MEDICINA DE PRECISIÓN

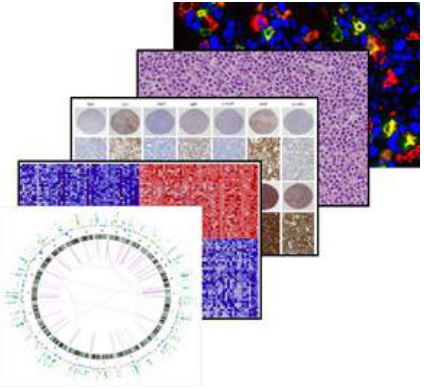
En la práctica clínica

ELOISA JANTUS LEWINTRE, PhD

Servicio Oncología Médica
Consorcio Hospital General Universitario de Valencia

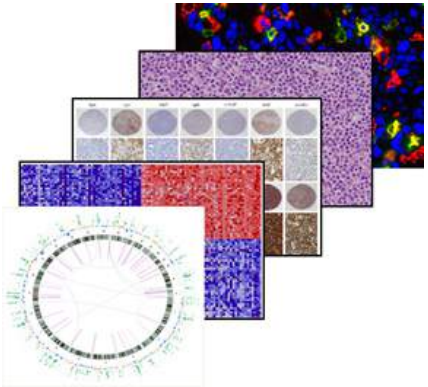
Laboratorio Oncología Molecular- FIHGUV

CIBERONC



MEDICINA DE PRECISIÓN

- Porqué … ?
- Qué …?
- Dónde …?
- Cómo … ?
- Quién … ?

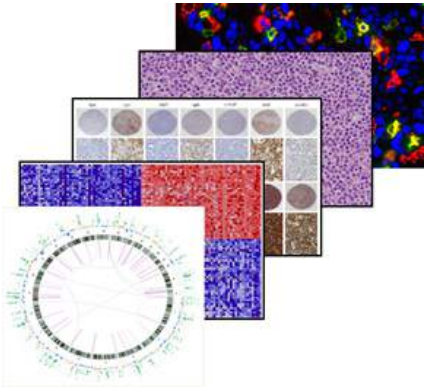


Fundación InstitutoRoche

Universitat de València

MEDICINA DE PRECISIÓN

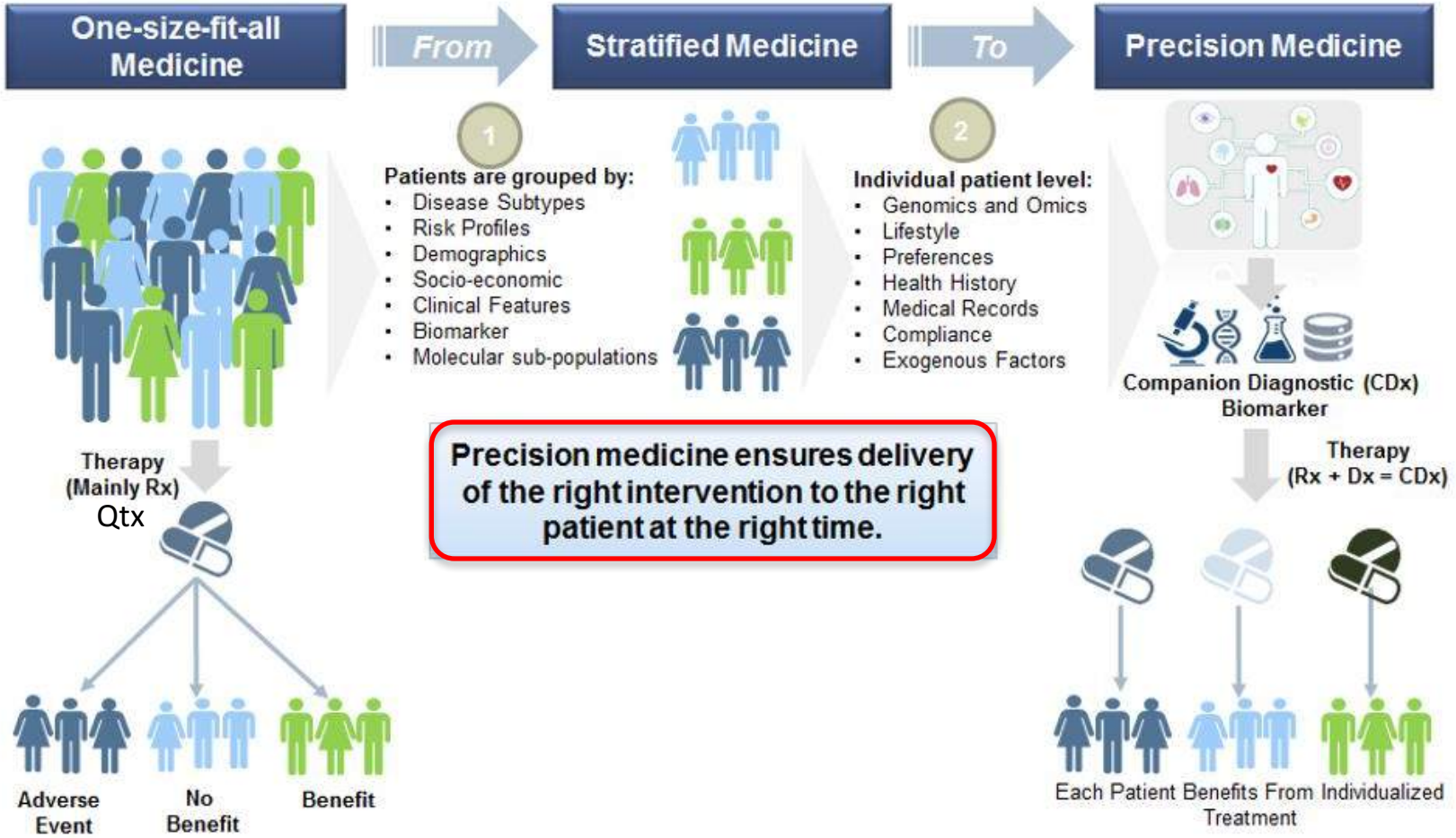
Porqué ?



Fundación InstitutoRoche

VNIVERSITAT DE VALÈNCIA

ONCOLOGÍA : camino hacia la PRECISIÓN



ONCOLOGIA DE PRECISIÓN : Ejemplo Cáncer de Pulmón

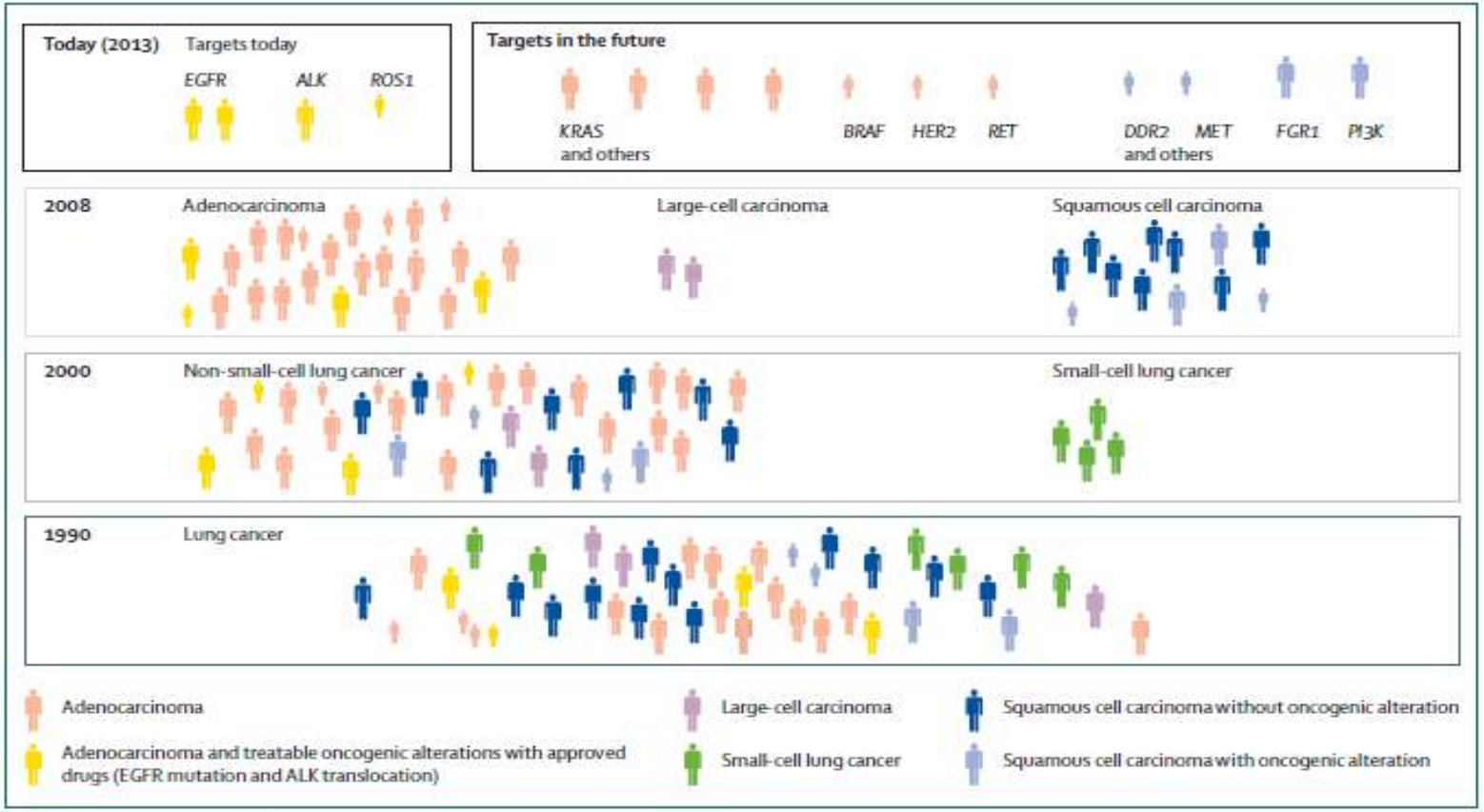
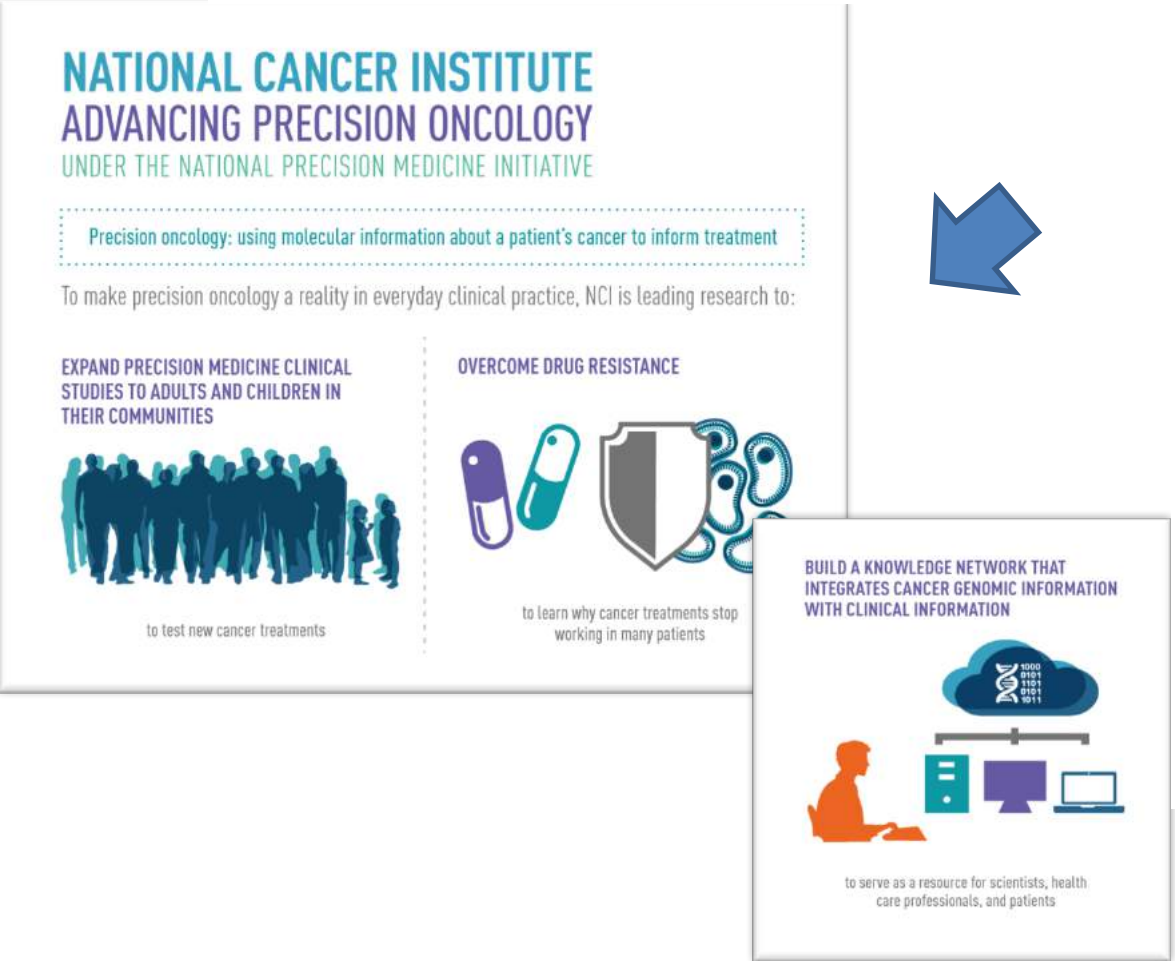


Figure 1: **Evolution of lung cancer histology over time**
The colours denote different histological subtypes; also see figure 2.

Reck et al, Lancet 2013

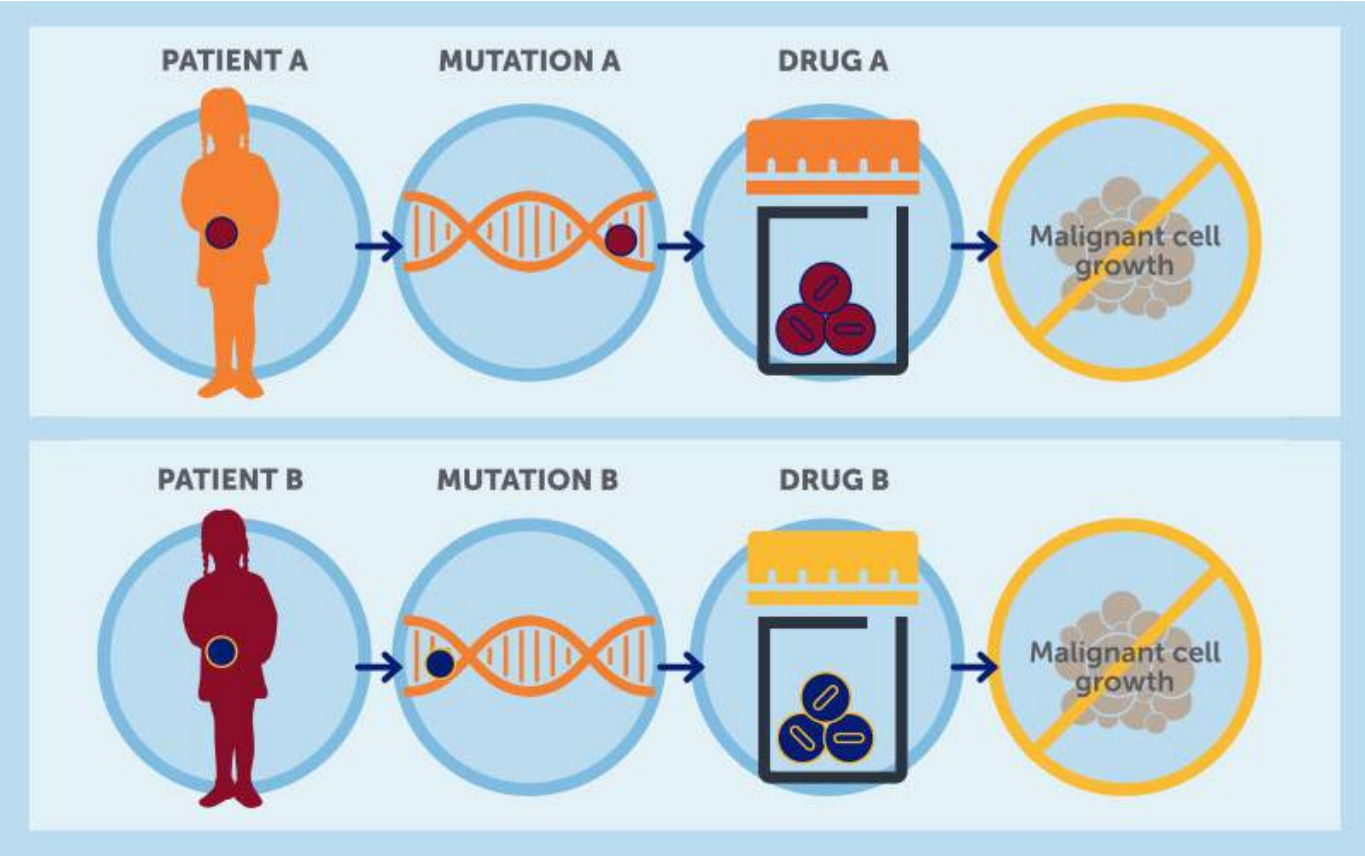
ONCOLOGIA DE PRECISIÓN



In the very near future, a patient's **cancer will be extensively characterized for mutations and other molecular abnormalities, and his or her treatment will be based on the identified molecular changes instead of where the cancer originated in the body**. This approach is part of what is called **precision** (or personalized) **medicine**.

To speed progress in this area, President Obama announced the **P**recision **M**edicine **I**nitiative® (PMI) on January 30, 2015. The PMI is a $215 million proposed investment to accelerate biomedical research and provide clinicians with new therapies and tools to select the treatments and interventions that will be most effective for individual patients

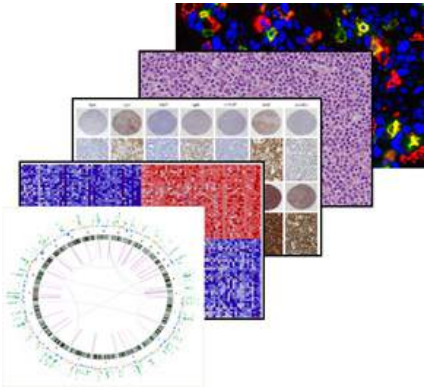
MEDICINA DE PRECISIÓN



Un nuevo modelo en el tratamiento del cáncer, donde las decisiones terapéuticas son guiadas por los **atributos moleculares de cada paciente**.

ONCOLOGÍA DE PRECISIÓN

Qué ?

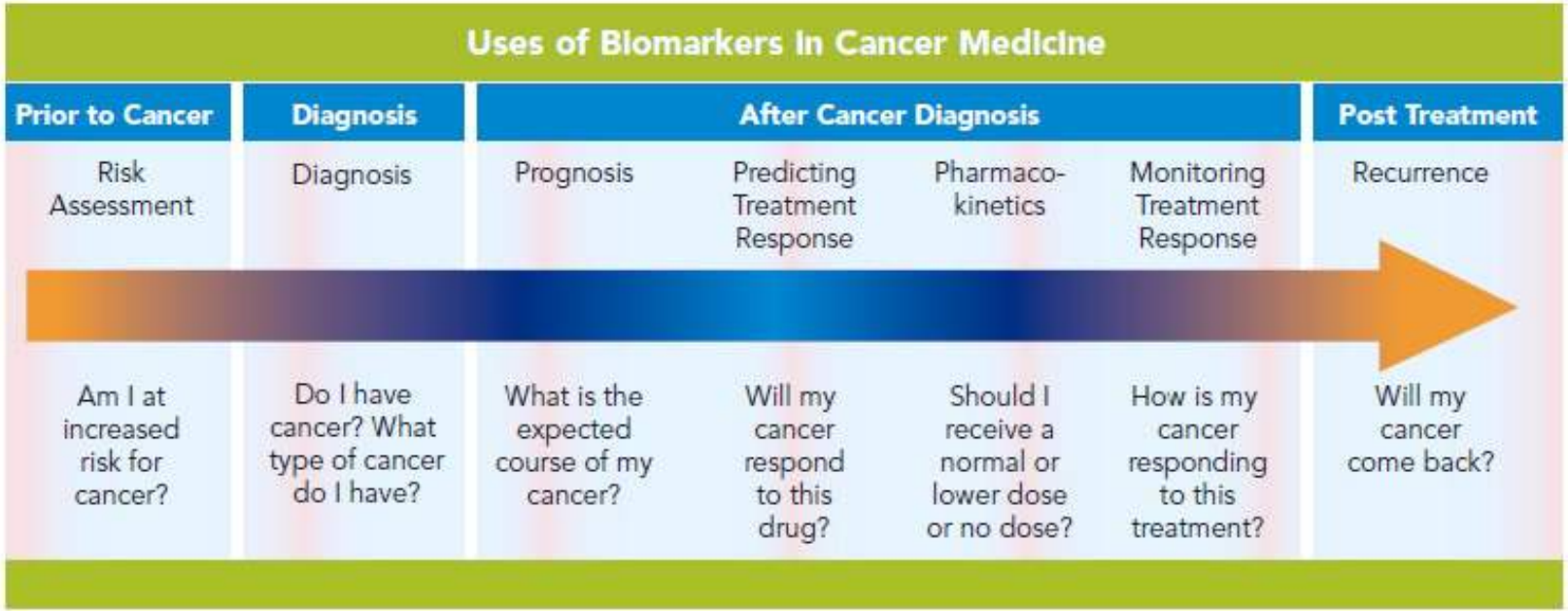


Fundación InstitutoRoche

Universitat de València

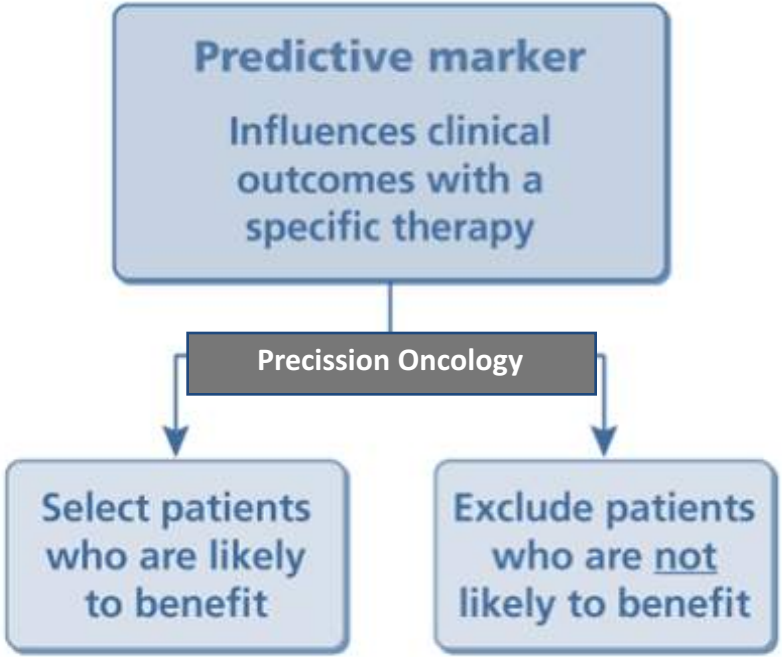
BIOMARCADOR

Característica que puede ser objetivamente medible y evaluable como un indicador de un proceso biológico normal, patológico o como respuesta farmacológica a una intervención terapéutica.



Biomarker Definitions Working Group, Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. Clin Pharmacol Ther 2001;69:89–95

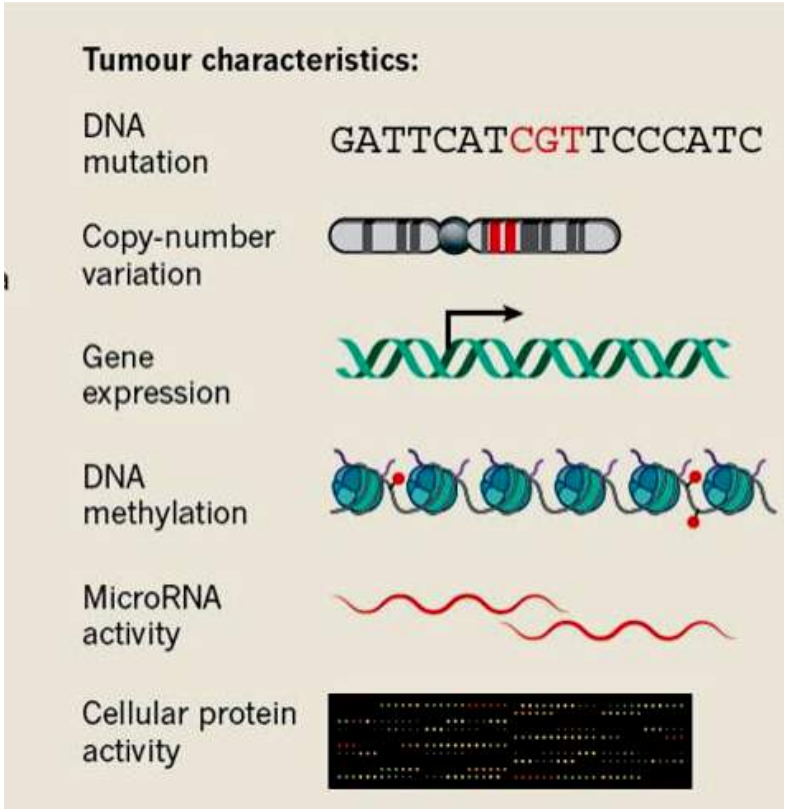
TIPOS DE BIOMARCADORES



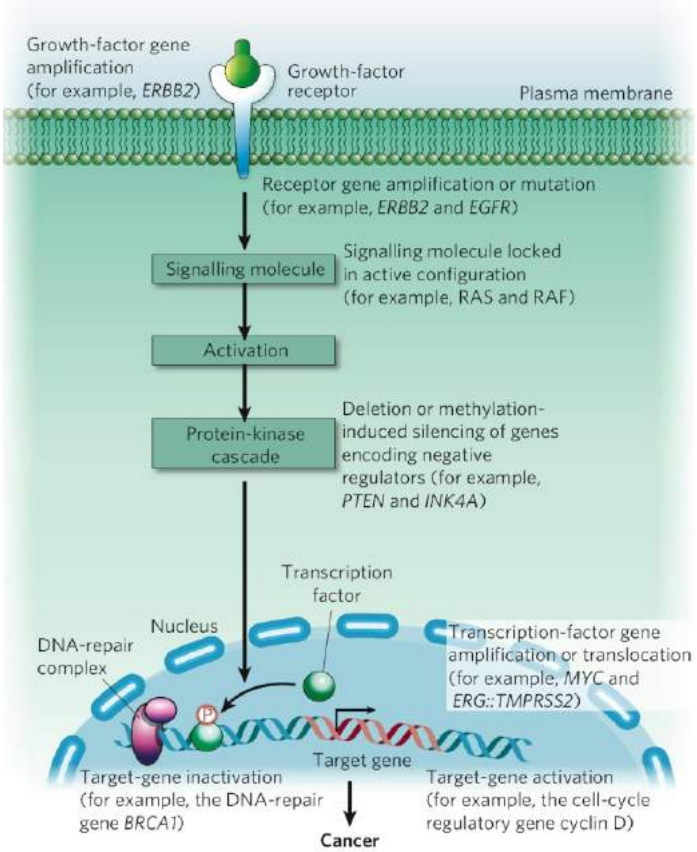
- Diagnóstico
- Pronóstico→ “efecto del tumor sobre la superv. del pte”
- Predictivo → “efecto del fármaco sobre la respuesta tumoral”

Biomarker Definitions Working Group, Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. Clin Pharmacol Ther 2001;69:89–95

BIOMARCADORES: Qué alteraciones buscamos?

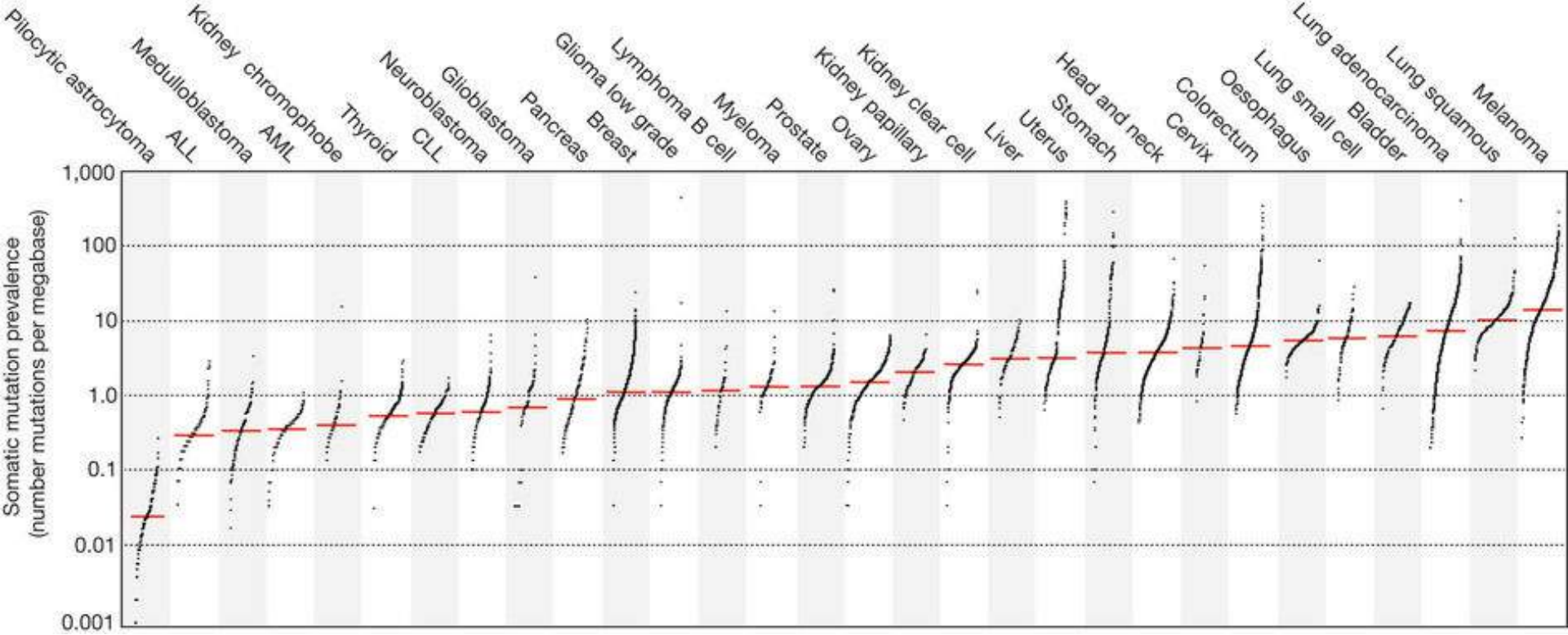


Hudson, T , Nature 502; 306-7, 2013



Chin, L. & Gray, J. *Insight Nature* 2008)

BIOMARCADORES: Qué alteraciones y cuántas buscamos?



Alexandrov L, Nature 2013

BIOMARCADORES: Qué alteraciones y cuántas buscamos?

GENOMAS DEL CÁNCER: complejos pero ….

CANCER LANDSCAPE

Small number of "mountains": genes altered in a high % of tumors

Larger number of "hills": genes altered infrequentely

>200 enfermedades

De **2 a 8** mutaciones "driver" **por tumor**

Dada su heterogeneidad **involucra 140 genes** (driver genes)

Vogelstein B.- Cancer Genome Landscape, Science 2013

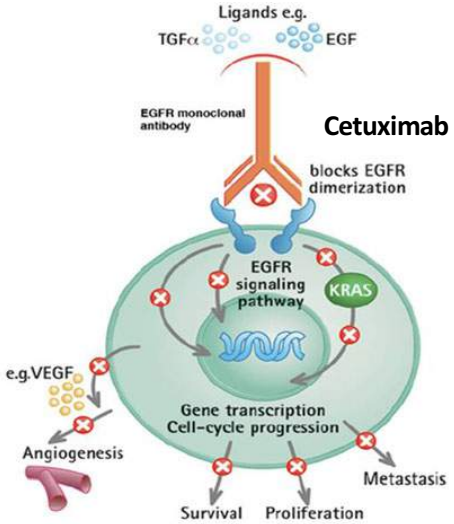
BIOMARCADORES: Que alteraciones buscamos?

Single biomarkers *vs.* signatures

Single biomarker

Colorectal cancer → ***KRAS* mutation**

Predictive biomarker for anti-EGFR therapy



Signature

mRNA signature of **70 genes**

that identify patients at risk of

breast cancer metastasis

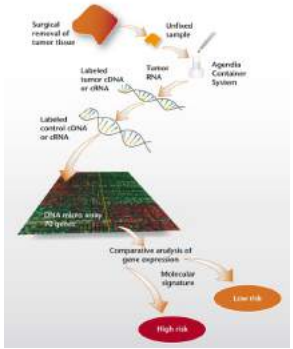


Table 2 Prognostic and diagnostic nucleic-acid based tumor biomarkers approved by the Center for Devices and Radiological Health (FDA)

Organ	Cancer	Biomarker target	Biomarker name	Tissue sampled	Method
Diagnosis					
Bladder	Bladder cancer	Aneuploidy for chromosomes 3, 7, 17 loss of 9p21	Vysis UroVysion Bladder Cancer Recurrence Kit	Urine	FISH
Breast	Breast cancer	MG, CK19	BLN assay	Sentinel lymph node	RT-PCR
Gastro-intestinal	Colorectal cancer	Multi-target DNA (aberrantly methylated BMP3 and NDRG4 promoter, mutant KRAS, and β-actin). Hemoglobin assay	Cologuard	Stool	PCR immunochemical assay for hemoglobin
Hemato-logical	B-cell chronic lymphocytic leukemia	alpha satellite (centromeric) region, 12p11.1-q11	CEP 12 SpectrumOrange Direct Labeled Fluorescent DNA Probe Kit	Peripheral blood	FISH
Ovary	Ovarian cancer	BRCA1 and 2 gene mutation	BRACAnalysis CDx	Blood	PCR
Prostate	Prostate cancer	PCA3	PROGENSA PCA3 Assay	Prostate biopsy, urine	PCR
Prognosis					
Breast	Breast cancer	58 gene RNA expression profile	Prosigna Breast Cancer Prognostic Gene Signature Assay	Tumor	nCounter system
		70-gene expression profile	Amsterdam 70-gene profile (MammaPrint)	Tumor	Agendia BV
		HER2	INFORM HER2 Dual ISH DNA Probe Cocktail; HER2 CISH pharmDxTM Kit DakoCytomation HER2 FISH pharmDx™ Kit	Tumor	ISH
		TOP2A	Dako TOP2A FISH PharmDx Kit	Tumor	FISH
Prostate	Prostate cancer	tPSA	NADiA ProsVue	Blood	PCR
Hemato-logical	Acute myeloid leukemia	EGR1	Vysis D7S486/CEP 7 FISH Probe Kit	Bone marrow peripheral blood	FISH
	B-cell chronic lymphocytic leukemia	TP53, ATM, D13S319 deletion D12Z3 gain	Vysis CLL FISH Probe Kit	Peripheral blood	FISH

BIOMARCADORES: Diagnósticos y pronósticos

Goossens et al, TCR 2015

BIOMARCADORES: Predictivos

Table 1 Predictive biomarkers in clinical use

Organ	Cancer	Biomarker and mechanism	Assay for measurement	Associated target and drug	Approximate proportion of positive tests	Stage of clinical validation	References
Breast	Breast cancer	HER2: oncogene overexpression	ISH, IHC	HER2: trastuzumab, pertuzumab, ado-trastuzumab emtansine	18-20%	In clinical use	(8-10)
		ER/PR: suggests sensitivity to endocrine therapy	IHC, LBA	ER: endocrine therapy (tamoxifen, aromatase inhibitors)	75%	In clinical use	(71)
Gastro-intestinal	Colorectal cancer	KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy	PCR	EGFR: cetuximab, panitumumab	40% mutated	In clinical use	(11,72)
	GIST	KIT: mutation leads to consitutional activation	IHC	BCR-ABL: imatinib	95%	In clinical use	(73)
	Esophago-gastric adenocarcinoma	HER2: oncogene overexpression	ISH, IHC	HER2: trastuzumab	7-22%	In clinical use	(70)
Hematological	Chronic myeloid leukemia	BCR-ABL: balanced t(9;22) leading to the formation of a constitutively active tyrosine kinase	Cytogenetics, FISH, RT-PCR	BCR-ABL: imatinib, dasatinib, nilotinib	>90%	In clinical use	(74)
	Acute promyelocytic leukemia	PML-RARa: balanced t(15;17) leading to aberrant retinoid receptor	Cytogenetics, FISH, RT-PCR	PML-RARa: All-trans retinoic acid	>90%	In clinical use	(75)

Goossens et al, TCR 2015

BIOMARCADORES: Predictivos

Lung	NSCLC	EGFR (HER1): mutations in tyrosine kinase domain	Sequencing, ISH	EGFR: Erlotinib, gefitinib, afatinib	15% adenocarcinomas in USA (higher in Asians, women and nonsmokers)	In clinical use	(76)
		ALK: Inversion in chromosome 2 leads to EML4-ALK fusion oncogene	FISH (IHC)	ALK: crizotinib, ceritinib (alectinib under development)	4% (mostly adenocarcinoma)	In clinical use	(77)
	Lung adenocarcinoma	Multiple genes:				Continued validation	(49)
		BRAF (V600E and non-V600E)	Multiplex sequencing	BRAF: AZD6244	2%		
		EGFR (HER1): mutations in tyrosine kinase domain		EGFR: erlotinib, gefitinib, afatinib, cetuximab	17%		
		HER2: oncogene overexpression		HER2: decinutubub, neratinib, lapatinib, trastuzumab	3%		
		KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy		KRAS: erlotinib, tivantinib, everolimus, ridaforalimus, AZD6244	25%		
		ALK: inversion in chromosome 2 leads to EML4-ALK fusion oncogene		ALK: crizotinib, ceritinib	8%		
		MET		MET: cizotinib	<1%		
Skin	Melanoma	BRAF V600: 80-90% V600E mutation, a downstream mediator of RAS, leads to downstream activation of MEK and ERK	Sequencing	BRAF: vemurafenib, dabrafenib	40-60%	In clinical use	(78)

HER2, human epidermal growth factor 2; (F)ISH, (fluorescence) in situ hybridization; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; LBA, ligand binding assay; MEK, mitogen-activated protein kinase kinase; EGFR, epidermal growth factor receptor; (RT-)PCR, (reverse transcription-) polymerase chain reaction; GIST, gastrointestinal stromal tumor; PML, promyelocytic leukemia gene; RARa, retinoic acid receptor-alpha; NSCLC, non-small cell lung cancer; ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; ERK, extracellular-signal-regulated kinases.

Goossens et al, TCR 2015

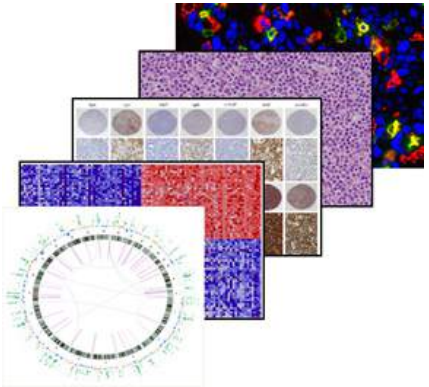
Dianas en Oncología: Ejemplo cáncer de pulmón

Genomic Alteration (i.e. driver event)	Available targeted agents with activity against driver event in lung cancer*
***EGFR* mutations**	erlotinib, gefitinib, afatinib, osimertinib
***ALK* rearrangements**	crizotinib, alectinib …
***HER2* mutations**	trastuzumab, afatinib
***BRAF* V600E mutations**	vemurafenib, dabrafenib + trametinib
***MET* amplification/mutation**	crizotinib
***ROS1* rearrangements**	crizotinib, alectinib
***RET* rearrangements**	cabozantinib
***TRK* rearrangements**	entrectinib

*Indicates recommended use in the NCCN Drugs and Biologics Compendium

ONCOLOGÍA DE PRECISIÓN

Dónde ?



Fundación InstitutoRoche

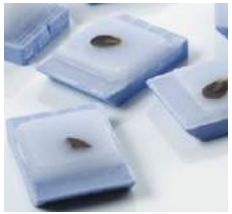
Universitat de València

Biomarcadores en cáncer : Dónde buscar?

Muestra de tejido tumoral

Acidos nucleicos **Proteinas** **Lipidos,** **Metabolitos**

EL PARADIGMA: *THE TISSUE IS THE ISSUE*



Las biopsias (FFPE) se consideran el estándar para las pruebas moleculares en la oncología de precisión

Sin embargo, en la práctica tiene algunas limitaciones

El procedimiento tradicional está bien establecido, pero ….

Invasive procedure

Obtain tumor tissue block

Selection bias

Tumor heterogeneity

Manual micro-dissection

DNA isolation & purification

Tissue not always accessible

Lo Frecuente — Una Rareza

Assessment of DNA quantity

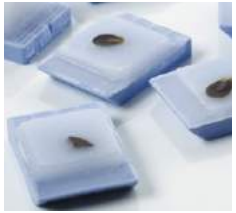
Assessment of gene status

Treatment decision

Archival tissue

Static, potentially outdated mutation profile, often degraded or unavailable

BIOPSIAS DE TEJIDO TUMORAL



Limitaciones

Limitaciones Biológicas

-La disponibilidad de tejido varía desde el 90% en CCR, 50-70% en cáncer de pulmón hasta un 0% en ciertos tumores (HCC, tumores cerebrales)

-Los tumores son heterogéneos (heterogeneidad intratumoral, entre primario y metastásico)

-Plasticidad de los tumores -> cambios fenotípicos y moleculares

-Baja prevalencia de ciertas mutaciones en ciertos tipos tumorales.

Limitaciones logísticas

-Baja calidad de DNA/ RNA

-Muestras invasivas, costosa, discomfort del paciente - > complicaciones en la toma de las muestras (2- 15%)

-Estática -> *snap shot*.

-No podemos ver los cambios tras terapia (exige rebiopsiar) -> biopsias seriadas son raras y difíciles de realizar de manera rutinaria

NECESIDAD de NUEVAS ESTRATEGIAS

BIOPSIA LÍQUIDA

Una **biopsia líquida** puede ser cualquier fluido biológico, como la sangre, orina, ascitis, líquido pleural, etc ., en el cual pueda determinarse un biomarcador y que al **igual que en una biopsia de tejido, es representativa del tejido/s en el que ese biomarcador se ha producido**

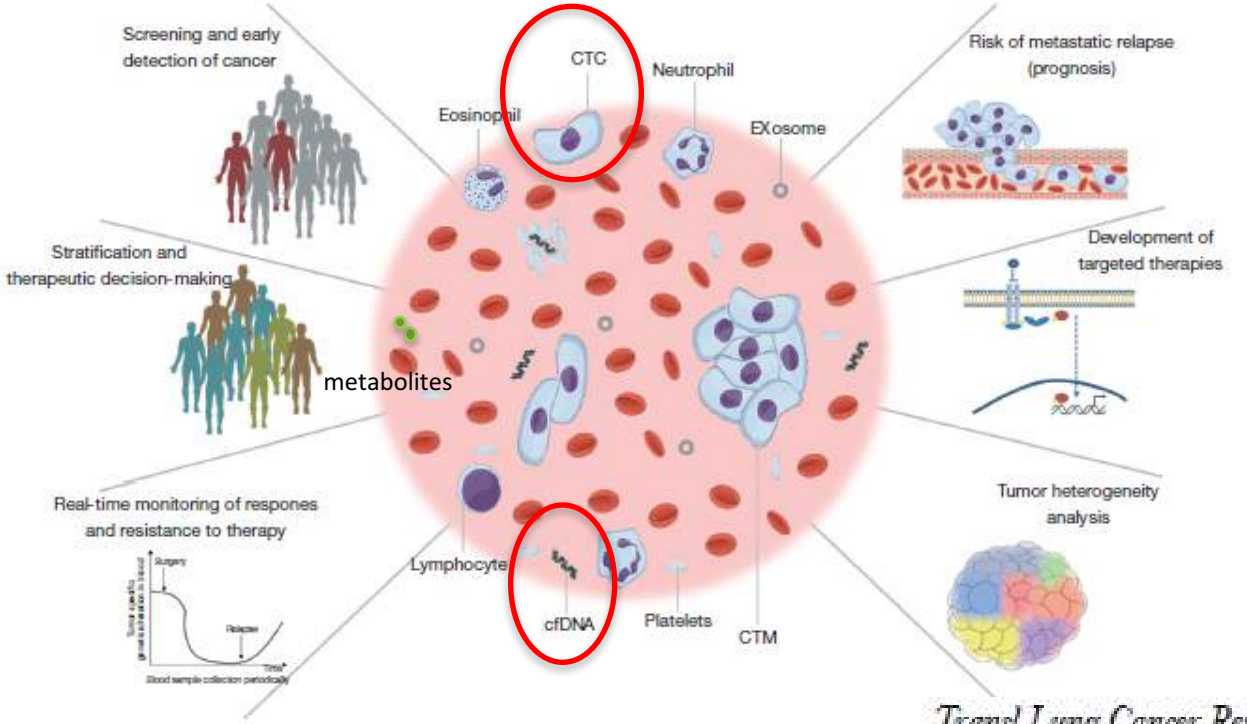
↑Cuando sea posible-> sangre

↓ Aún es necesario trabajar en: estandarización pre-analítica (recolección, procesamiento, condiciones de almacenamiento)

Circulating tumor cells versus circulating tumor DNA in lung cancer—which one will win?

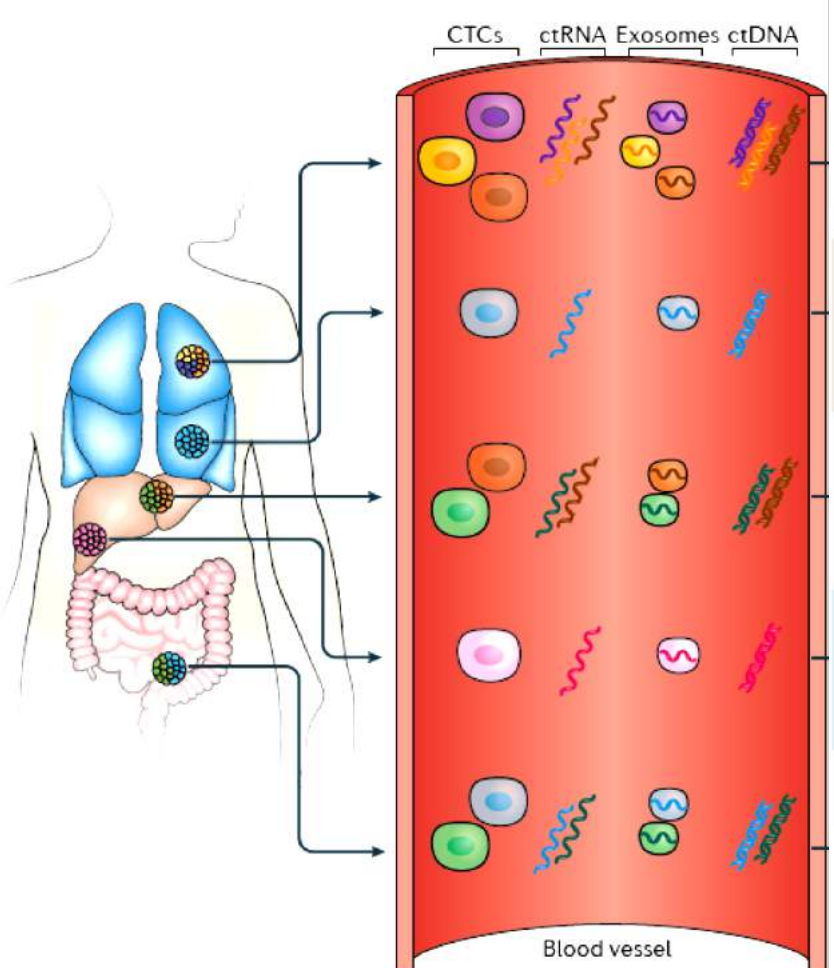
Silvia Calabuig-Fariñas[1,2*], Eloísa Jantus-Lewintre[1,3*], Alejandro Herreros-Pomares[1,3], Carlos Camps[1,4,5]

Calabuig-Fariñas et al. CTC *vs.* cfDNA

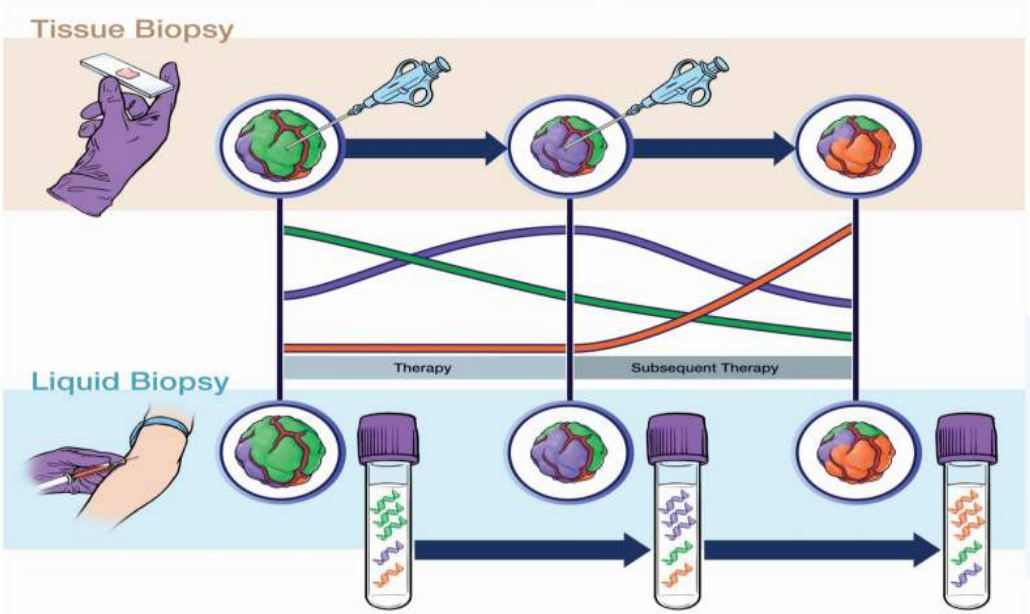


Transl Lung Cancer Res 2016;5(5):466-482

BIOPSIA LIQUIDA: Por qué ?



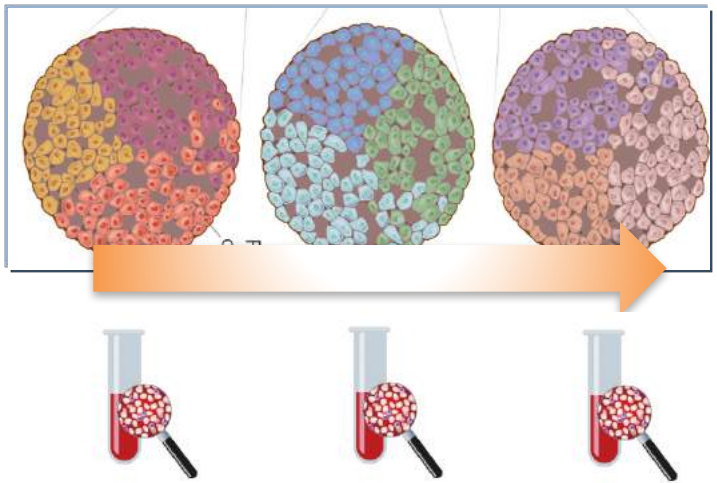
HETEROGENEIDAD

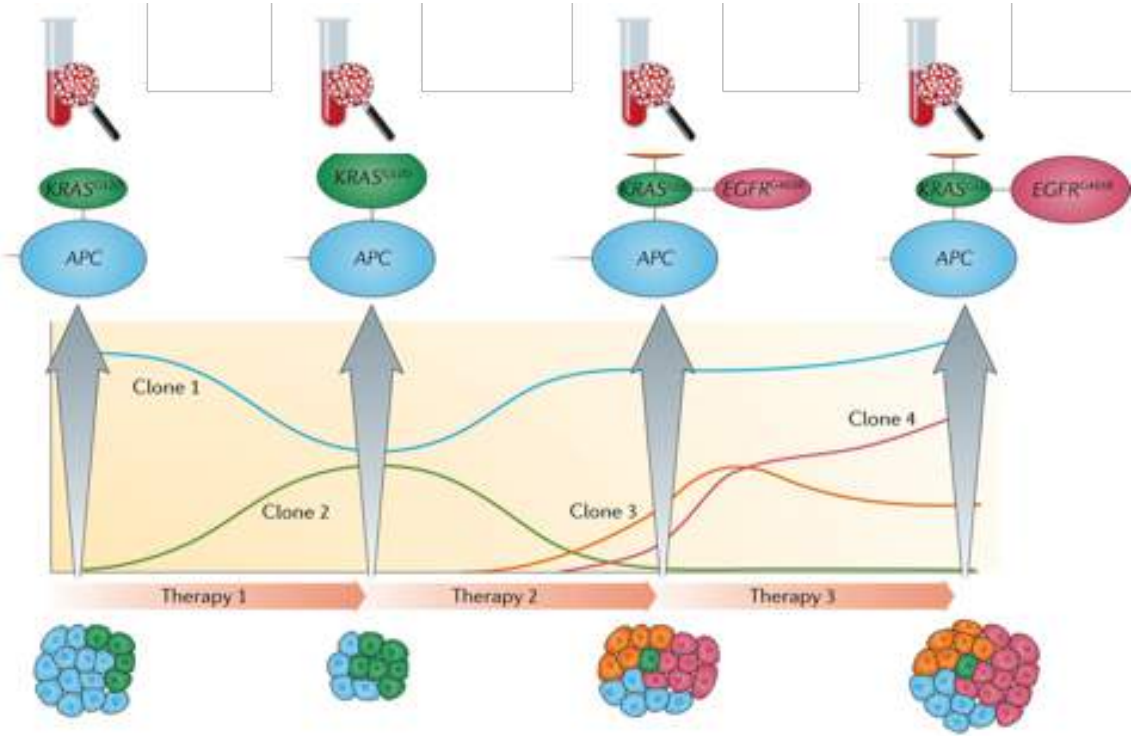


Siravegna et al , Nat Rev Clin Oncol , 2017

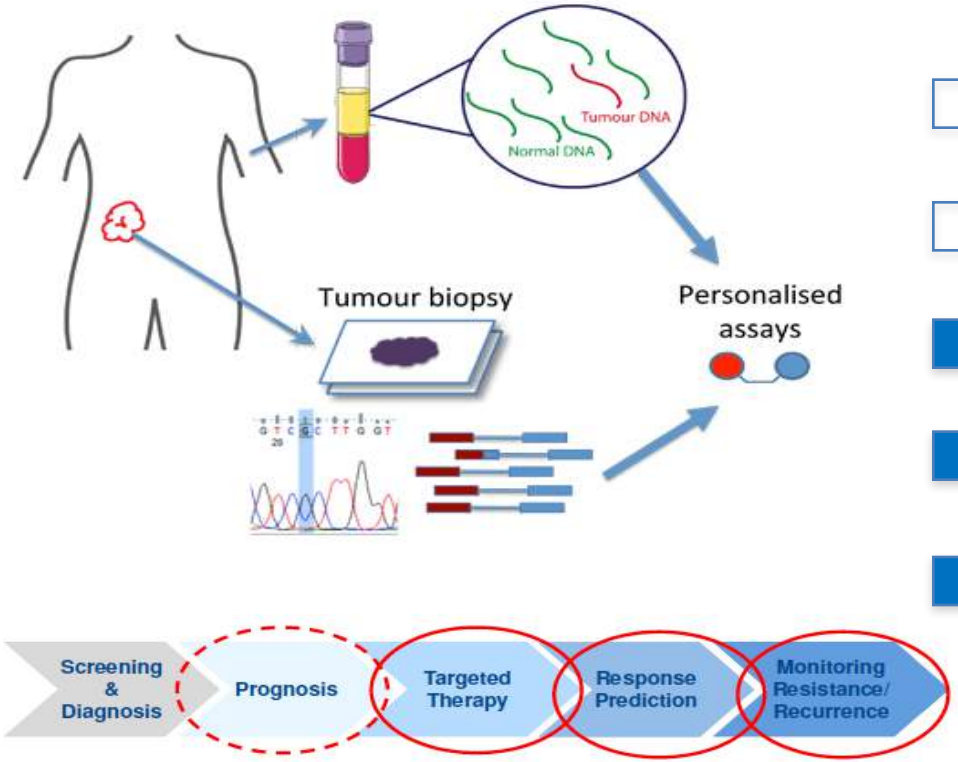
BIOPSIA LIQUIDA: Por qué ?

PLASTICIDAD -> Cambios evolutivos





BIOPSIA LIQUIDA: Para qué ?



↑ Posibilidad de monitorizar la carga tumoral (marcador subrogado).

↑ ↓ Detección temprana de enfermedad
Enfermedad mínima residual.

↑ Detección de estado mutacional

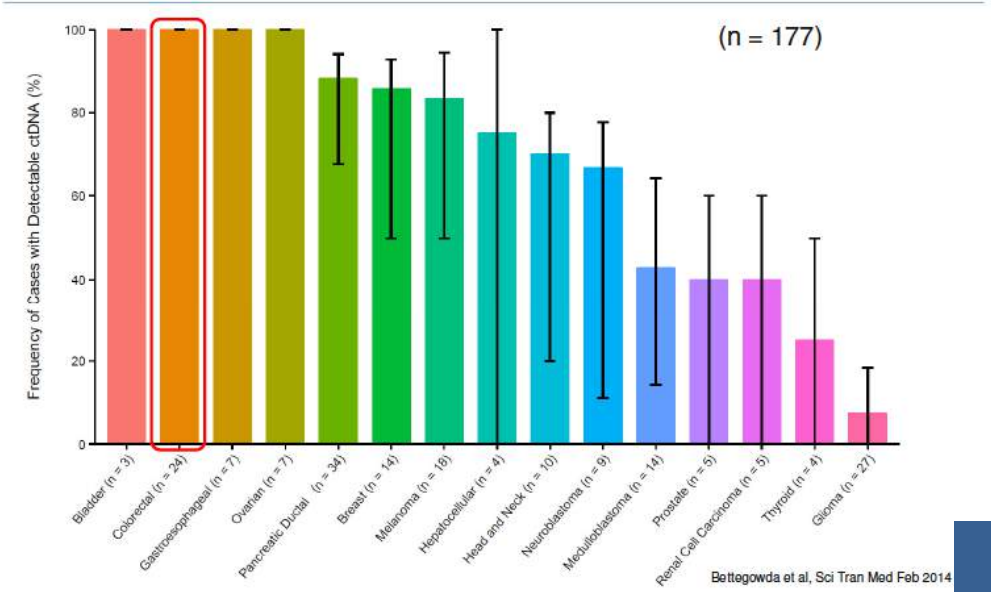
↑ Monitorización tto. Detección temprana de resistencias

↑ Permiten obtener una mejor representación
-De todo el tumor (heterogeneidad tumoral)
-De toda la enfermedad (tumor + metástasis)

Lovly et al. My Cancer Genome 2016

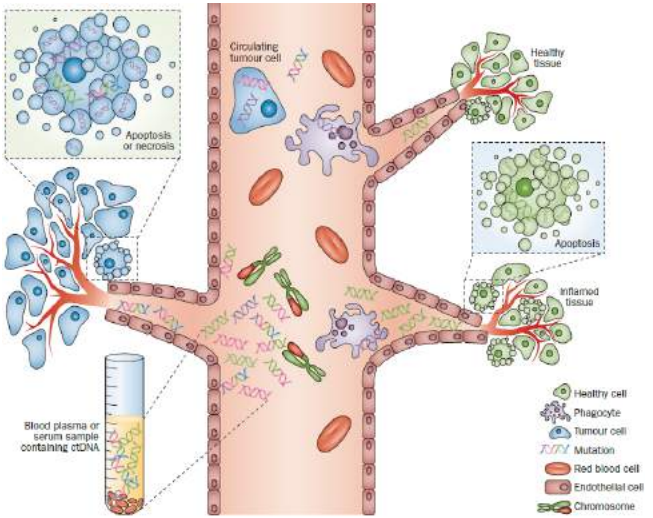
BIOPSIA LIQUIDA: ASPECTOS A CONSIDERAR

- La cantidad de ctDNA en plasma puede variar, dependiendo de:
  - Tipo de tumor
  - Estadio tumoral (localizado, diseminado)
  - Carga tumoral



Bettegowda et al, Sci Tran Med Feb 2014

- Existe una gran proporción de DNA no tumoral circulando en el plasma, y solo pequeñas cantidades de ctDNA (que puede contener mutaciones)

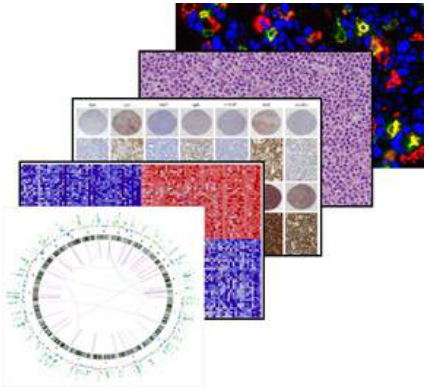


SE REQUIEREN TECNICAS DE GRAN SENSIBILIDAD

BIOPSIA LIQUIDA: ASPECTOS A CONSIDERAR

ONCOLOGÍA DE PRECISIÓN

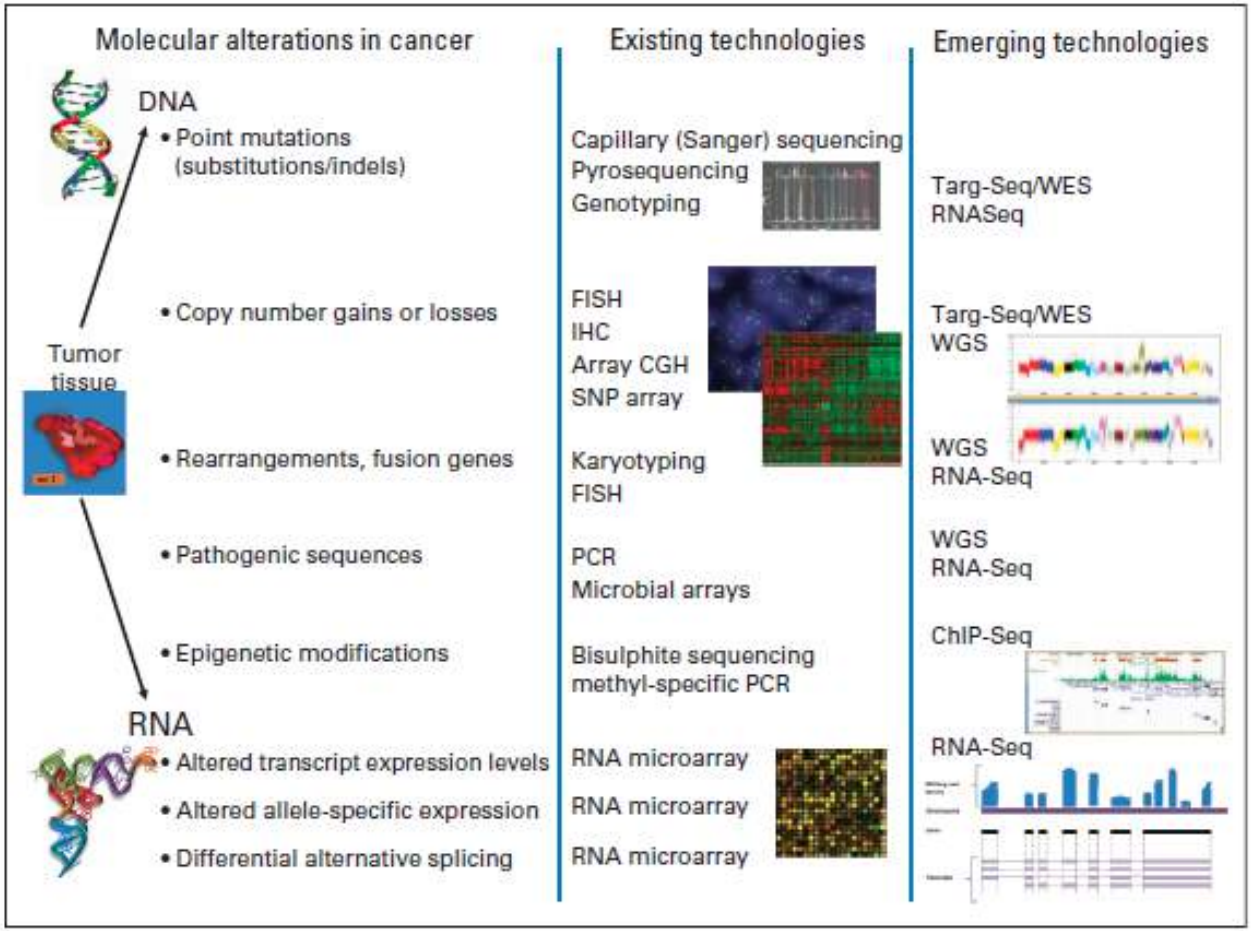
Cómo ?

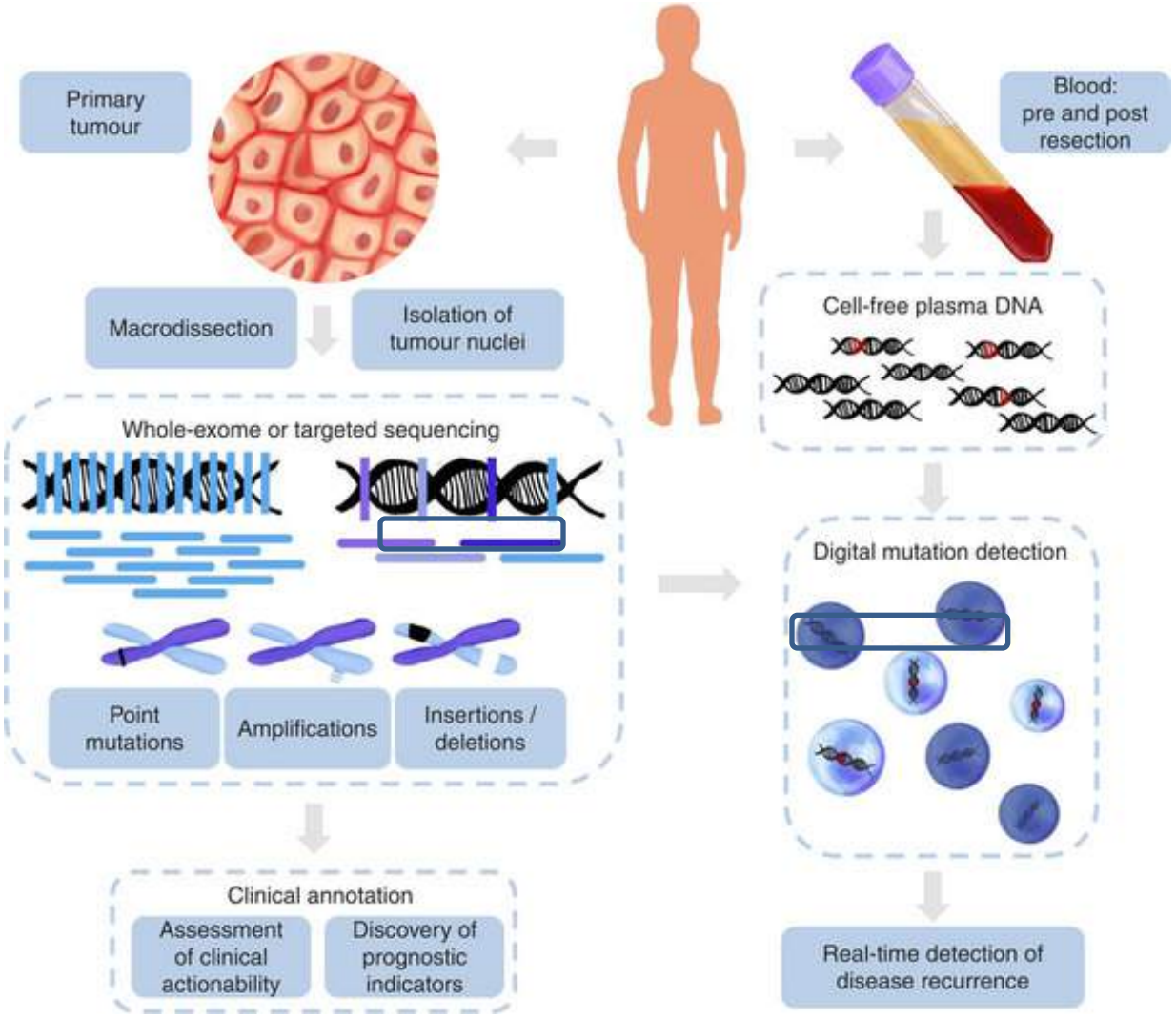


Fundación InstitutoRoche

VNIVERSITAT DE VALÈNCIA

Tecnologías para él análisis de biomarcadores




Primary tumour
Blood: pre and post resection
Macrodissection
Isolation of tumour nuclei
Cell-free plasma DNA
Whole-exome or targeted sequencing
Digital mutation detection
Point mutations
Amplifications
Insertions / deletions
Clinical annotation
Assessment of clinical actionability
Discovery of prognostic indicators
Real-time detection of disease recurrence

Metodología

Technique	Sensitivity	Optimal Application
Sanger sequencing	> 10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation seqeuncing	2%	Tumor tissue
Quantative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
BEAMing, PAP, Digital PCR, TAM-Seq	0.01% or lower	ctDNA, rare variants in tumor tissue

- Qué técnica?
- Cuál/ Cuáles y cuántos genes debo analizar?
- Sensibilidad?
- Problemas de estandarización
- Control de calidad

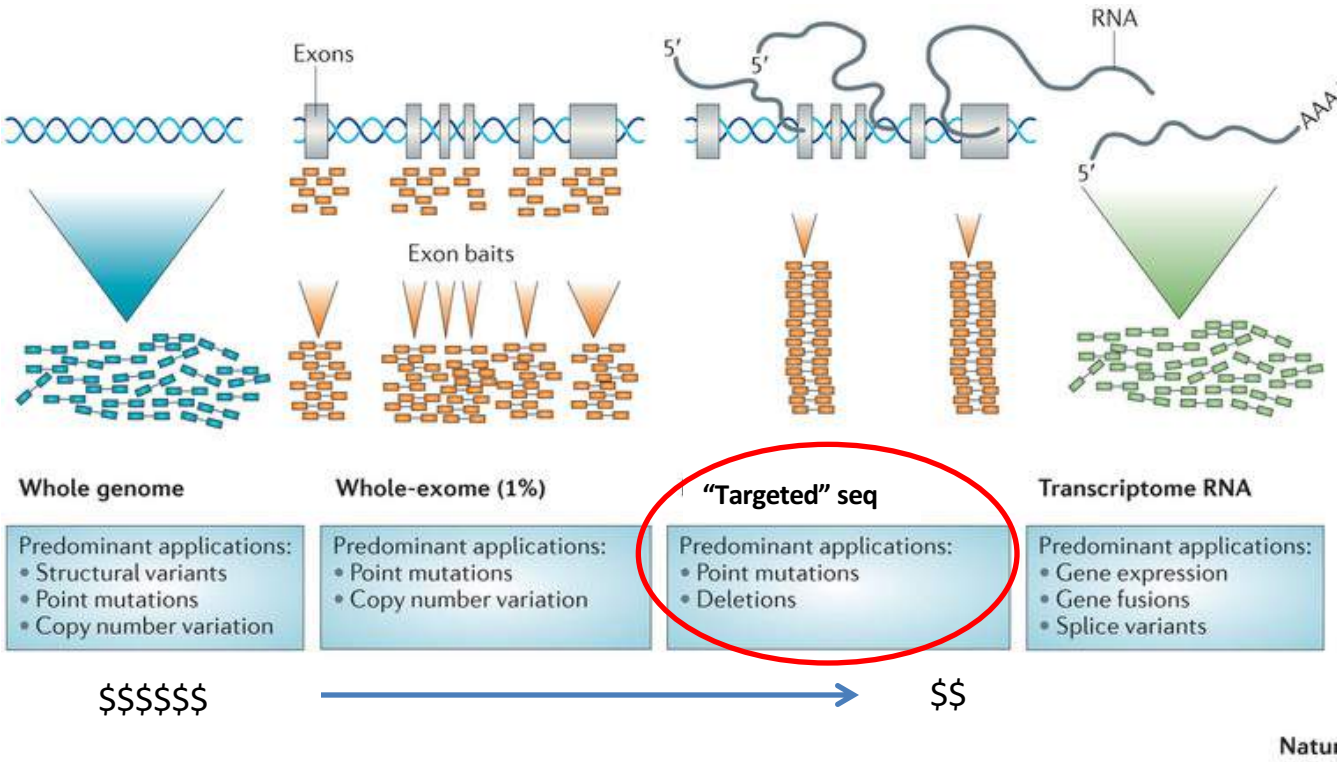
Diaz and Bardelli.- J Clin Oncol 2014

Metodología

Technique	Sensitivity	Optimal Application
Sanger sequencing	> 10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation seqeuncing	2%	Tumor tissue
Quantative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
BEAMing, PAP, Digital PCR, TAM-Seq	0.01% or lower	ctDNA, rare variants in tumor tissue

Diaz and Bardelli.- J Clin Oncol 2014

NGS: que tipo de información genera??



Richard Simon & Sameek Roychowdhury; Nature Reviews Drug Discovery 12, 358–369 (2013)

METODOLOGIAS: NGS (+++ información)

Table 1: Gene Content on TruSight Tumor 15

AKT1	*GNA11*	*NRAS*
BRAF	*GNAQ*	*PDGFRA*
EGFR	*KIT*	*PIK3CA*
ERBB2	*KRAS*	*RET*
FOXL2	*MET*	*TP53*

Oncomine Solid Tumor DNA Kit

Oncomine Solid Tumor Fusion Transcript Kit

EGFR, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7, TP53	*ALK, RET, ROS1, NTRK1*
22 genes	4 fusions

Categorized by somatic alteration type — **52 genes** — **Categorized by published relevance**

Hotspot mutations
AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1, SMO

Focal CNV gains
ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA, PIK3CA

Fusion drivers
ABL1, AKT3, ALK, AXL, BRAF, EGFR, ERBB2, ERG, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, MET, NTRK1, NTRK2, NTRK3, PDGFRA, PPARG, RAF1, RET, ROS1

Labels
ALK, BRAF, EGFR, ERBB2, KRAS, NRAS, ROS1

Guidelines
ALK, BRAF, EGFR, ERBB2, KIT, KRAS, MET, NRAS, PDGFRA, RET, ROS1

Known drug targets
ABL1, AKT1, AKT3, ALK, AR, AXL, BRAF, CCND1, CDK4, CDK6, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, MYC, MYCN, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PPARG, RAF1, RET, ROS1, SMO

Oncomine Comprehensive

143/161 genes: Several used in multiple applications (hotspot, CNV, driver fusion)

Categorized by somatic alteration type — **143/161 genes** — **Categorized by relevant evidence**

- **73/87** Genes with hotspot mutations
- **49/43** Genes with focal CNV gains
- **26/48** Genes with Full CDS for DEL mutations
- **22/51** Gene-fusion drivers

- **9/9** Genes on **35/36** Labels
- **12** Genes in **17/19** Guidelines
- **81/117** Genes are used in **>800** global clinical trials

NGS: que tipo de información genera??

Nuestra experiencia

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

TruSight® Tumor 15

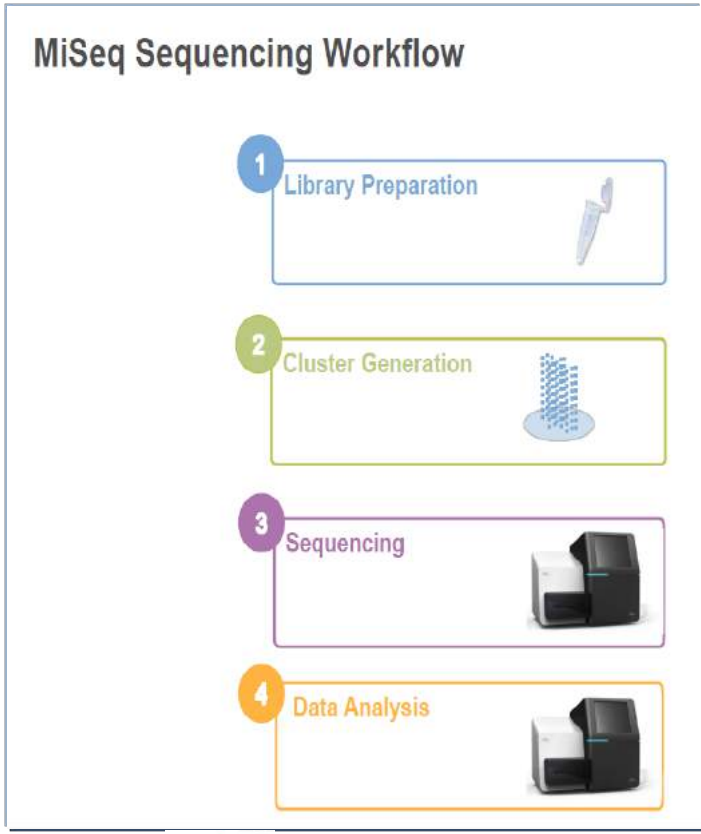
DNA716297

Sample Name: DNA716297
Run Name: 25102016
Run ID: 161025_M04274_0005_000000000-ALALW
Report Definition: TST_15-ReportDefinition-v1
Report Generated On: 2016-10-26 20:32

Variants identified as specified in the report definition

Detected SNVs, Insertions, and Deletions

Gene	Amino Acid Change	Variant Type	Nucleotide Change	Variant Frequency	Transcript
EGFR	p.Leu747_Thr751del	inframe deletion	c.2238_2252delATTAAGAGAAGCAAC	0.165	ENST00000275493
TP53	p.Arg337Leu	missense variant	c.1010G>T	0.221	ENST00000269305



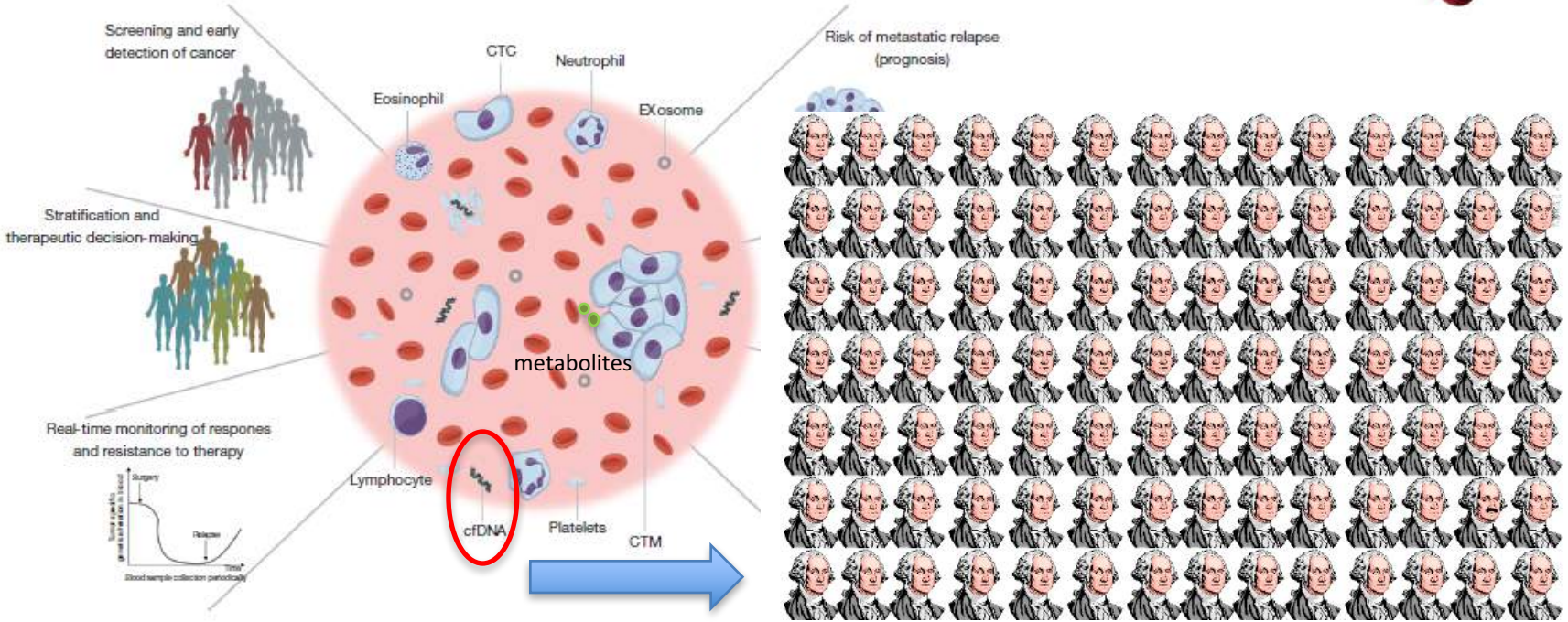
DATA STORAGE

Camps & Jantus Lab, 2016

Circulating tumor cells versus circulating tumor DNA in lung cancer—which one will win?

Silvia Calabuig-Fariñas[1,2*], Eloísa Jantus-Lewintre[1,3*], Alejandro Herreros-Pomares[1,3], Carlos Camps[1,4,5]

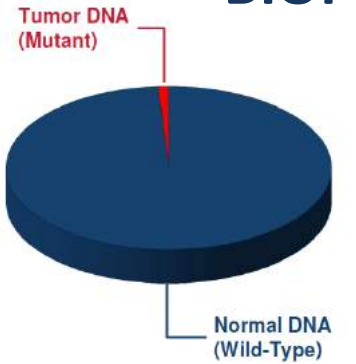
Calabuig-Fariñas et al. CTC *vs.* cfDNA



Transl Lung Cancer Res 2016;5(5):466–482

BIOPSIA LÍQUIDA: Aproximaciones metodológicas

ctDNA



FROM YES/ NO

TO **How much**

Biology of release

Tumor | Metastasis | Healthy/inflamed tissue

Apoptosis | Necrosis | Secretion

Circulating tumor DNA

Structural rearrangements

Mutations

Plasma DNA

Copy number aberrations

Methylation

Tumor fraction of cfDNA

Low tumor fraction <1% >10% High tumor fraction

Detection of ctDNA

- dPCR (Ref. 65,66)
- BEAMing (Ref. 52)
- Safe-SeqS (Ref. 68)
- Capp-Seq (Ref. 67)
- TAm-Seq (Ref. 71)

Sensitivity

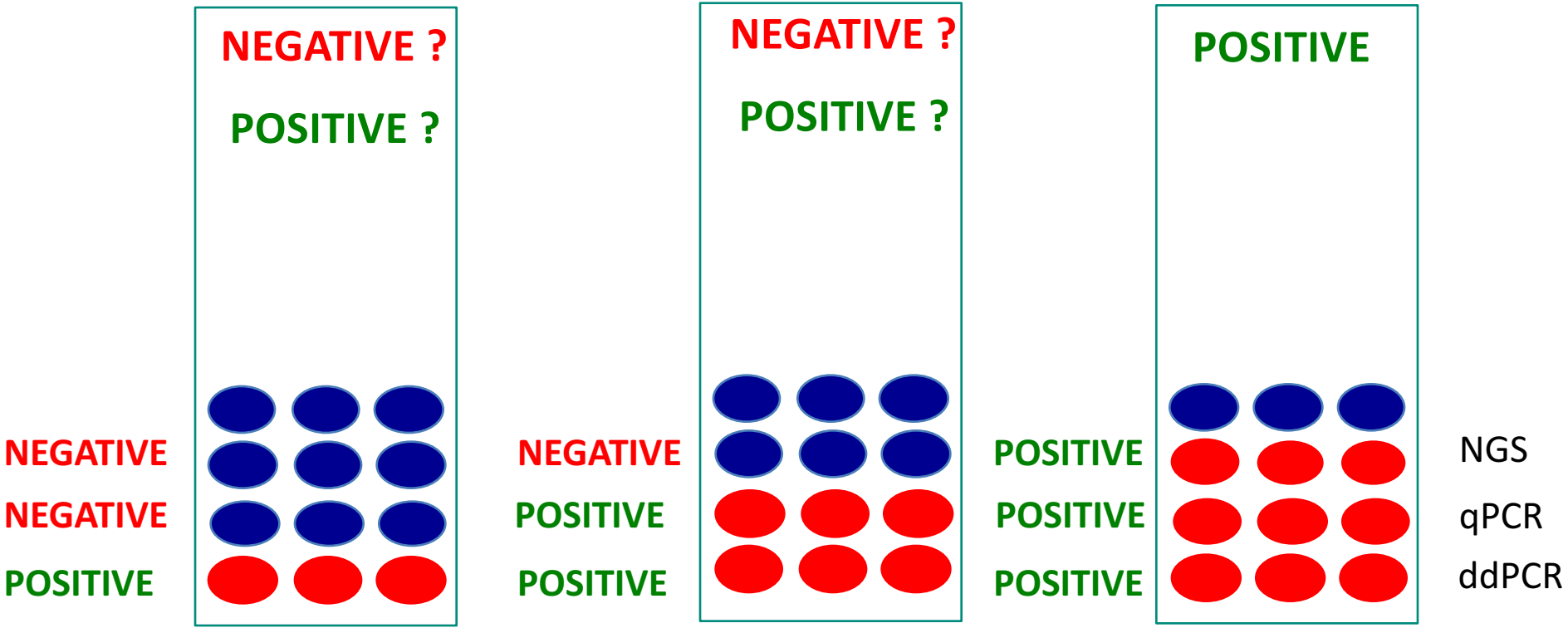
- WGS (Ref. 11)
- Exome (Ref. 72)
- Plasma-Seq (Ref. 69)
- PARE (Ref. 46)
- Whole Methylome (Ref. 10)

Clinical value

Diagnosis | Prognosis | Treatment

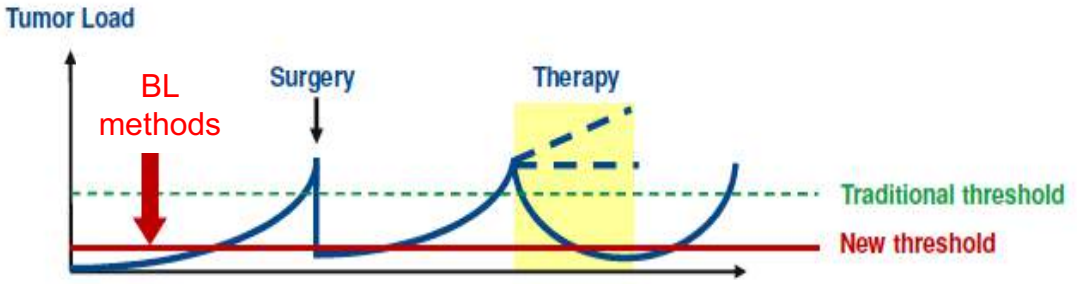
Heitzer, Clin Chem 2015

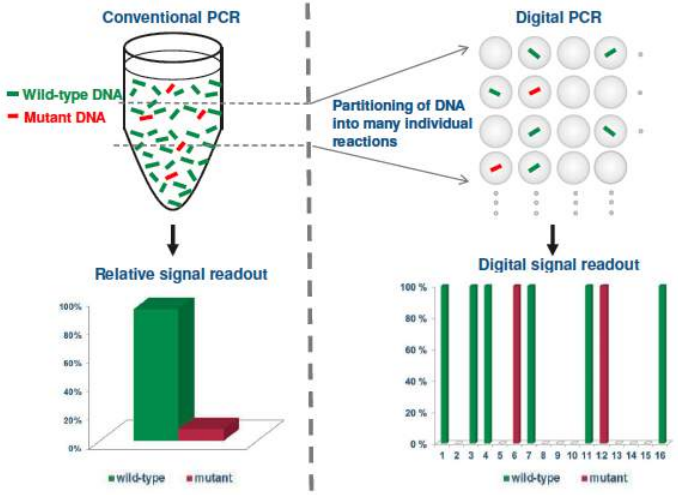
ASPECTOS METODOLÓGICOS TIENEN IMPLICACIONES



Threshold of positivity impacts in conclusions
Clinical relevance of “subclonal” detection

BIOPSIA LÍQUIDA: Métodos sensibles



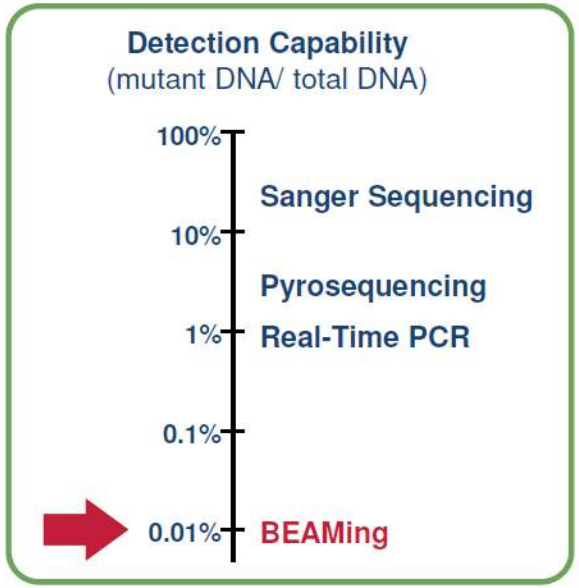


Vogelstein *et al.* PNAS 1999; mod. Diehl *et al* Curr Opin Oncol. 2007

We must be SCRUPULOUS in the analytical phase to avoid erroneous results

Clinically irrelevant molecular changes due to the high sensitivity

BEAMing Lab Requirements and Contamination Prevention



Li et al, Nature Methods, 2006

Unidirectional Workflow

Laboratory Space Setup

Pre-Analytical Area	Pre-PCR Lab	Post-PCR Lab
• Blood Draw • Plasma Preparation • Not required, but highly recommended	• DNA Extraction • Multiplex PCR Setup	• Amplification (Thermal Cycler) Area • Emulsion PCR Setup • Breaking and Hybridization Area • Detection (Flow Cytometry) Area

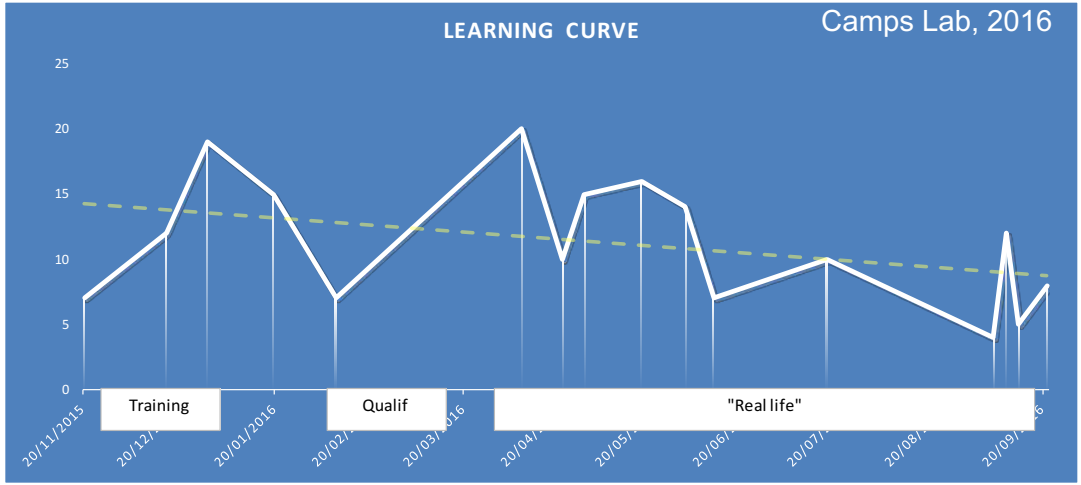
BIOPSIA LÍQUIDA: IMPLEMENTACION

ctDNA

Training

Qualification

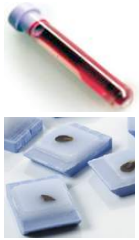
Learning curve



Validation

CONCORDANCIA DE LA DETECCIÓN DE MUTACIONES

RAS EN PLASMA vs TEJIDO EN CCRm



Estudio restrospectivo → 1. Plasma almacenado, previo tto (protocolo propio)
2. Determinación en tejido (técnica estandar: Pirosecuenciación)

N=30 CRC Stage IV

		Tissue RAS status		
		MUT	WT	Total
Plasma RAS status	MUT	19	1	20
	WT	2	7	9
	Total	21	8	29

1 sample= invalid (low DNA)

$$\textbf{Overall agreement} = \frac{a+d}{a+b+c+d} = \frac{(19+7)}{29} = \rightarrow \textbf{89,7\%}$$

$$\textbf{Positive agreement} = \frac{a}{a+c} = \frac{19}{21} = \rightarrow \textbf{90,5\%}$$

$$\textbf{Negative agreement} = \frac{d}{d+b} = \frac{7}{8} = \rightarrow \textbf{87,5\%}$$

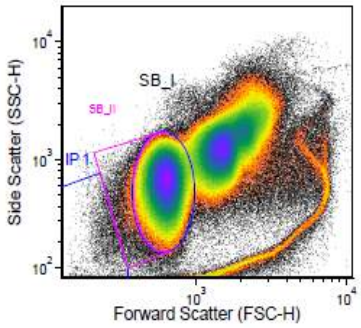
Camps & Jantus Lab, 2016

BEAMing Flow Cytometry Analysis
KR2Cdn12_KR2Cdn13_2016_02_16_V1.0P

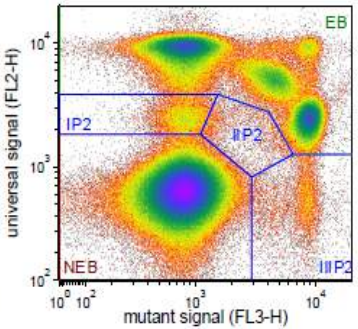
sysmex

File name:	2016_05_19_12_44_11_PLACA_PACIENTES_5_19052016-B01-29170000Sample2KR2_Cdn12__					
Run ID	Well	Sample name	Tested Mutation	Sample	Class	Cytometer
PLACA_PACIENTES_5_19052016	B01	29170000	KR2_Cdn12__	Sample2	sample	1508002743
mutation detected						

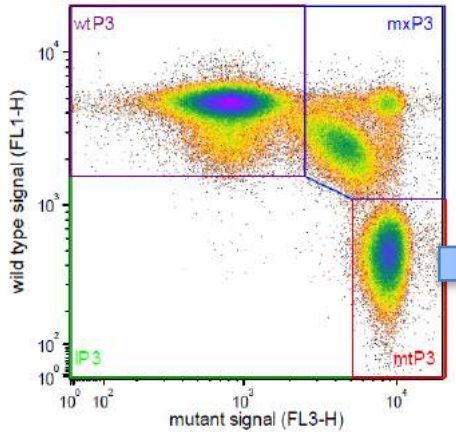
Single beads



Extended beads



Wild type vs. mutant beads



KRAS-12 MUT

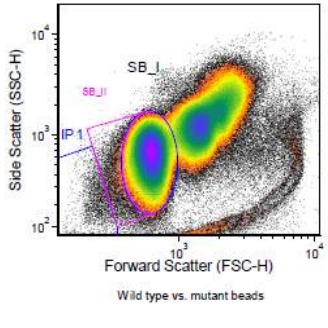
AP14 (mutant beads)	43582	Pass
AP15 (mutant fraction)	28,042%	Pass
MVC Valid?		PASS
Test Status		mutation detected

BEAMing Flow Cytometry Analysis
KR2Cdn12_KR2Cdn13_2016_02_16_V1.0P

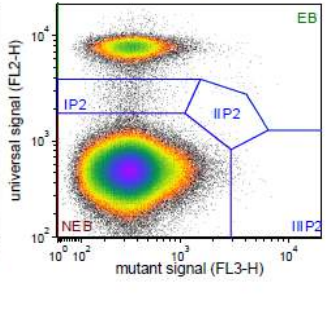
sysmex

File name:	2016_05_19_13_15_26_PLACA_PACIENTES_5_19052016-F02-36000000Sample6KR2_Cdn13__f					
Run ID	Well	Sample name	Tested Mutation	Sample	Class	Cytometer
PLACA_PACIENTES_5_19052016	F02	36000000	KR2_Cdn13__	Sample6	sample	1508002743
no mutation detected						

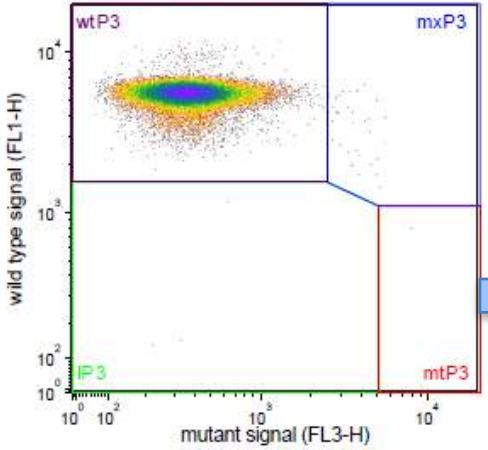
Single beads



Extended beads



Wild type vs. mutant beads

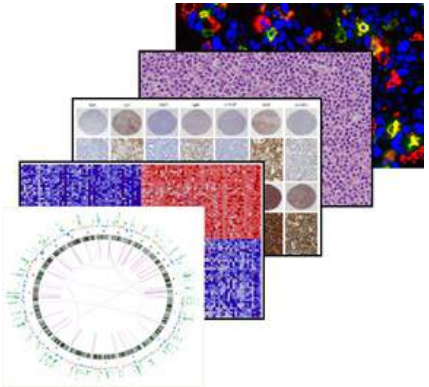


KRAS-13 WT

AP14 (mutant beads)	1	Pass
AP15 (mutant fraction)	0,003%	Pass
MVC Valid?		PASS
Test Status		no mutation detected

ONCOLOGÍA DE PRECISIÓN

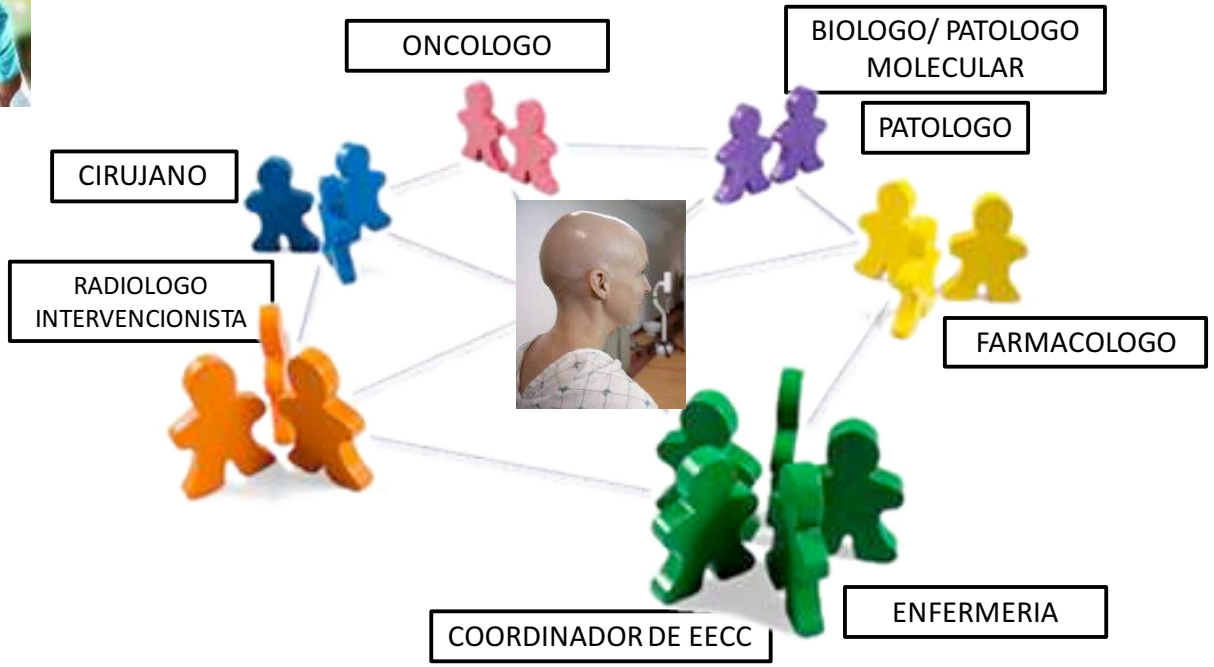
Quién ?



Fundación InstitutoRoche

Universitat de València

EQUIPOS MULTIDISCIPLINARES



MUESTRAS ADECUADAS
DATOS CLINICOS

Clinical data

Blood samples

TECNOLOGIA

Cube 6i flow cytometer

illumina

ion torrent

Roche

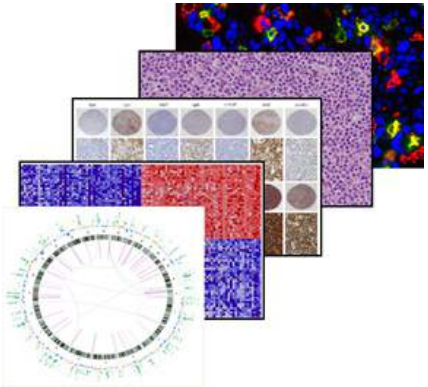
CALIDAD

EMQN

The European Molecular Genetics Quality Network

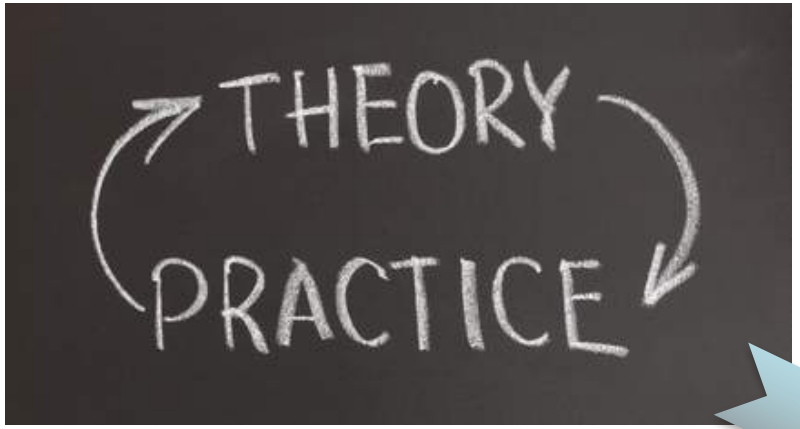
ONCOLOGÍA DE PRECISIÓN

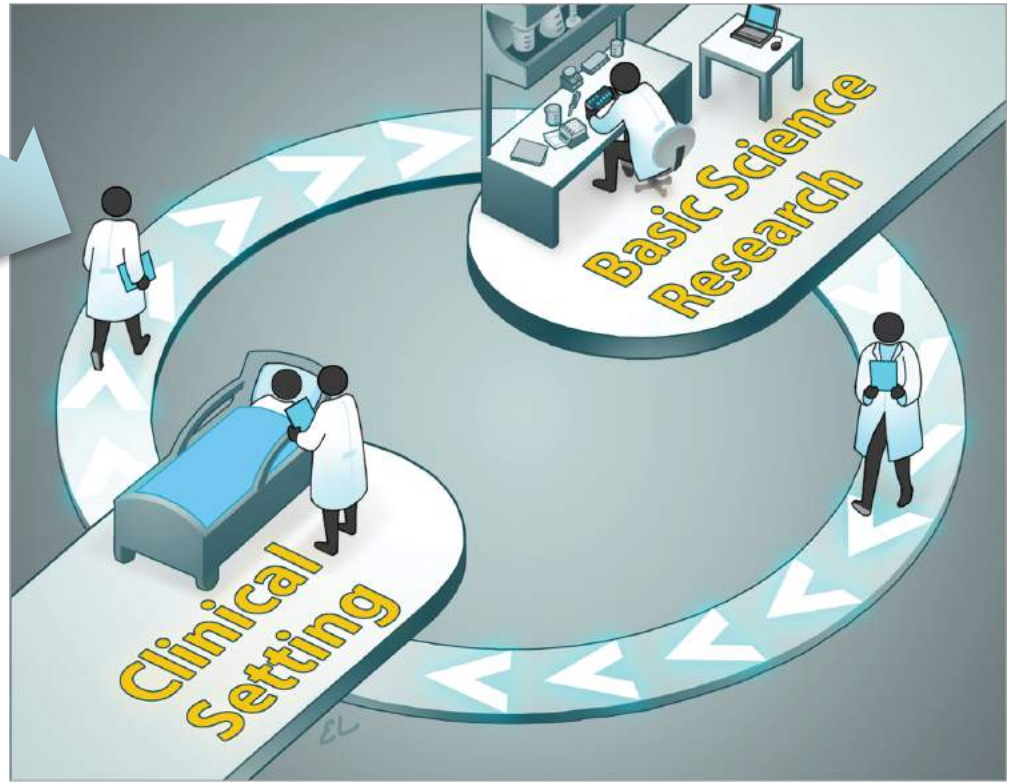
…. ?



Fundación InstitutoRoche

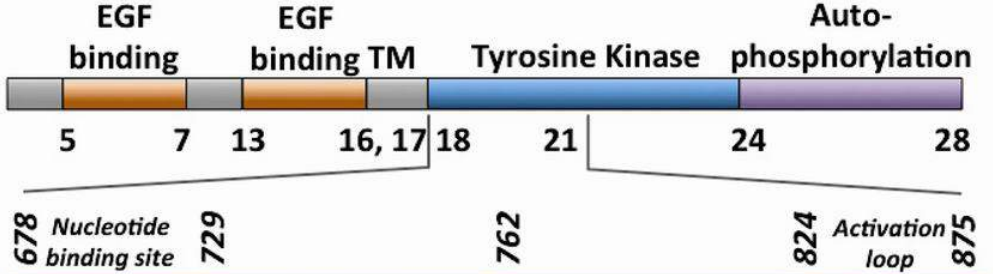
VNIVERSITAT DE VALÈNCIA


THEORY
PRACTICE

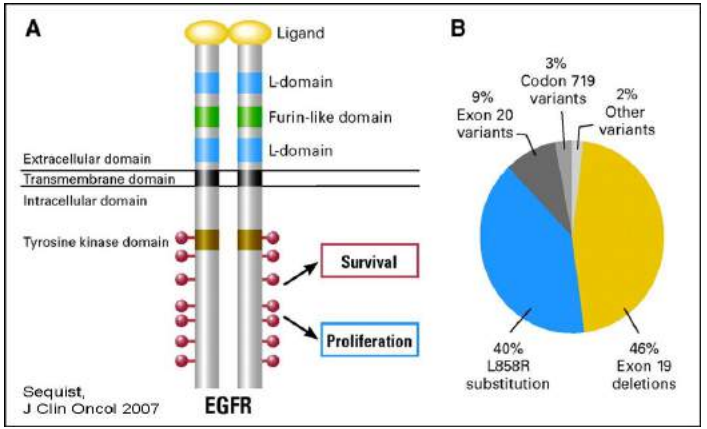

Basic Science Research
Clinical Setting

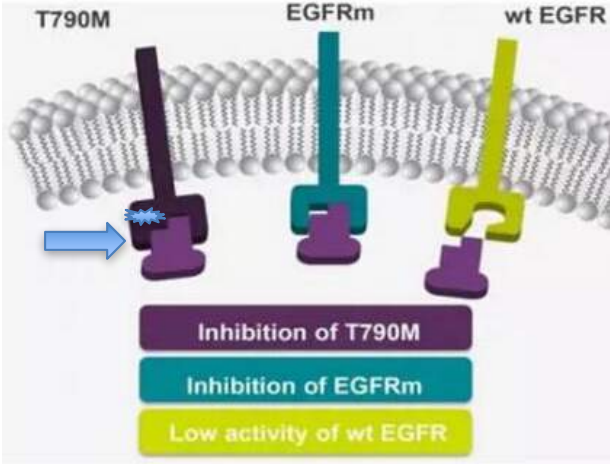
BIOMARCADORES: Terapias dirigidas

EGFR

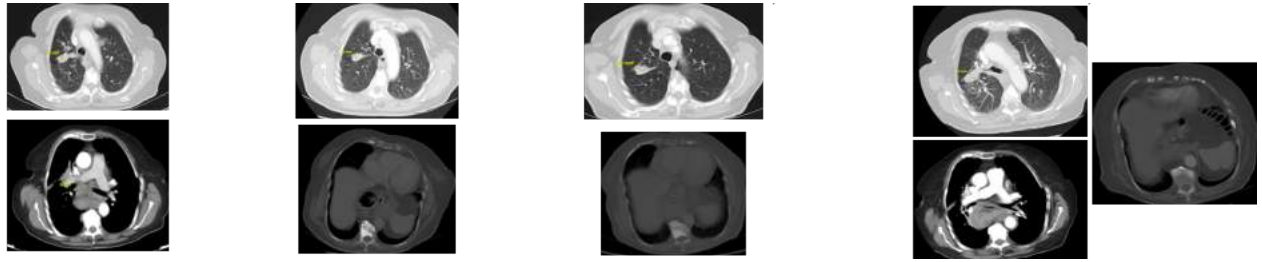


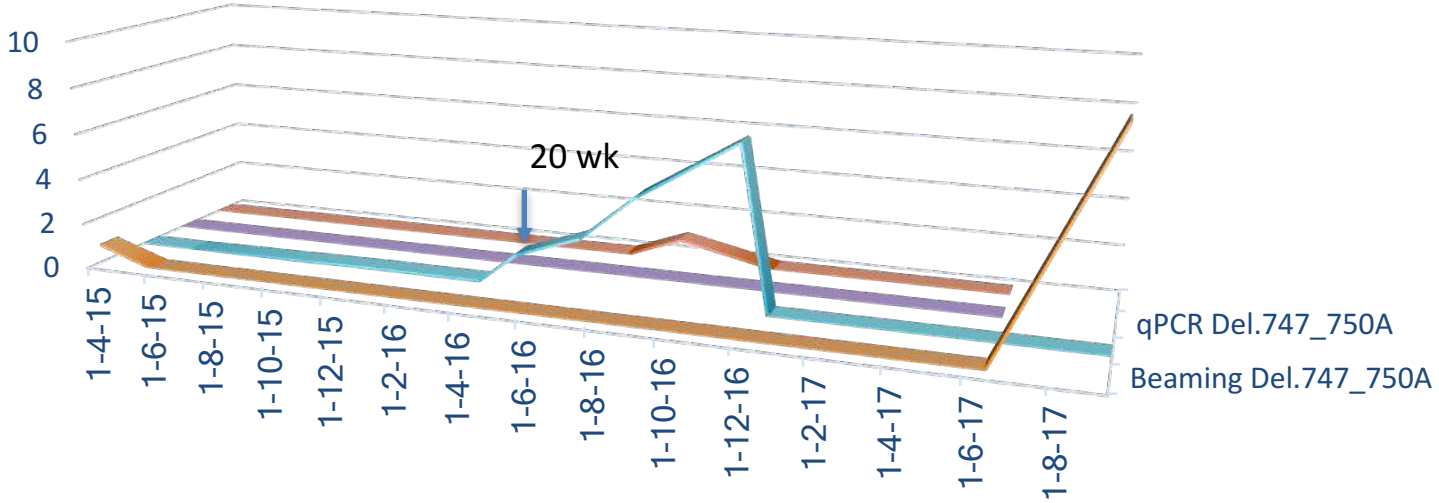
EXON 18	EXON 19	EXON 20	EXON 21
G719X (3%)	LREA deletion (45%)	V765A (<1%)	L858R (40%)
	VAIKEL insertion (1%)	T783A (<1%)	L861X (2%)
	L747S (<1%)	V774A (<1%)	T854A (<1%)
	D761Y (<1%)	S784P (<1%)	A871E (<1%)
		T790M *	
		Exon 20 insertion (4%)	
		V769M (<1%)	
		V769M (<1%)	





CASE 1: EGFR mut +

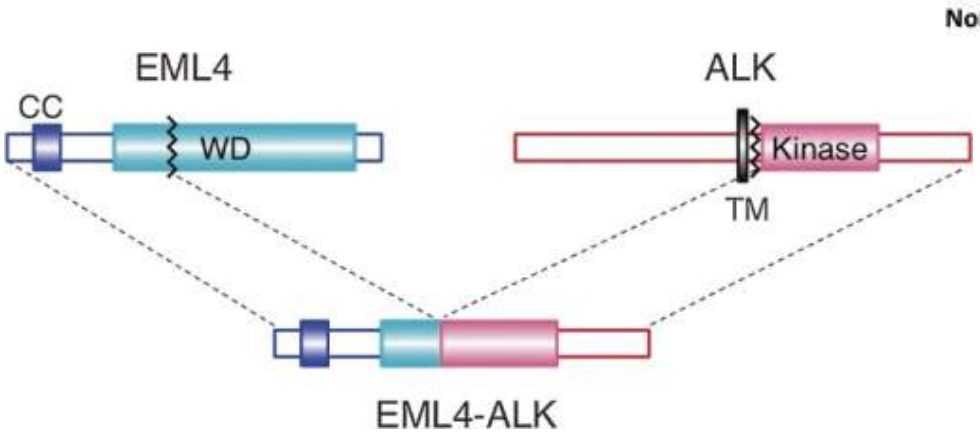


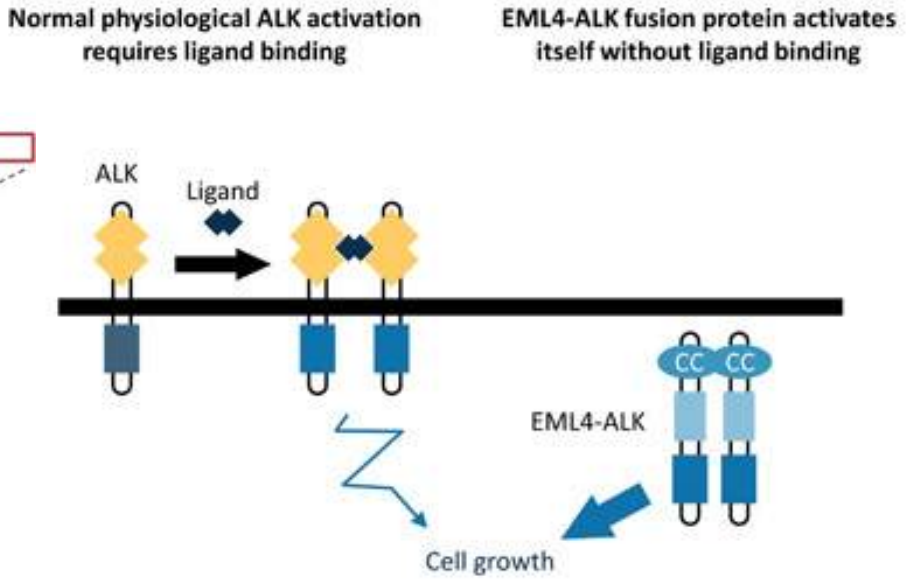


1st generation EGFR-TKI → 3rd generation EGFR-TKI

BIOMARCADORES: Terapias dirigidas

ALK





CASO 2: ALK + (traslocación EML4-ALK)

06/2016
Varón, 47 años, fumador.
CPNM, estadio IV, Mtx óseas, y ganglionares
ALK + por IHC y FISH

07/2016
Lesión encía/mandíbula

Diagnóstico: Junio 2016

Estudio inmunohistoquímico:

ALK: Positivo.
ROS1: Negativo.

24/06/2016 15:33 | Ampliación Diagnóstica (

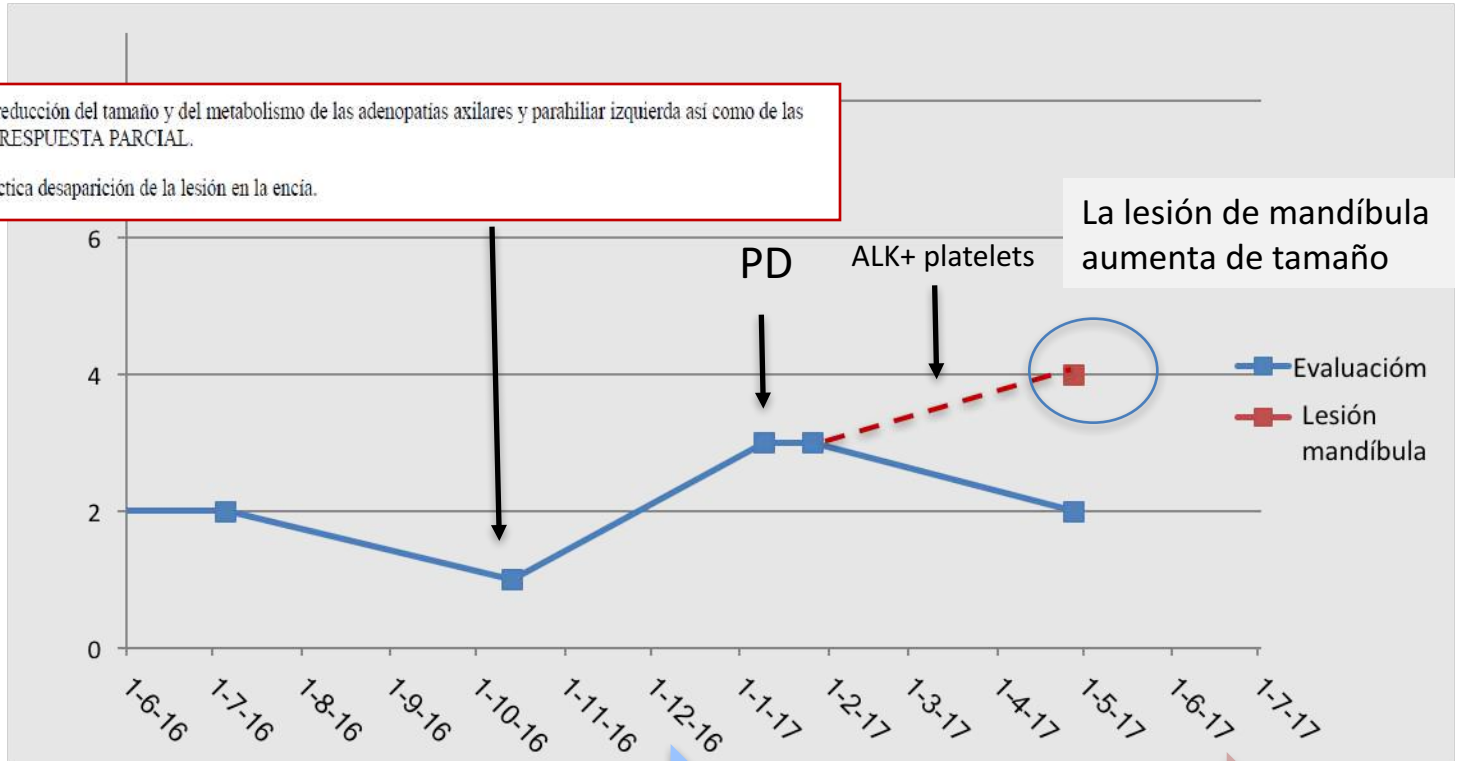
FISH ALK (2p23): POSITIVO

TKI (1º generación, Crizotinib)

CASO 2: ALK + (traslocación EML4-ALK)

PET-TAC: Marcada reducción del tamaño y del metabolismo de las adenopatías axilares y parahiliar izquierda así como de las metástasis óseas. --> RESPUESTA PARCIAL.

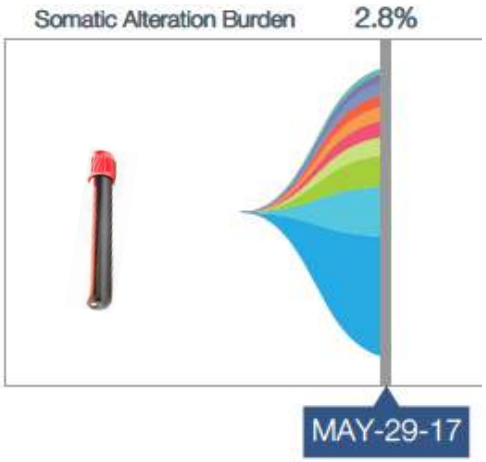
A la exploración, práctica desaparición de la lesión en la encía.



TKI (1º generación, Crizotinib)

TKI(2º generación, Brigatinib)

Jantus & Camps Lab



11 Total Somatic Alteration(s) Detected

6 with Associated Therapy
3 Associated with Lack of Response
Multiple Clinical Trials Available

Summary of Somatic Alterations & Associated Treatment Options

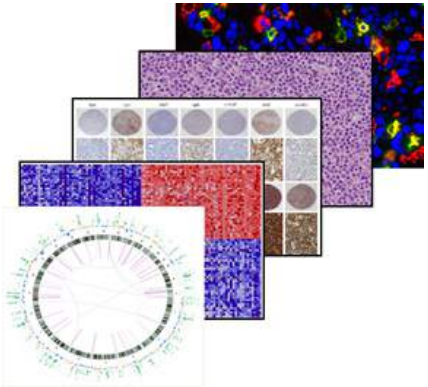
The percentage of altered cell-free DNA (% cfDNA) circulating in blood is related to the unique tumor biology of each patient. Factors that may affect the % cfDNA of detected somatic alterations include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, and treatment.

Alteration		% cfDNA or Amplification	FDA Approved in Indication *see page 4*	Available for Use in Other Indications *see page 4*	Clinical Drug Trials *see page 12*
Relevant for Therapy Selection					
ALK	*EML4-ALK fusion*	2.8	None	None	Trials Available
	G1202R	0.9	Lack of Response: Alectinib, Brigatinib, Ceritinib, Crizotinib	None	Trials Available
	F1174V	0.1	Lack of Response: Ceritinib, Crizotinib	None	Trials Available
	F1174C	0.05	Lack of Response: Ceritinib, Crizotinib	None	Trials Available

3 mutaciones de resistencia a Crizotinib y Brigatinib detectadas

ONCOLOGÍA DE PRECISIÓN

…. ?



Fundación InstitutoRoche

VNIVERSITAT DE VALÈNCIA

COMITÉ MOLECULAR DE TUMORES

COMITÉ MOLECULAR DE TUMORES

Diagnosis

Follow up

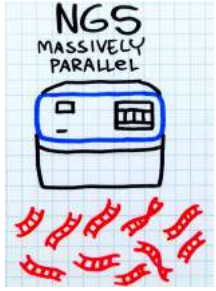
Progression

If...

Initial biopsy insufficient, unavailable, or undergenotyped

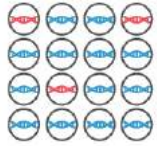
Molecular alteration detected

quantification



Targeted therapy 1

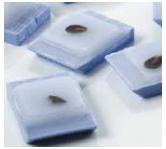
Molecular alteration detected



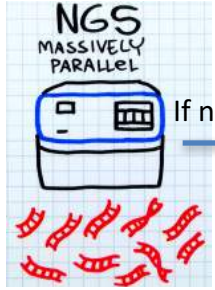
quantification

Targeted therapy 2

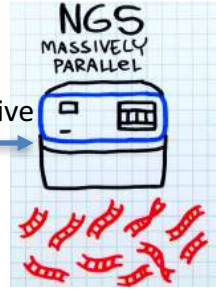
1st option

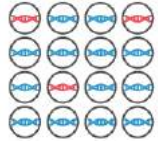


2nd option



If negative

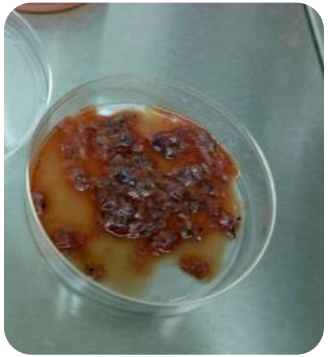




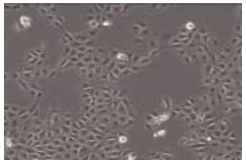
quantification

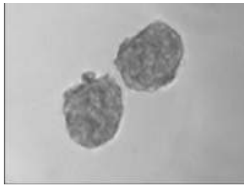
Jantus-Lewintre, 2018

Cultivos 3D - organoides : PRESENTE Y FUTURO



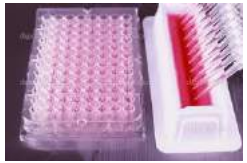
2D cultures

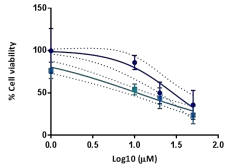


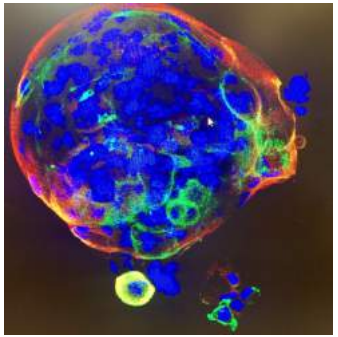


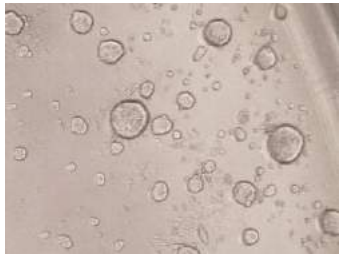
3D cultures
Tumorspheres

Drug Screening









EN RESUMEN…. La oncología de precisión

Aumenta tasas de supervivencia

Permite tratamientos mas seguros dirigido contra dianas (disminuyen efectos adversos)

Elimina el uso de tratamientos innecesarios o no efectivos

Mejora la eficiencia del sistema (no usar recursos en quienes no se van a beneficiar)

http://www.aseica.es/noticias/comprometidos-con-la-investigacion-en-cancer/

excelencia oncología molecular

www.oncologiamolecularvalencia.es

Eloisa Jantus Lewintre

jantus_elo@gva.es