 30322949 DOI: [10.1158/1535-7163.MCT-18-0591](#)

Abstract

The critical T790M mutation in *EGFR*, which mediates resistance to first- and second-generation EGFR tyrosine kinase inhibitors (TKI; gefitinib, erlotinib, and afatinib), has facilitated the development of third-generation mutation-selective EGFR TKIs (rociletinib and osimertinib). We previously reported heterogeneous afatinib-resistant mechanisms, including emergence of T790M-*EGFR*, and responses to third-generation EGFR TKIs. Here, we used afatinib-resistant lung adenocarcinoma cells [AfaR (formerly AFR3) cells], carrying exon 19 deletion/T790M in *EGFR* To identify the novel resistance mechanisms in post-afatinib treatment, RocR1/RocR2 and OsiR1/OsiR2 cells were established using increasing concentrations of rociletinib and osimertinib, respectively. Attenuation of exon 19 deletion and T790M was confirmed in both rociletinib-resistant cells; in addition, *EGFR* and *KRAS* amplification was observed in RocR1 and RocR2, respectively. Significant *KRAS* amplification was observed in the osimertinib-resistant cell lines, indicating a linear and reversible increase with increased osimertinib concentrations in OsiR1 and OsiR2 cells. OsiR1 cells maintained osimertinib resistance with *KRAS* amplification after osimertinib withdrawal for 2 months. OsiR2 cells exhibited *KRAS* attenuation, and osimertinib sensitivity was entirely recovered. Phospho-EGFR (Y1068) and growth factor receptor-bound protein 2 (GRB2)/son of sevenless homolog 1 (SOS1) complex was found to mediate osimertinib resistance in OsiR1 cells with sustained *KRAS* activation. After 2 months of osimertinib withdrawal, this complex was dissociated, and the EGFR signal, but not the GRB2/SOS1 signal, was activated. Concomitant inhibition of MAPK kinase and EGFR could overcome osimertinib resistance. Thus, we identified a heterogeneous acquired resistance mechanism for third-generation EGFR TKIs, providing insights into the development of novel treatment strategies.

***KRAS* amplificado en resistencia a Osimertinib**