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The role of comprehensive genomic profiling in solid tumour biomarker testing

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The Role of Genomics in Personalized Medicine

- Genomics is moving to the forefront of medicine because new technologies are transforming health care:
  - preventive care,
  - determining treatment options,
  - reproductive health counseling, as well as in screening for infertility and newborn genetic disorders.
  - treating global outbreaks like COVID-19,
  - … and the development of advanced therapeutics for diseases such as cancer.

Access, the main problem with genomic medicine

- **Understanding**: i.e a broad lack of molecular-clinical understanding
- **Cost**: i.e insurance barriers.

The Role of CIMA LAB Diagnostics

- CIMA LAB Diagnostics is the genetic and phenotypic diagnostic laboratory of the Clínica Universidad de Navarra (CUN).

https://www.unav.edu/web/cimalab

The Role of CIMA LAB Diagnostics

- Improve the quality of medical care, by making the newest biomarkers available to medical professionals to support diagnosis.
  - Quality Control & Assurance (QC & QA)
  - Turnaround time (TAT)
  - Newest technology.

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MEDICAL LABORATORY

MiSeq, Illumina

Ion S5, Life Technologies

NextSeq, Illumina

Ion Chef™

SeqStudio Genetic Analyzer

QuantStudio Absolute Q digital PCR

- Collaborate in research projects and clinical trials.

For Research use only. Not for use in diagnostics prodecures

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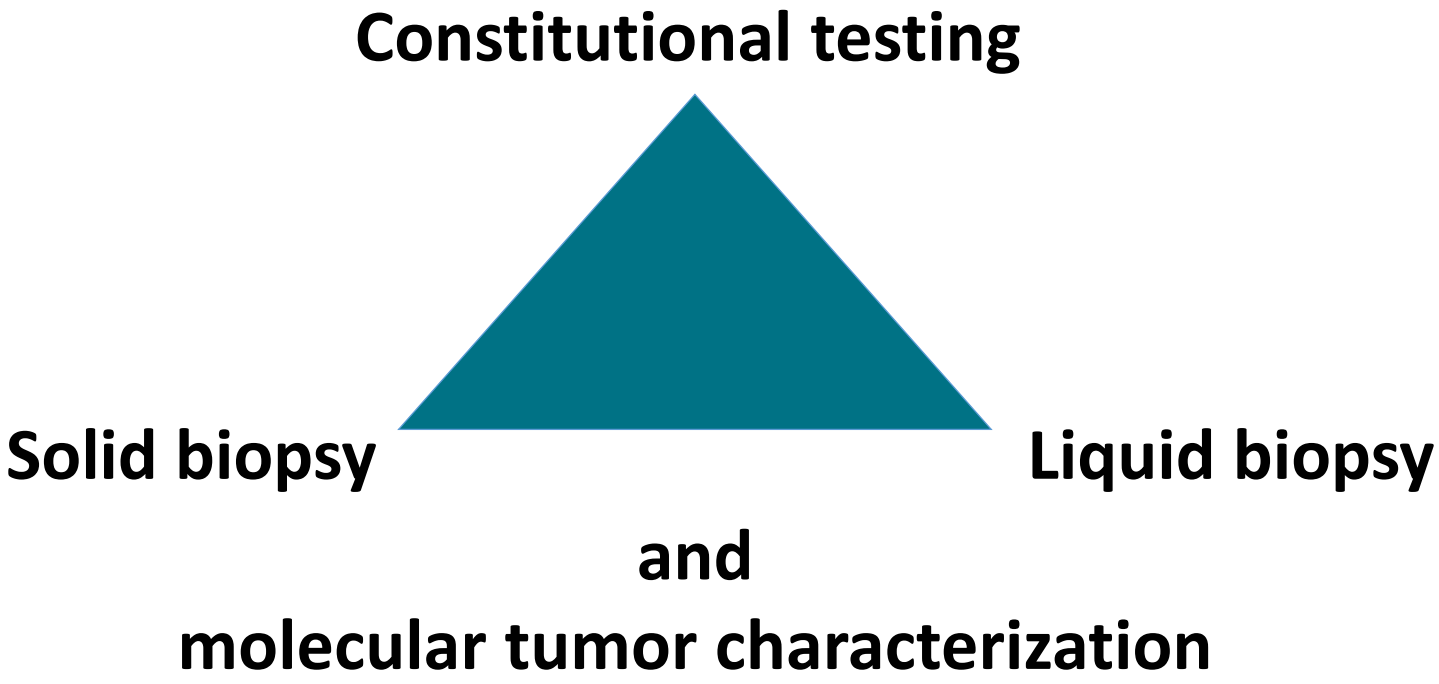
Three-angle strategy to advance in cancer management



Three-angle strategy to advance in cancer management

1.- Constitutional testing

- NGS-gene panel testing
  - ISO15189 accredited in house 91 PanCancer gene panel
    - Internal and external control samples including commercial samples (i.e., Coriell and Seracare) and proficiency testing programs (i.e., EMQN).

- Whole-exome or whole-genome sequencing

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LABORATORIO DE DIAGNÓSTICO GENÉTICO Y FENOTÍPICO INTEGRAL
COMPREHENSIVE GENETIC AND PHENOTYPIC DIAGNOSTIC LABORATORY

LAB. 1.05 - LABORATORIO DE TUMORES SÓLIDOS Y ENFERMEDADES HEREDITARIAS

SÍNDROMES DE SUSCEPTIBILIDAD HEREDITARIA A CÁNCER (91 GENES)

Este test es una prueba de secuenciación dirigida a **91 genes de predisposición hereditaria a distintos tipos de cáncer,** que facilita el correcto diagnóstico y manejo de pacientes cuyos antecedentes personales y familiares sugieren la presencia de un componente hereditario, permitiendo establecer medidas de seguimiento y reducción de riesgo en las personas portadoras.

Características del test

El diseño de este panel permite la detección de mutaciones puntuales y variaciones en el número de copias (CVNs) en la secuencia codificante completa de genes relacionados con diferentes tipos de cáncer hereditario: colorrectal, gástrico, pancreático, renal y de tracto urinario, de tiroides, de mama y/u ovario, melanoma, o paraganglioma/feocromocitoma.

El panel incluye el análisis de los siguientes genes:

APC, AKT1, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDH1, CDK4, CDKN1B, CDKN2A, CEPS2, CHEK2, CTNNA1, DICER1, DIS3L2, EGLN1, ENG, EPCAM, EPHX1, FAM175A, FANI, FANCC, FH, FLCN, GALNT12, GPC3, GREM1, HOXB13, KIF1B, KIT, MAP3K6, MAX, MC1R, MEN1, MET, MITF, MLH1, MLH3, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, NFKB12, NTHL1, PALB2, PALLD, PDGFRA, PIK3CA, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTEN, RAD50, RAD51C, RAD51D, RB1, RET, RINT1, RPS20, SCG5, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEMA4A, SMAD4, SMARCA4, SMARCB1, SPINK1, STK11, TERT, TMEM127, TP53, TSC1, TSC2, VHL, WRN, WT1, XRCC2 y XRCC4.

Tipo de muestra

El estudio se lleva a cabo, previo consentimiento informado del paciente, sobre una muestra de 10 mL de sangre periférica anticoagulada en EDTA.

Para otro tipo de muestra, consultar con el laboratorio.

La muestra será enviada junto con la hoja de petición disponible en: www.cimalabdiagnostics.es

Plazo de respuesta

El informe con la interpretación de los resultados será entregado en el plazo de 25 días hábiles por correo encriptado al facultativo solicitante.

CONTACTO:

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EMQN
The European Molecular Genetics Quality Network

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Three-angle strategy to advance in cancer management

2.- Solid biopsy tumor characterization

- NGS-gene panel testing
  - 161- gene panel

Hotspot genes				Full-length genes			Copy number genes	
AKT1	FOXL2	MET	AKT2	ATM	TP53	MSH6	AKT1	PPARG
ALK	GATA2	MTOR	AKT3	BAP1	TSC1	NBN	AR	TERT
AR	GNA11	MYD88	AXL	BRCA1	TSC2	NOTCH2	CCND1	AKT2
ARAF	GNAQ	NFE2L2	CCND1	BRCA2	ARID1A	NOTCH3	CCNE1	AKT3
BRAF	GNAS	NRAS	CDK6	CDKN2A	ATR	PALB2	CDK4	ALK
BTK	HNF1A	PDGFRA	ERCC2	FBXW7	ATRX	PMS2	CDK6	AXL
CBL	HRAS	PIK3CA	FGFR4	MSH2	CDK12	POLE	EGFR	BRAF
CDK4	IDH1	PPP2R1A	H3F3A	NF1	CDKN1B	RAD50	ERBB2	CCND2
CHEK2	IDH2	PTPN11	HIST1H3B	NF2	CDKN2B	RAD51	FGFR1	CCND3
CSF1R	JAK1	RAC1	MAP2K4	NOTCH1	CHEK1	RAD51B	FGFR2	CDK2
CTNNB1	JAK2	RAF1	MDM4	PIK3R1	CREBBP	RAD51C	FGFR3	CDKN2A
DDR2	JAK3	RET	MYC	PTCH1	FANCA	RAD51D	FGFR4	CDKN2B
EGFR	KDR	RHEB	MYCN	PTEN	FANCD2	RNF43	FLT3	ESR1
ERBB2	KIT	RHOA	NTRK1	RB1	FANCI	SETD2	IGF1R	FGF19
ERB83	KNSTRN	SF3B1	NTRK2	SMARCB1	MLH1	SLX4	KIT	FGF3
ERBB4	KRAS	SMO	PDGFRB	STK11	MRE11A	SMARCA4	KRAS	NTRK1
ESR1	MAGOH	SPOP	PIK3CB				MDM2	NTRK2
EZH2	MAP2K1	SRC	ROS1				MDM4	NTRK3
FGFR1	MAP2K2	STAT3	SMAD4				MET	PDGFRB
FGFR2	MAPK1	U2AF1	TERT				MYC	PIK3CB
FGFR3	MAX	XPO1	TOP1				MYCL	RICTOR
FLT3	MED12						MYCN	TSC1
							PDGFRA	TSC2
							PIK3CA	

- 4.5% with inherited pathogenic sequences

LAB. 1.05 - LABORATORIO DE TUMORES SÓLIDOS Y ENFERMEDADES HEREDITARIAS

ESTUDIO DE BIOMARCADORES CON VALIDEZ CLÍNICA EN TUMORES SÓLIDOS DE ADULTOS (161 GENES)

En la era de la medicina personalizada es esencial disponer de herramientas que nos permitan conocer el panorama genético completo de cada tumor, para poder así ofrecer la mejor opción terapéutica a cada paciente.

Ventajas del panel para tumores sólidos de adultos

Partiendo de tejido tumoral, este test es una prueba de secuenciación masiva (*Next Generation Sequencing*: NGS) dirigida a **161 genes relevantes en los tumores sólidos de adultos,** por ser de valor diagnóstico, pronóstico, predictivo de respuesta o ser criterio de inclusión o exclusión en ensayos clínicos.

La profundidad de secuenciación permite identificar poblaciones tumorales presentes a frecuencias a partir del 5%.

Este panel es una herramienta útil también para la caracterización molecular de tumores sincrónicos y así poder establecer si estos tumores están relacionados o no (tumor primario vs metástasis).

Dirigir el panel a un elevado número de genes permite, además del estudio de los genes frecuentemente alterados en cáncer, la posibilidad de explorar otros que probablemente van a tener terapias dirigidas en fase de ensayo clínico o una indicación fuera de ficha, ampliando así las opciones de tratamiento del paciente.

El panel incluye el análisis de los genes de la siguiente forma:

(1) **Mutaciones puntuales en hotspots:** *AKT1, AKT2, AKT3, ALK, AR, ARAF, AXL, BRAF, BTK, CBL, CCND1, CDK4, CDK6, CHEK2, CSF1R, CTNNB1, DDR2, EGFR, ERB83, ERBB2, ERBB4, ERCC2, ESR1, EZH2, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FOXL2, GATA2, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDR, KIT, KNSTRN, KRAS, MAGOH, MAP2K1, MAP2K2, MAP2K4, MAPK1, MAX, MDM4, MED12, MET, MTOR, MYC, MYCN, MYD88, NFE2L2, NRAS, NTRK1, NTRK2, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PPP2R1A, PTPN11, RAC1, RAF1, RET, RHEB, RHOA, ROS1, SF3B1, SMAD4, SMO, SPOP, SRC, STAT3, TERT, TOP1, U2AF1 y XPO1.*

(2) **Mutaciones puntuales en la secuencia codificante completa de los siguientes genes:** *ARID1A, ATM, ATR, ATRX, BAP1, BRCA1, BRCA2, CDK12, CDKN1B, CDKN2A, CDKN2B, CHEK1, CREBBP, FANCA, FANCD2, FANCI, FBXW7, MLH1, MRE11A, MSH2, MSH6, NBN, NF1, NF2, NOTCH1, NOTCH2, NOTCH3, PALB2, PIK3R1, PMS2, POLE, PTCH1, PTEN, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RB1, RNF43, SETD2, SLX4, SMARCA4, SMARCB1, STK11, TP53, TSC1 y TSC2.*

(3) **Amplificaciones de los siguientes genes**: *AKT1, AKT2, AKT3, ALK, AR, AXL, BRAF, CCND1, CCND2, CCND3, CCNE1, CDK2, CDK4, CDK6, CDKN2A, CDKN2B, EGFR, ERBB2, ESR1, FGF19, FGF3, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, IGF1R, KIT, KRAS, MDM2, MDM4, MET, MYC, MYCL, MYCN, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PPARG, RICTOR, TERT, TSC1 y TSC2.*

(4) **Reordenamientos (traslocaciones/fusiones) de los siguientes genes:** *ALK, AXL, BRAF, EGFR, ERBB2, ERG, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, NTRK1, NTRK3, PDGFRA, PPARG, RAF1, RET, ROS1, AKT2, AR, BRCA1, BRCA2, CDKN2A, ERBB4, ESR1, FGR, FLT3, JAK2, KRAS, MDM4, MET, MYB, MYBL1, NF1, NOTCH1, NOTCH4, NRG1, NTRK2, NUTM1, PDGFRB, PIK3CA, PRKACA, PRKACB, PTEN, RAD51B, RB1, RELA, RSPO2, RSPO3 y TERT.*

Tipo de muestra

La muestra requerida para esta prueba son dos tubos eppendorf por caso con 4-5 cortes de 5 micras cada uno (con contenido tumoral >50%).

Para otro tipo de muestra, consultar con el laboratorio.

La muestra será enviada junto con la hoja de petición disponible en: www.cimalabdiagnostics.es

Plazo de respuesta

El informe con la interpretación de los resultados será entregado en el plazo de 15 días hábiles por correo encriptado al facultativo solicitante.

CONTACTO:

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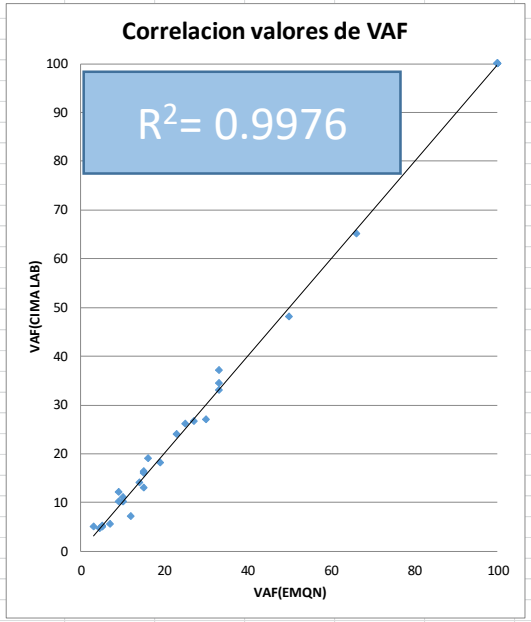
Three-angle strategy to advance in cancer management

2.- Solid biopsy tumor characterization requires validation, so control samples

- Internal control samples
- External control samples:
  - Proficiency testing programs,
    - for example EMQN

QCEAA.XXX	MB	Variante	VAF(EMQN)	VAF(CIMALAB)
QCE18.001	MB10459	NM_005228.5(EGFR):c.2155G>Ap.(Gly719Ser)	33	33
QCE18.001	MB10459	NM_004985.5(KRAS):c.35G>A p.(Gly12Asp)	50	48
QCE18.001	MB10461	NM_005228.5(EGFR):c.2390G>C p.(Cys797Ser)	15	13
QCE18.001	MB10461	NM_005228.5(EGFR):c.2369C>Tp.(Thr790Met)	15	16
QCE18.001	MB10461	NM_005228.5(EGFR):c.2573T>Gp.(Leu858Arg)	25	26
QCE18.001	MB10464	NM_005228.5(EGFR):c.2582T>A p.(Leu861Gln)	5	5
QCE18.001	MB10465	NM_005228.5(EGFR):c.2236_2250del p.(Glu746_Ala750del)	12	7.1
QCE18.001	MB10466	NM_005228.5(EGFR):c.2155G>A p.(Gly719Ser)	33	37
QCE18.001	MB10468	NM_004333.4(BRAF):c.1799T>A p.(Val600Glu)	66	65
QCE19.004	MB13952	NM_000546.5(TP53):c.643A>G p.(Ser215Gly)	100	100
QCE19.004	MB13954	NM_002524.5(NRAS):c.182A>T p.(Gln61Leu)	4.5	4.66
QCE19.004	MB13954	NM_004333.6(BRAF):c.1798_1799delinsAA p.(Val600Lys)	5	5.2
QCE19.004	MB13954	NM_005228.5(EGFR):c.2390G>C p.(Cys797Ser)	7	5.5
QCE19.004	MB13954	NM_000546.5(TP53):c.643A>G p.(Ser215Gly)	100	100
QCE19.004	MB13956	NM_004985.5(KRAS): c.436G>C p.(Ala146Pro)	23	23.9
QCE19.004	MB13956	NM_005228.5(EGFR):c.2303G>T p.(Ser768Ile)	27	26.64
QCE19.004	MB13956	NM_004333.6(BRAF):c.1798_1799delinsAA p.(Val600Lys)	33	34.35
QCE19.004	MB13956	NM_000546.5(TP53):c.643A>G p.(Ser215Gly)	100	100
QCE20.001	MB17520	NM_005228.5(EGFR):c.2369C>Tp.(Thr790Met)	5	5
QCE20.001	MB17520	NM_002524.5(NRAS):c.351G>T p.(Lys117Asn)	9	10
QCE20.001	MB17520	NM_005228.5(EGFR):c.2573T>Gp.(Leu858Arg)	10	10
QCE20.001	MB17520	NM_004985.5(KRAS): c.34_36delinsTGG p.(Gly12Trp)	10	11
QCE20.001	MB17520	NM_006218.4(PIK3CA):c.3140A>G p.(His1047Arg)	16	19
QCE20.001	MB17520	NM_000222.2(KIT): c.1504_1509dup p.(Ala502_Tyr503dup)	30	27
QCE20.001	MB17520	NM_000546.5(TP53):c.643A>G p.(Ser215Gly)	100	100
QCE20.001	MB17522	NM_000546.5(TP53):c.643A>G p.(Ser215Gly)	100	100
QCE20.001	MB17524	NM_004333.6(BRAF):c.1798_1799delinsAA p.(Val600Lys)	3	5
QCE20.001	MB17524	NM_000222.2(KIT):c.1961T>C p.(Val654Ala)	10	10
QCE20.001	MB17524	NM_002524.5(NRAS):c.183A>C p.(Gln61His)	9	12
QCE20.001	MB17524	NM_004985.5(KRAS): c.351A>C p.(Lys117Asn)	14	14
QCE20.001	MB17524	NM_000546.5(TP53):c.404G>T p.(Cys135Phe)	15	16.23
QCE20.001	MB17524	NM_000546.5(TP53):c.643A>G p.(Ser215Gly)	100	100
QCE20.001	MB17524	NM_005228.5(EGFR):c.2300_2308dupCCAGCGTGGp.(Ala767_Val769dup)	19	18.1



Three-angle strategy to advance in cancer management

2.- Solid biopsy tumor characterization requires validation, so control samples

- Internal control samples
- External control samples:
  - Proficiency testing programs,
    - for example EMQN
  - Commercial samples:
    - SeraCare samples –
      - FFPE Fusion RNA

NGS Data:

RNA Fusion	NGS Average Unique Start Sites per Fusion	NGS Average Unique Reads per Fusion*
CCDC6-RET	147	639
CD74-ROS1	93	320
EGFR variant III	70	143
EGFR-SEPT14	233	574
EML4-ALK	132	667
ETV6-NTRK3	193	728
FGFR3-BAIAP2L1	131	911
FGFR3-TACC3	200	1587
KIF5B-RET	167	1001
LMNA-NTRK1	202	1661
MET Exon 14 Skipping	93	166
NCOA4-RET	99	513
PAX8-PPARG1	90	366
SLC34A2-ROS1	97	410
SLC45A3-BRAF	95	2972
TFG-NTRK1	185	1182
TMPRSS2-ERG	88	2298
TPM3-NTRK1	187	862

*Total number of reads per sample was 3.86M.

Approval:

Prepared By — Date 1/24/2019

QA Verified By — Date 2/7/19

18/18 fusions

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2.- Solid biopsy tumor characterization requires validation, so control samples

- Internal control samples
- External control samples:
  - Proficiency testing programs,
    - for example EMQN
  - Commercial samples:
    - SeraCare samples –
      - FFPE Fusion RNA
      - FFPE Tumor DNA

Comprehensive genomic profiling (CGP) is an important part of an integrated clinical management of cancer patients, where cancer patients harboring variants/biomarkers of clinical utility are determined by highly multiplexed targeted NGS assay testing. Precise analysis of these patient samples requires high quality sample-to-result assay workflow controls to guide and validate the accurate identification of these actionable variants.

LGC SeraCare has developed a highly multiplexed Compromised FFPE Tumor DNA reference material imbibing "patient-like" characteristics to support end-to-end NGS workflows performed by clinical labs in the analysis of cancer patient samples. This product consists of 17 genes and 34 variants, incorporating all variant types – SNVs, INDELs, CNVs, and SVs. These variants were precisely quantitated by digital PCR and targeted NGS against a single well-characterized genomic background (GM24385).

- Highly multiplexed FFPE Tumor DNA reference material
- Contains 34 variants in 17 genes
- All variant types – SNVs (18), INDELs (10), CNVs (3) and SVs (3)
- For use in sample-to-result NGS workflows to analyze for mutation-positive variants in patient samples
- Manufactured in GMP-compliant ISO 13485 certified facility

Gene List			
ATK1	EGFR	MYC	CD74-ROSI
ALK	ERBB2	NRAS	NCOA4-RET
BRAF	KIT	PIK3CA	EML4-ALK
BRCA1	KRAS	TP53	
BRCA2	MET		

(18/18 SNVs)

(3/3 CNVs)

(7/10 indels)

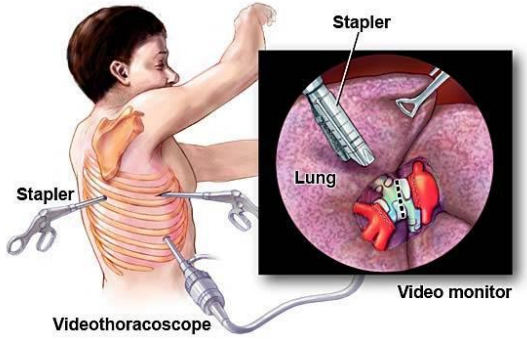
* 2 within homopolimeric regions

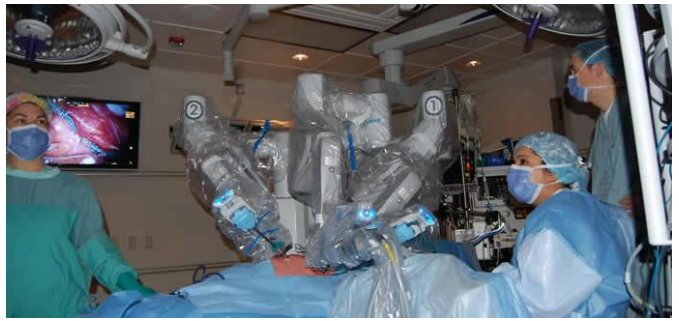
* 24 base del instead of 18 base del

Three-angle strategy to advance in cancer management

2.- Solid biopsy tumor characterization has its own limitations

- Might not be an option – patient condition or tumor size/localization
- It's an invasive procedure
- Not enough for the increasing request of molecular tests
- Might not reflect completely tumor heterogeneity or tumor evolution
  - i.e., drug treatments produce molecular changes in tumor cells



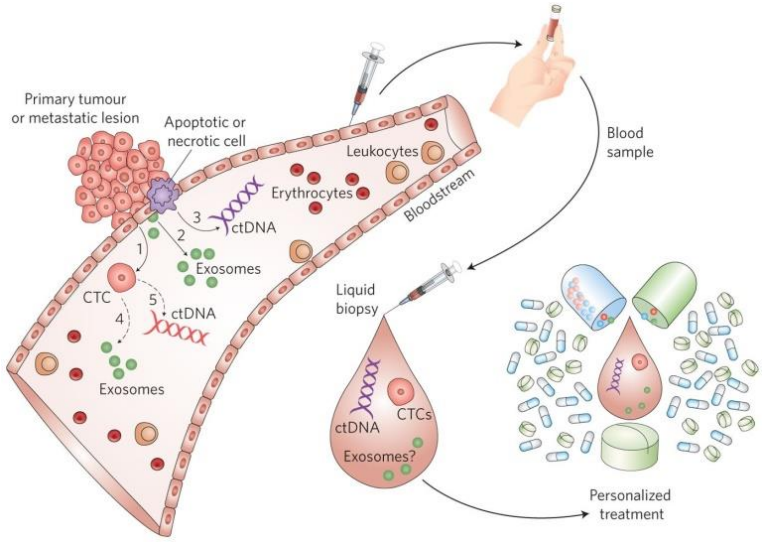


www.lhsc.on.ca/

Three-angle strategy to advance in cancer management

3.- Liquid biopsy tumor characterization could be an alternative

- Characterizing the components that the tumor releases:
  - Circulating tumor cells (CTCs),
  - Pieces of cel-free DNA and RNA (cfDNA and cfRNA)
  - Exosomes, platelets, ...
- Advantages:
  - Time and cost reduction from sample to results
  - Early disease detection
  - Study of tumor latency
  - Real-time evaluation of treatment response
  - Evaluation of tumor heterogeneity and dynamics
  - Non-invasive characterization of primary tumors and metastases



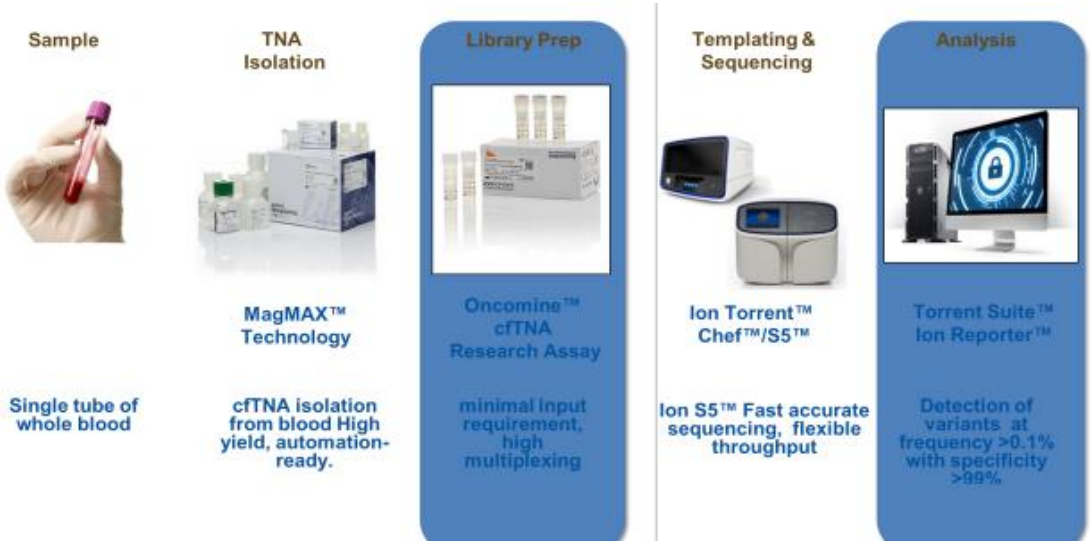
Alix-Panabières *et al.*, (2017) **Nature Biomedical Engineering**

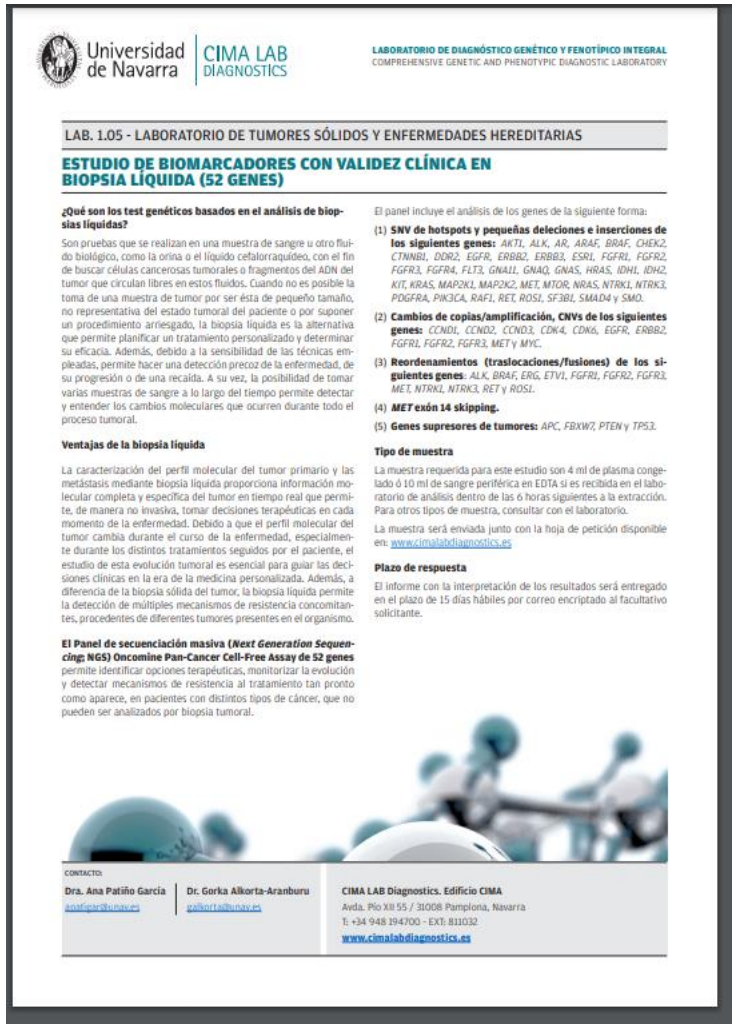
FDA approves liquid biopsy NGS for multiple cancers and biomarkers

Three-angle strategy to advance in cancer management

3.- Liquid biopsy tumor characterization

- NGS-gene panel testing
  - 52 gene panel, only hot spots, CNV, fusions.





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COMPREHENSIVE GENETIC AND PHENOTYPIC DIAGNOSTIC LABORATORY

LAB. 1.05 - LABORATORIO DE TUMORES SÓLIDOS Y ENFERMEDADES HEREDITARIAS

ESTUDIO DE BIOMARCADORES CON VALIDEZ CLÍNICA EN BIOPSIA LÍQUIDA (52 GENES)

¿Qué son los test genéticos basados en el análisis de biopsias líquidas?

Son pruebas que se realizan en una muestra de sangre u otro fluido biológico, como la orina o el líquido cefalorraquídeo, con el fin de buscar células cancerosas tumorales o fragmentos del ADN del tumor que circulan libres en estos fluidos. Cuando no es posible la toma de una muestra de tumor por ser ésta de pequeño tamaño, no representativa del estado tumoral del paciente o por suponer un procedimiento arriesgado, la biopsia líquida es la alternativa que permite planificar un tratamiento personalizado y determinar su eficacia. Además, debido a la sensibilidad de las técnicas empleadas, permite hacer una detección precoz de la enfermedad, de su progresión o de una recaída. A su vez, la posibilidad de tomar varias muestras de sangre a lo largo del tiempo permite detectar y entender los cambios moleculares que ocurren durante todo el proceso tumoral.

Ventajas de la biopsia líquida

La caracterización del perfil molecular del tumor primario y las metástasis mediante biopsia líquida proporciona información molecular completa y específica del tumor en tiempo real que permite, de manera no invasiva, tomar decisiones terapéuticas en cada momento de la enfermedad. Debido a que el perfil molecular del tumor cambia durante el curso de la enfermedad, especialmente durante los distintos tratamientos seguidos por el paciente, el estudio de esta evolución tumoral es esencial para guiar las decisiones clínicas en la era de la medicina personalizada. Además, a diferencia de la biopsia sólida del tumor, la biopsia líquida permite la detección de múltiples mecanismos de resistencia concomitantes, procedentes de diferentes tumores presentes en el organismo.

El Panel de secuenciación masiva (*Next Generation Sequencing*; NGS) Oncomine Pan-Cancer Cell-Free Assay de 52 genes permite identificar opciones terapéuticas, monitorizar la evolución y detectar mecanismos de resistencia al tratamiento tan pronto como aparece, en pacientes con distintos tipos de cáncer, que no pueden ser analizados por biopsia tumoral.

El panel incluye el análisis de los genes de la siguiente forma:

(1) **SNV de hotspots y pequeñas deleciones e inserciones de los siguientes genes:** *AKT1, ALK, AR, ARAF, BRAF, CHEK2, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, RAF1, RET, ROS1, SF3B1, SMAD4* y *SMO*.

(2) **Cambios de copias/amplificación, CNVs de los siguientes genes:** *CCND1, CCND2, CCND3, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, MET* y *MYC*.

(3) **Reordenamientos (traslocaciones/fusiones) de los siguientes genes**: *ALK, BRAF, ERG, ETV1, FGFR1, FGFR2, FGFR3, MET, NTRK1, NTRK3, RET* y *ROS1*.

(4) ***MET* exón 14 skipping.**

(5) **Genes supresores de tumores:** *APC, FBXW7, PTEN* y *TP53*.

Tipo de muestra

La muestra requerida para este estudio son 4 ml de plasma congelado ó 10 ml de sangre periférica en EDTA si es recibida en el laboratorio de análisis dentro de las 6 horas siguientes a la extracción. Para otros tipos de muestra, consultar con el laboratorio.

La muestra será enviada junto con la hoja de petición disponible en: www.cimalabdiagnostics.es

Plazo de respuesta

El informe con la interpretación de los resultados será entregado en el plazo de 15 días hábiles por correo encriptado al facultativo solicitante.

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Avda. Pío XII 55 / 31008 Pamplona, Navarra
T: +34 948 194700 - EXT: 811032
www.cimalabdiagnostics.es

EMQN
The European Molecular Genetics Quality Network

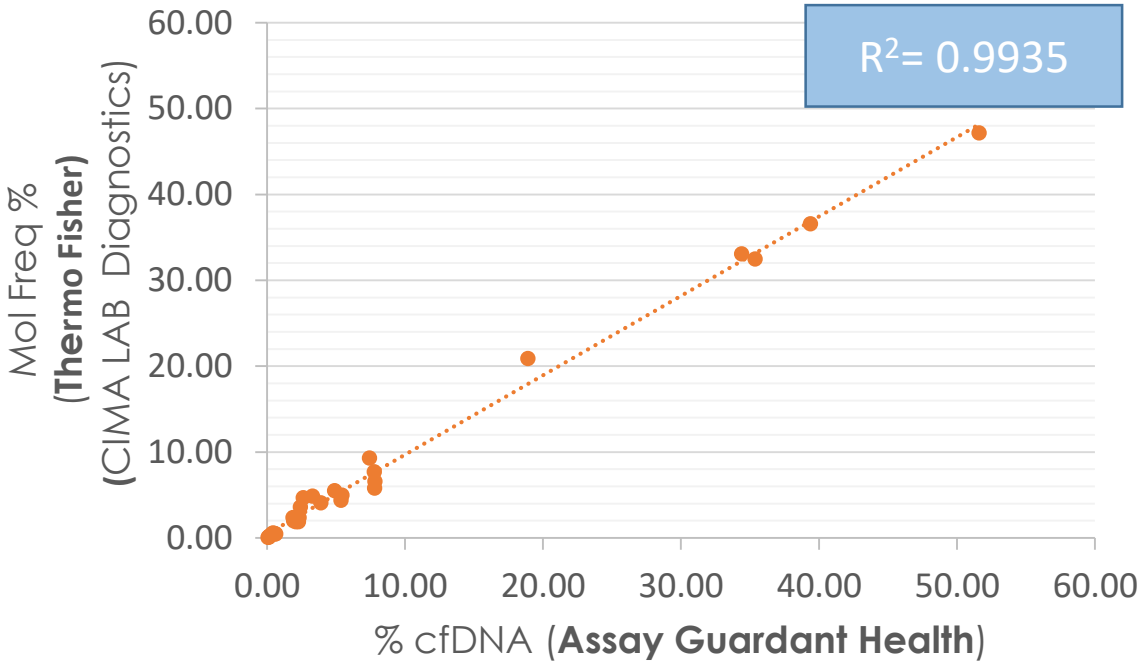
ISO 9001:2015 CERTIFIED

ISO 15189 ACCREDITATION
MEDICAL LABORATORY

Three-angle strategy to advance in cancer management

3.- Liquid biopsy tumor characterization requires validation, so control samples

- Internal control samples
  - Reproducibility of the variant allele frequency
  - Same allele frequency values among 28 SNVs identified by both solutions



Three-angle strategy to advance in cancer management

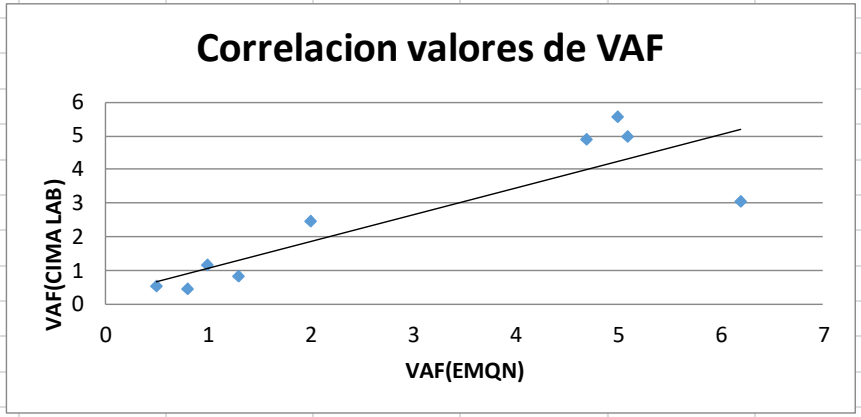
3.- Liquid biopsy tumor characterization requires validation, so control samples

- External control samples:
  - Proficiency testing programs,
    - for example EMQN

But

R^2= 0.7513

QCEAA.XXX	MB	Variante	VAF(EMQN)	VAF(CIMALAB)
QCE18.006	MB11732	NM_005228.5(EGFR):c.2236_2250delp.(Glu746_Ala750del)	1.3	0.78
QCE18.006	MB11734	NM_005228.5(EGFR):c.2369C>T; p.(Thr790Met)	5.1	4.93
QCE18.006	MB11734	NM_005228.5(EGFR):c.2573T>G; p.(Leu858Arg)	4.7	4.86
QCE18.006	MB11735	NM_005228.5(EGFR):c.2236_2250delp.(Glu746_Ala750del)	6.2	3
QCE18.006	MB11736	NM_005228.5(EGFR):c.2369C>T; p.(Thr790Met)	0.8	0.4
QCE18.006	MB11736	NM_005228.5(EGFR):c.2573T>G; p.(Leu858Arg)	0.5	0.5
QCE20.006	MB17722	NM_005228.5(EGFR): c.2155G>A p.(Gly719Ser)	5	5.53
QCE20.006	MB17723	NM_005228.5(EGFR): c.2236_2250del p.(Glu746_Ala750del)	2	2.44
QCE20.006	MB17723	c.2369C>T p.(Thr790Met)	1	1.11
QCEAA.XXX	MB	Variante	VAF(EMQN)	VAF(CIMALAB)
QCE18.006	MB11733	NA	NA	NA
QCE20.006	MB17721	NA	NA	NA



Three-angle strategy to advance in cancer management

3.- Liquid biopsy tumor characterization requires validation, so control samples

- External control samples:
  - Proficiency testing programs,
    - for example EMQN

Eliminating the indel

R^2= 0.9762

QCEAA.XXX	MB	Variante	VAF(EMQN)	VAF(CIMALAB)
QCE18.006	MB11732	NM_005228.5(EGFR):c.2236_2250delp.(Glu746_Ala750del)	1.3	0.78
QCE18.006	MB11734	NM_005228.5(EGFR):c.2369C>T; p.(Thr790Met)	5.1	4.93
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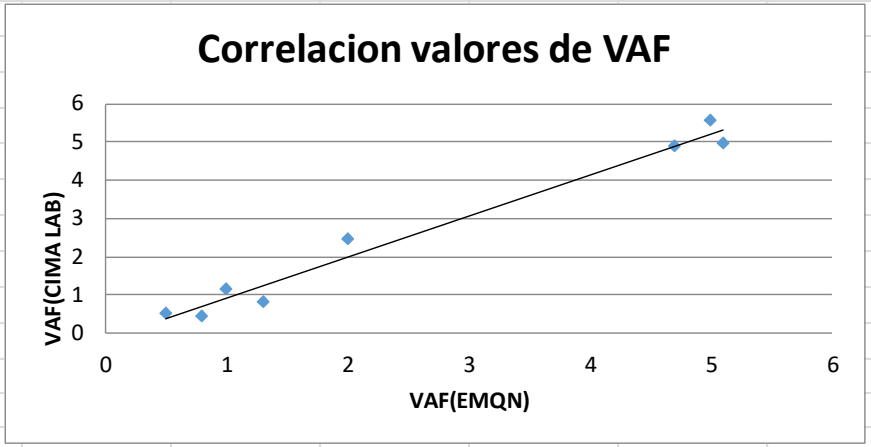
QCE18.006	MB11736	NM_005228.5(EGFR):c.2573T>G; p.(Leu858Arg)	0.5	0.5
QCE20.006	MB17722	NM_005228.5(EGFR): c.2155G>A p.(Gly719Ser)	5	5.53
QCE20.006	MB17723	NM_005228.5(EGFR): c.2236_2250del p.(Glu746_Ala750del)	2	2.44
QCE20.006	MB17723	c.2369C>T p.(Thr790Met)	1	1.11



Universidad de Navarra | CIMA LAB DIAGNOSTICS

Three-angle strategy to advance in cancer management

Conclusions

- Three-angle strategy to advance in cancer management
  - Constitutional testing,
  - Solid and liquid biopsy molecular tumor characterization

- However, inherited challenges:

  Knowledge is growing, so how can we provide more comprehensive information?

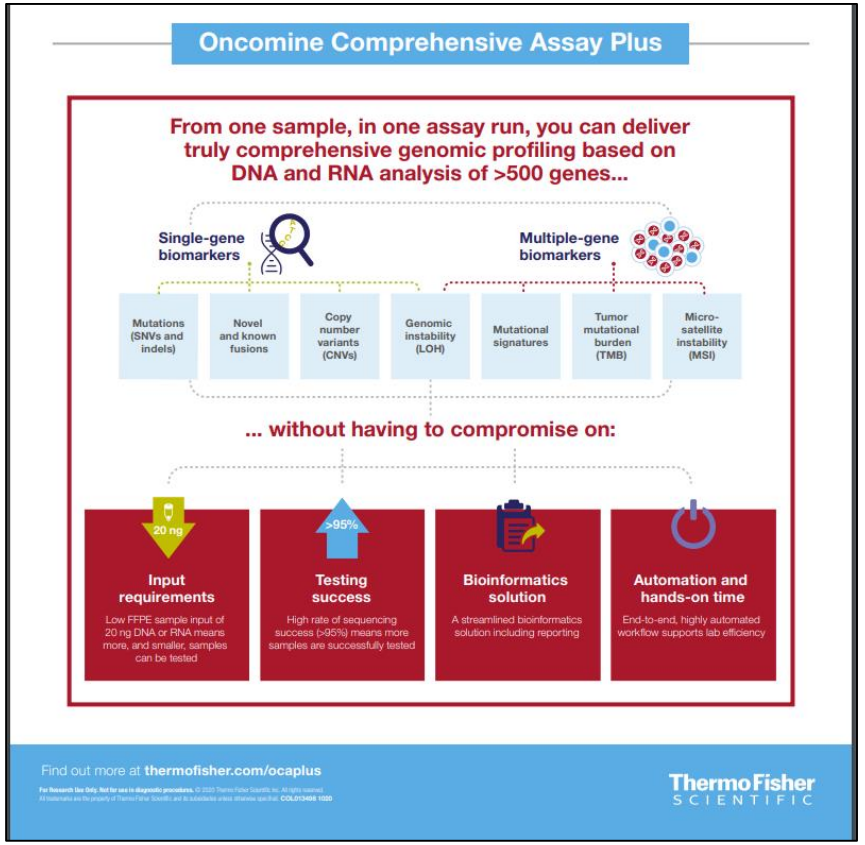
  But, cost is also growing, so how can we reduce the cost?
  - In house *versus* commercial company testing
  - Insurance companies
  - Pharmaceutical companies, research projects,…

Oncomine Comprehensive Assay Plus
(solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

- NGS-gene panel testing
  - 500- gene panel
  - Genes associated with inherited cancer
  - Multiple-gene markers:
    - MSI
    - TMB
    - HRD
    - Mutational signatures
  - Comprehensive Genomic Profiling

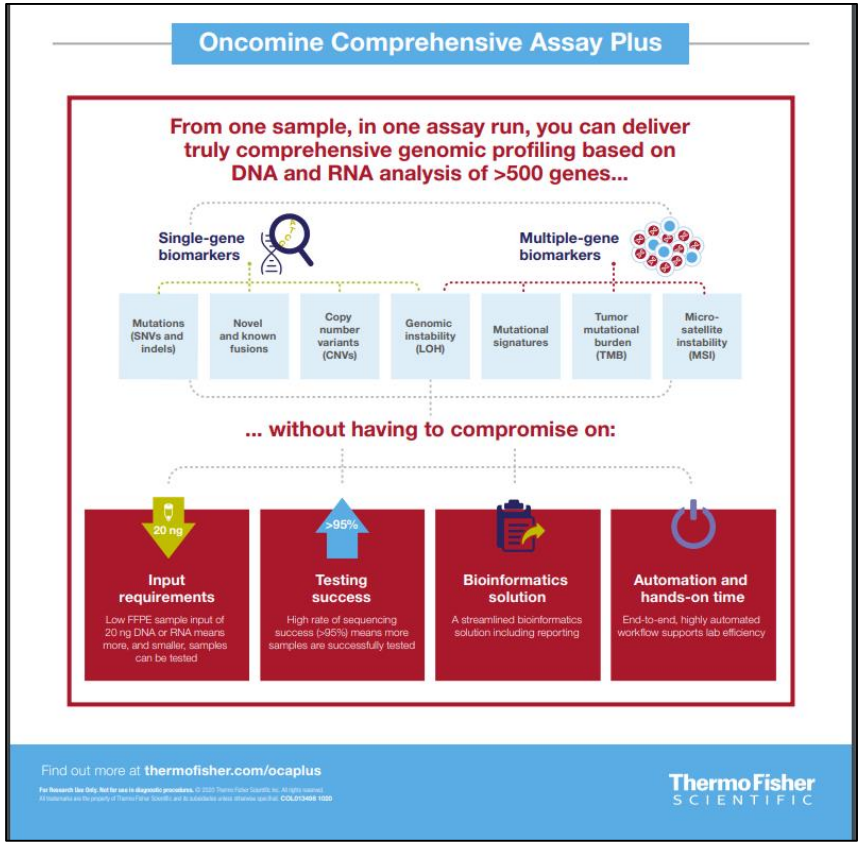


Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

- ISO15189 accreditation process has been activated within our laboratory:
  - Participated in EMQN proficiency testing
  - Validated in-house using the same SeraCare samples used for OCA validation, and results are identical.
- Processed samples:
  - Research projects including clinical trials
  - Clinical assistance.



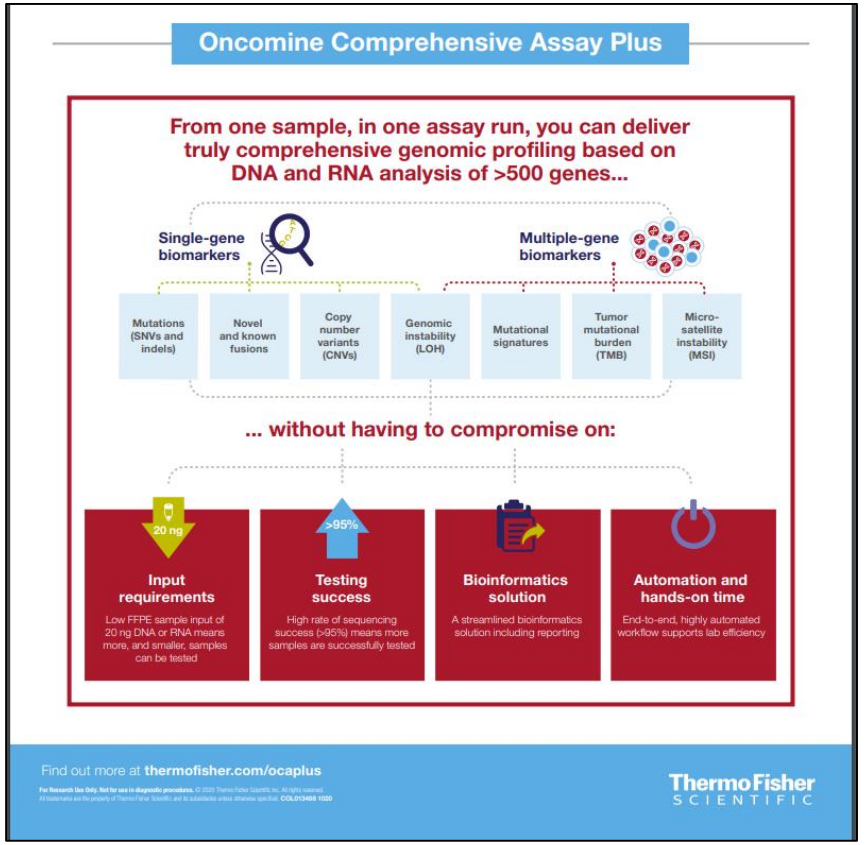
Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

- Three examples that clearly state we can we be RIGTH NOW:
  - Case A: Lung cancer
  - Case B: Glioblastoma
  - Case C: Colon cancer

- What do we need next?



Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

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    - Mutational signatures
  - Comprehensive Genomic Profiling

- Case A: Lung cancer (MB20388, TMF00530)

- 2021 OCA: NTRK positive
- Now, OCAplus: …

Three-angle strategy to advance in cancer management

NTRK fusion-positive cancer!!!!

2*.- Solid biopsy tumor characterization

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  - Comprehensive Genomic Profiling

- Case A: Lung cancer (MB20388, TMF00530)

- 2021 OCA: NTRK positive
- Now, OCAplus: …

EML4:: NTRK3

Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

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  - 500- gene panel
  - Genes associated with inherited cancer
  - Multiple-gene markers:
    - MSI
    - TMB
    - HRD
    - Mutational signatures
  - Comprehensive Genomic Profiling

- Case B: Glioblastoma (MB18609, TMF00530)

- 2019 TMB study: TMB-High
- Now, with OCAplus: …

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

- NGS-gene panel testing
  - 500- gene panel
  - Genes associated with inherited cancer
  - Multiple-gene markers:
    - MSI
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    - HRD
    - Mutational signatures
  - Comprehensive Genomic Profiling

- Case B: Glioblastoma (MB18609, TMF00530)

- 2019 TMB study: TMB-High
- Now, with OCAplus: …

Mutation Load (Mutations/Mb)=187.77

Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

Analysis	Sample	MSI Status	MSI Score	MSI Coverage	MSI Algorithm version	MSI QC	
TMF00530_4_MB18609_c10390_2021-10-08-10-36-45-243	TMF00530_4_MB18609_DNA	MSS	1.28	100706	MSI_IR 2.0.2		

POLE-mutated cancer!!!!

Mutations in the exonuclease domain of the DNA polymerase epsilon (POLE) gene

- Case B: Glioblastoma (MB18609, TMF00530)

- 2019 TMB study: TMB-High
- Now, with OCAplus: …

Othee studies; for example, A.B. Schrock et al., 2017

"Mutations of the DNA polymerase epsilon (POLE) can lead to a hypermutated tumor phenotype, in the absence of microsatellite instability (MSI). Exceptional responses to ICPIs in POLE-mutated endometrial adenocarcinoma (EA), colorectal (CRC), and glioblastoma (GBM) are described."

Mutation Load (Mutations/Mb)=187.77

Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

- NGS-gene panel testing
  - 500- gene panel
  - Genes associated with inherited cancer
  - Multiple-gene markers:
    - MSI
    - TMB
    - HRD
    - Mutational signatures
  - Comprehensive Genomic Profiling

- Case C: Colon cancer (MB10300, TMF00530)

- 2016 MSI study: MSI-High
- Now, with OCAplus: …

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

- NGS-gene panel testing
  - 500- gene panel
  - Genes associated with inherited cancer
  - Multiple-gene markers:
    - MSI
    - TMB
    - HRD
  - Comprehensive Genomic Profiling

- Case C: Colon cancer (MB10300, TMF00530)

- 2016 MSI study: MSI-High
- Now, with OCAplus: …

Analysis	Sample	MSI Status	MSI Score	MSI Coverage	MSI Algorithm version	MSI QC		
TMF00530_8_MB10300_c2922_2021-10-13-17-25-32-352	TMF00530_8_MB10300_DNA	MSI-High	56.59	139923	MSI_IR 2.0.2			

Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

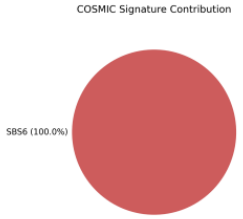
Mutation Signature Identification Report

Sample Name: TMF00530_8_MB10300_DNA

Analysis Name: TMF00530_8_MB10300_DNA_20211013152724159

Summary

Enriched Signature	Cosine Similarity	Signature Description
SBS6	0.733	Associated with defective DNA mismatch repair (with microsatellite instability, MSI)



Mutation Signature Identification Report

Sample Name: TMF00530_8_MB10300_DNA

Analysis Name: TMF00530_8_MB10300_DNA_20211013152724159

Signature specific gene mutations

SBS6

Locus	Type	Gene	Frequency	Protein
chr2:47637291	SNV	MSH2	48.25	p.Ser142Ter
chr2:47703631	SNV	MSH2	21.97	p.Arg711Ter

Inherited cancer!!!!

- Case C: Colon cancer patient (MB10300, TMF00530)

- 2016 MSI study: MSI-High
- Now, with OCAplus: …

- HRD

Analysis	Sample	MSI Status	MSI Score	MSI Coverage	MSI Algorithm version	MSI QC		
TMF00530_8_MB10300_c2922_2021-10-13-17-25-32-352	TMF00530_8_MB10300_DNA	MSI-High	56.59	139923	MSI_IR 2.0.2			

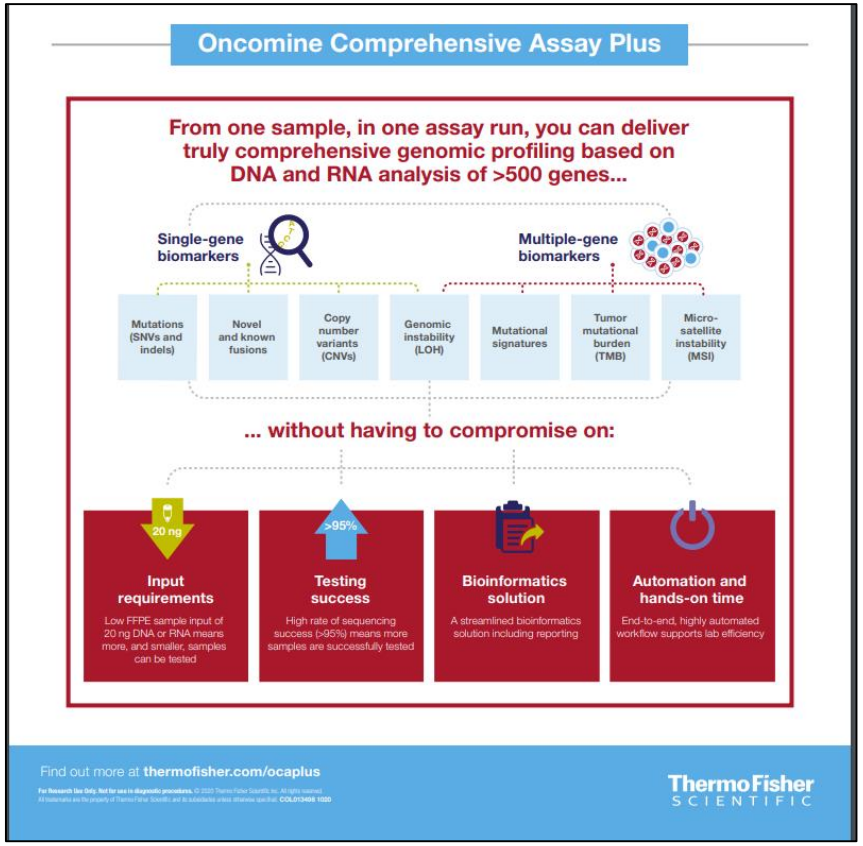
- Comprehensive Genomic Profiling

Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

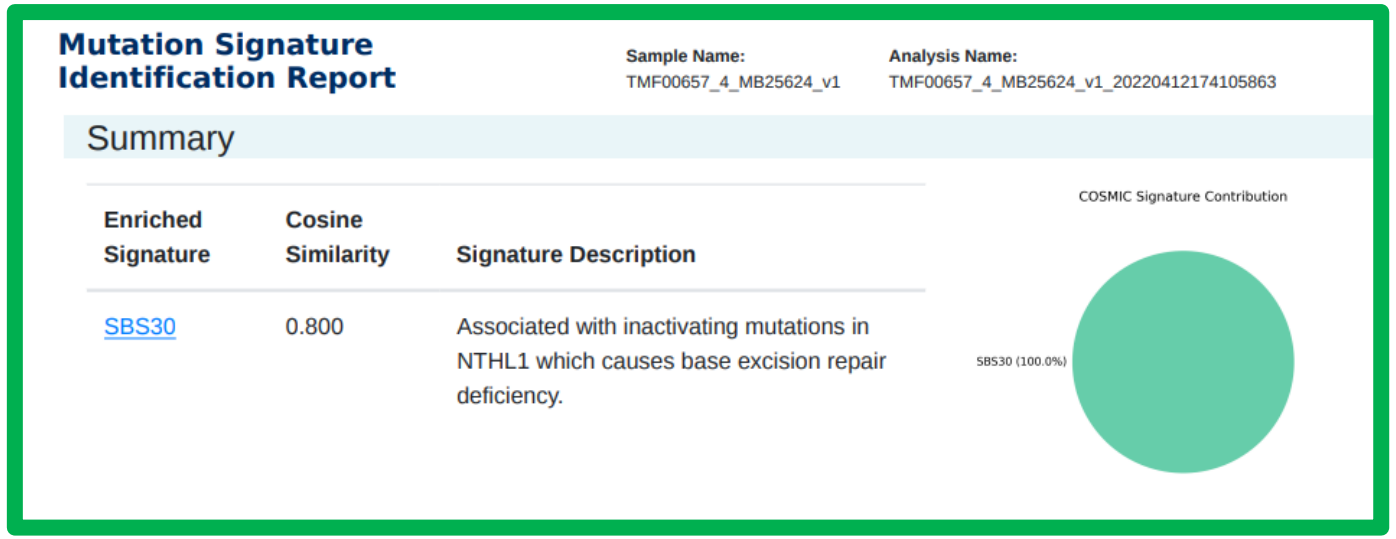
- Three examples that clearly state we can we be RIGTH NOW:
  - Case A: Lung cancer
  - Case B: Glioblastoma
  - Case C: Colon cancer
  - …
- What do we need next?
  - Metanalysis and follow-up some of the clearly identified, for example, signatures



Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization



Mutation Signature Identification Report

Sample Name: TMF00657_4_MB25624_v1

Analysis Name: TMF00657_4_MB25624_v1_20220412174105863

Summary

Enriched Signature	Cosine Similarity	Signature Description
SBS30	0.800	Associated with inactivating mutations in NTHL1 which causes base excision repair deficiency.

Signature specific gene mutations

No signature specific gene mutations found.

The *NTHL1* gene, related to hereditary predisposition to cancer, is not included in OCAplus

Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Three-angle strategy to advance in cancer management

2*.- Solid biopsy tumor characterization

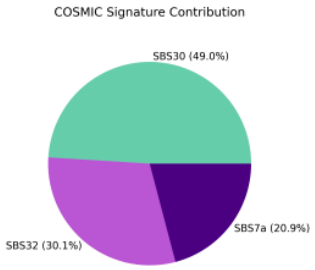
Mutation Signature Identification Report

Sample Name: TMF00667_2_MB25788_v1

Analysis Name: TMF00667_2_MB25788_v1_20220429141426271

Summary

Enriched Signature	Cosine Similarity	Signature Description
SBS30	0.906	Associated with inactivating mutations in NTHL1 which causes base excision repair deficiency.
SBS7a	0.758	Found in cancers of the skin from sun exposed areas due to ultraviolet light. Possible due to either of cyclobutane pyrimidine dimers or 6-4 UV photoproducts but with no clear evidence.
SBS32	0.719	Associated with immunosuppresant azathioprine treatment.



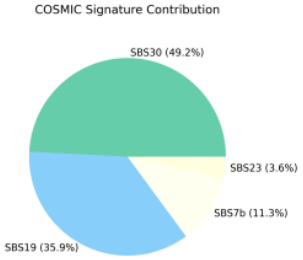
Mutation Signature Identification Report

Sample Name: TMF00667_3_MB25789_v1

Analysis Name: TMF00667_3_MB25789_v1_20220429141433382

Summary

Enriched Signature	Cosine Similarity	Signature Description
SBS30	0.775	Associated with inactivating mutations in NTHL1 which causes base excision repair deficiency.
SBS19	0.760	Unknown
SBS7b	0.744	Found in cancers of the skin from sun exposed areas due to ultraviolet light.
SBS23	0.737	unknown



Oncomine Comprehensive Assay Plus (solid tumor biopsies)

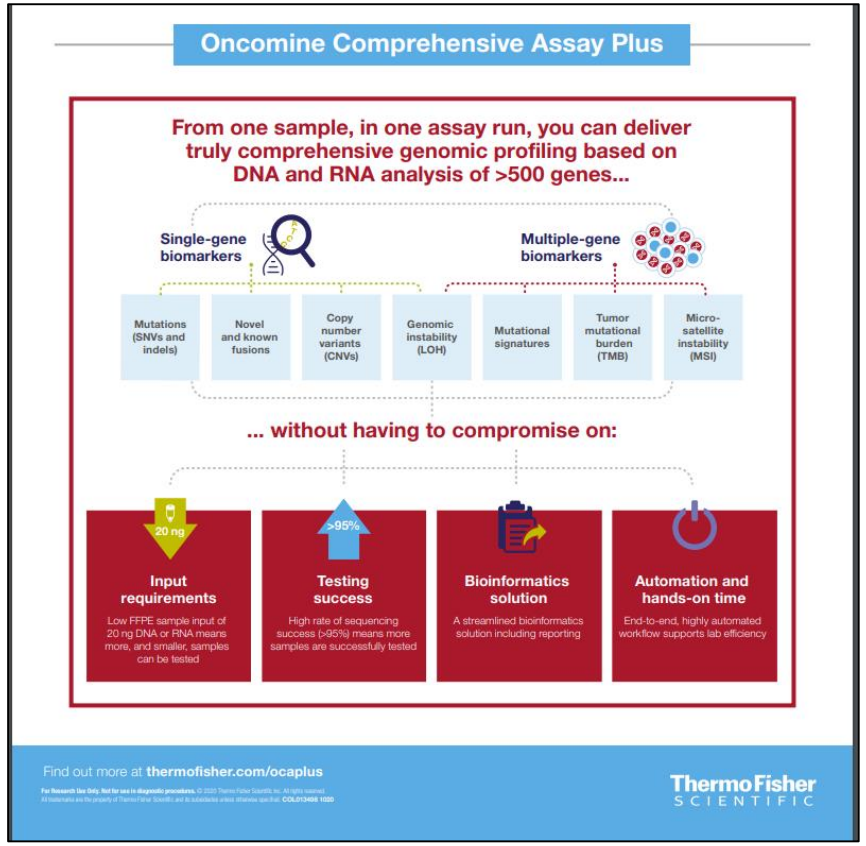
Three-angle strategy to advance in cancer management

Conclusions

- Three-angle strategy to advance in cancer management
  - Constitutional testing,
  - Solid and liquid biopsy molecular tumor characterization

- Inherited challenges – work in progress!

  Oncomine Comprehensive Assy Plus is **improving access to genomic medicine** by:
  - Providing a more comprehensive tumor characterization
  - Reducing both sample and cost burden



Oncomine Comprehensive Assay Plus (solid tumor biopsies)

Access, the main problem with genomic medicine

Thanks & Questions

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galkorta@unav.es

https://www.unav.edu/web/cimalab